Metagenomics reveal structure and function of extremely acidic sulfur oxidizing cave wall biofilms

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Community genomic analyses provide a link between microbial metabolic potential and the biogeochemistry of an environment. In this study we use metagenomics, rRNA methods, culturing, and lipid analyses to explore the community structure and function of extremely acidic (pH 0-1) 'snottite' biofilms from sulfidic caves. Snottites form on cave walls and ceilings in areas where gypsum weathering crusts isolate microbial activity from limestone buffering. Full cycle rRNA methods have shown previously that snottites have very low biodiversity and are dominated by relatives of *Acidithiobacillus thiooxidans*, Thermoplasmatales-group archaea, and *Acidimicrobium spp*.

In order to explore snottite community metabolism, we pyrosequenced 12 megabases (Mb) of metagenomic DNA from a sample collected in the Frasassi cave system, Italy. Taxonomic classification of phylogenetic marker genes in the metagenome agrees with the community structure determined independently using rRNA methods. Unassembled metagenome reads were annotated to COG and GO catagories. Overrepresented functions include cation transport and lipid biosynthesis genes that offer clues about how snottite populations survive in the extremely the low pH of the biofilm matrix. Near-complete genomic coverage of the dominant *Acidithiobacillus* phylotype allowed for the identification of specific sulfur oxidation, carbon fixation, and nutrient uptake mechanisms. The dominant archaea were discovered to be wall-less members of the 'G-plasma' clade in the Thermoplasmatales. Despite lower genome coverage, available data suggest that both the G-plasma and *Acidimicrobium* phylotypes are heterotrophs.

We recently obtained two larger metagenomic datasets (roughly 200 Mb each) from snottites collected in the Frasassi (F) and nearby Acquasanta (AS) cave systems. While both datasets are dominated by *Acidithiobacillus spp*., AS snottites contain a large proportion of archaea related to *Ferroplasma* spp., while F snottites are made up of a more diverse community including 'G-plasma', *Acidimicrobium*, and rare taxa. Comparative analysis of all three metagenomes will provide insight into functions and adaptations unique to each community, as well as further define a metagenomic signature of acidic and/or sulfur-oxidizing environments.

Organic toxicity may be a factor during stimulation of biogenic methane from coal

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In order to meet the world's growing energy need there is increasing interest in stimulating microbial methane production from geopolymers such as coal and shale in existing natural gas wells and plays. One possible approach for *in situ* stimulation of microbial methane production involves the accelerated release of soluble organic substances from coal. In microcosms designed to evaluate gas production and the potential for enhanced biogas production in subbituminous coal (Wilcox group, south Texas, and Wyodak-Anderson in the Powder River Basin), we showed that there are three steps involved in generating methane from coal: 1) the release of long chain fatty acids (LCFA), long chain alkanes (LCA), and single ring aromatics (SRA) to solution, (2) the degradation of these intermediates to methane precursors (primarily acetate), and (3) the generation of methane. Degradation of LCFA, LCA, and SRA has been previously observed in methanogenic consortia, but some of these compounds can also be bacteriocidal (toxic to microorganisms). In the TX coal microcosms, acetate accumulated and was not converted to methane until the concentration of soluble intermediates was significantly reduced. Furthermore, although methanogens were present, their growth was inhibited when the concentration of the soluble intermediates was high, and the population of hydrogenotrophic methanogens decreased as LCFA in solution increased. The methanogenic consortium WBC-2, which generated methane from coal, was used to study toxic effects of LCFA, LCA, and SRA. The addition of Ca^{+2} increased methane generation by forming organic salts, effectively decreasing solution levels of some organics. The accelerated release of coal organics to solution may be a viable approach to stimulating biogenic methane renewal, however the toxicity of organic intermediates will need to be mitigated if this approach is to be successful.