

Searching for a Thermodynamically Predicted Novel Microbial Metabolism in the Sulfidic Karst Caves at Frasassi, Italy

Research Results

A field expedition to the Frasassi Caves (Ancona, Italy) took place from July 8 – July 28. Four separate field sites within the caves were visited: Ramo Sulfureo, Pozzo dei Cristalli, Grotta Bella, and Grotta Nuova di Rio Garrafo at Acquasanta Terme. At each site, geochemical and biological samples and measurements were collected. pH, temperature, conductivity, oxidation/reduction potential were measured within the streams. ENMET meters were used to measure gas concentrations in the air, including H₂S, O₂, NH₃, O₂, CH₄, SO₂, and CO₂. Draeger tubes were used to collect more precise measurements of gaseous H₂S, NH₃, SO₂, and CO₂. Water samples were collected for measurements of dissolved gases, dissolved inorganic carbon, metals, and concentrations of various cations and anions. Measurements of these samples using ion chromatography, gas chromatography, and inductively coupled plasma mass spectrometry are ongoing. These geochemical measurements will be converted to chemical activities and will be used to calculate Gibbs energy yields of various redox reactions that may serve as microbial metabolisms at each site.

Sediment samples were collected at Ramo Sulfureo and Pozzo dei Cristalli. Samples were collected for microbial cultivation, metagenomic sequencing, and transcriptomic sequencing. Sediment was used to inoculate media for the enrichment of sulfur disproportionators under several treatment conditions. As the enrichments progress, samples will be taken for transcriptomics to determine how gene expression changes under each treatment.

Snottites (extremely acidic microbial biofilms) were collected at Ramo Sulfureo, Grotta Bella, Grotta Nuova di Rio Garrafo, and Pozzo dei Cristalli. Snottites were collected for microbial cultivation and metagenomic sequencing. Samples for cultivation were used to inoculate media designed to enrich for potential sulfur comproportionating microorganisms. Sulfur comproportionation ($3\text{H}_2\text{S}_{(\text{aq})} + \text{SO}_4^{2-} + 2\text{H}^+ \rightarrow 4\text{S}^0 + 4\text{H}_2\text{O}$) is an as-of-yet undiscovered microbial metabolism that is energy-yielding under the geochemical conditions present at Frasassi. These enrichments are still in progress, and are being monitored for increases in cell density and decreases in sulfate and sulfide concentration.

DNA is currently being extracted from all biological samples, including ongoing enrichments. The 16S rRNA gene will be amplified and sequenced from these DNA samples to assess microbial diversity, and metagenomes will be sequenced from snottites, sediments, and enrichments for the reconstruction of microbial genomes. Comparisons of microbial diversity within snottites and sediments between all sites will be analyzed and novel microbial species will be characterized and described.