GENOMIC EVIDENCE OF CHEMOTROPHIC METABOLISMS IN DEEP-DWELLING CHLOROFLEXI CONFERRED BY ANCIENT HORIZONTAL GENE TRANSFER EVENTS. L.M. Momper,[1] C.M. Magnabosco,[2] J.P. Amend[3] and G.P. Fournier[1], [1]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, (momper@mit.edu) [2]Center for Computational Biology, Simons Foundation [3]Department of Earth and Planetary Sciences, University of Southern California

Introduction: If there is or ever was life on an extraterrestrial planetary body, evidence of that life would likely be found below the surface. Some subsurface environments on Earth can be seen as analogs to the subsurface ecosystems on other rocky planets. In Earth's deep subsurface biosphere (DSB), water, organic carbon and chemical energy are often extremely scarce. However, it has been shown that archaea and bacteria are able to persist in the DSB to at least 3.5 km below surface [1]. Understanding how organisms persist, and on what energetics they subsist, is critical in predicting which extraterrestrial bodies may harbor similar, extant or extinct, life.

To address these questions we investigated four deep terrestrial subsurface environments: one legacy mine in South Dakota, USA, and three mines in South Africa. Boreholes within these mines provided access to fluids buried beneath the earth's surface and sampled depths as great as 3.1 km below surface. Geochemical data were collected from deeply-circulating anoxic subsurface fluids concomitantly with DNA for metagenomic and genomic sequencing. We examined genomes of the ancient and deeply branching phylum *Chloroflexi* for chemolithotrophic metabolic capabilities and interrogated the geochemical drivers behind those metabolisms with thermodynamic modeling of reaction energetics at each site.

Results: A total of 19 *Chloroflexi* genomes greater than 75% complete were identified and analyzed from the four subsurface sites. Genes for nitrate reduction (*nar*) were found in 10 of the 13 South Africa *Chloroflexi* but were completely absent from genomes collected in South Dakota. Indeed, nitrate reduction was among the most energetically favorable chemolithotrophic reactions in South African fluids (10^{-14} kJ $cell^{-1}$ sec^{-1} per mol of reactant) [2, 3]. Conversely, genes for nitrite and nitrous oxide reduction (*nrfD, nirG* and *nosZ*) were found in three of the six genomes collected in South Dakota, but not in the South Africa genomes. The canonical genes implicated in sulfate reduction (*dsrAB*) were found in genomes from both geographical locations. Thermodynamic modeling of sulfate reduction in South Dakota and South Africa fluids showed sulfate reduction was energetically favorable with ferrous iron and/or hydrogen as electron donors (-10 to -20 kJ/mol e- in South Dakota and -10 to -13 kJ/cell/sec, in South Africa fluids) [3, 4].

Evidence for horizontal gene transfer events. We examined the origin of genes conferring chemolithotrophic metabolic capabilities in the 19 *Chloroflexi* genomes. We discovered evidence for horizontal gene transfer (HGT) from the phylum *Firmicutes* for both *nrfD* and *dsrAB* and HGT of *nosZ* from within the *Proteobacteria.* Retention of these genes in *Chloroflexi* lineages indicates this conferred an advantageous metabolism in terrestrial subsurface environments. We are using molecular dating techniques and phylogenetic comparative methods to constrain the timing of these HGT events on a geologic timescale.

Broader implications: Studying microbial communities in extreme environments on Earth will help us to form testable hypotheses about the existence of communities on extraterrestrial bodies. Combining geochemical metadata with genomic and metagenomic analyses may enable us to predict what metabolic pathways would be likely under extraterrestrial geochemical conditions.

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References:

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