

**METAGENOMIC INSIGHTS INTO S(0) PRECIPITATION IN A TERRESTRIAL SUBSURFACE LITHOAUTOTROPHIC ECOSYSTEM.** T. L. Hamilton<sup>1</sup>, D. S. Jones<sup>2</sup>, L. Tsao<sup>3</sup>, I. Schaperdoth<sup>3</sup>, J. L. Macalady<sup>3</sup>, <sup>1</sup>Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA, trinity.hamilton@uc.edu, <sup>2</sup>Department of Earth Sciences, University of Minnesota, Minneapolis, MN, USA; <sup>3</sup>Department of Geosciences and the Penn State Astrobiology Research Center (PSARC), The Pennsylvania State University, University Park, PA 16802, USA.

**Introduction:** The Frasassi cave system in Italy is host to isolated lithoautotrophic ecosystems characterized by sulfur-oxidizing biofilms with up to 50% S(0) by mass. The net contributions of microbial taxa in the biofilms to production and consumption of S(0) are poorly understood and have implications for understanding the formation of geological sulfur deposits as well as the ecological niches of sulfur-oxidizing autotrophs. Filamentous *Sulfurovum*-like (Epsilonproteobacteria) populations are among the principal biofilm architects in Frasassi streams, colonizing high-sulfide, low-oxygen niches relative to other major biofilm-forming populations such as “*Candidatus Thiobacillus barengensis*”-like populations (Gammaproteobacteria). The *Sulfurovum* clade contains only a few isolates and “*Ca. Thiobacillus barengensis*” is poorly characterized despite a number of environmental sequences affiliated with both of these taxa retrieved from sulfidic caves and springs. Here, we employed metagenomic sequencing to probe the metabolic potential of these poorly characterized strains in S(0)-rich biofilms to better define their role in subsurface biogeochemical cycles.

Sequencing of eight biofilm samples revealed diverse Epsilonproteobacteria and abundant “*Ca. Thiobacillus barengensis*”-like sequences. Analyses of the assembled data indicate that the dominant populations of Epsilon- and Gammaproteobacteria are autotrophic and thus putatively provide organic carbon to the isolated subsurface ecosystem. After binning, we retrieved 4 nearly-complete genomes of *Sulfurovum*-like organisms and a “*Ca. Thiobacillus barengensis*”-like spp.. Multiple homologs of sulfide-quinone reductases, together with incomplete or absent Sox pathways, suggest a prominent role for *Sulfurovum*-like populations in S(0) precipitation via sulfide oxidation. In contrast, we recovered homologs of the complete complement of Sox proteins affiliated with “*Ca. Thiobacillus barengensis*”-like populations and less abundant *Sulfuricurvum* and *Arcobacter* spp., suggesting these populations are capable of complete oxidation of sulfide to sulfate. The genomes (and metagenomes) provide new insights into the ecological success of these organisms in sulfidic cave waters and indicate a role for *Sulfurovum*-like populations in S(0)-production in subsurface environments.