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# Microbially mediated $FeS_2$ formation from FeS

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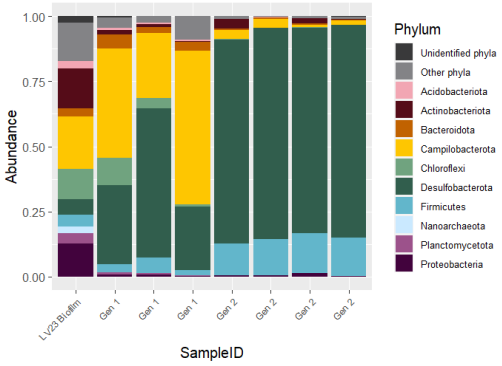


Dive exploration and geobiological investigation of a permanently stratified, carbonate-hosted karst aquifer previously revealed that it hosts a microbial ecosystem based on lithoautotrophy, and that the ecosystem is taxonomically and geochemically coherent on a kilometer scale. The deep layer of the aquifer is euxinic and features decimeter- to meter-scale, hanging biofilms containing abundant pyrite framboids. In an effort to understand potential microbial involvement in pyrite precipitation, we used the biofilm material to inoculate an enrichment medium containing FeS, $H_2S$ and $CO_2$ as sole carbon and energy sources. The enrichments produce $FeS_2$ microspheroids in close association with cells in an extracellular polymeric substance (EPS) matrix. Notably, no $FeS_2$ is produced in uninoculated or inoculated killed controls, showing that FeS conversion to $FeS_2$ on a months-long timescale at 25 °C requires microbial activity. The enrichments do not produce detectable methane and very few 16S rRNA sequences affiliated with known methanogenic or anaerobic methanotroph (ANME) taxa have been retrieved in amplicon libraries constructed from enrichment DNA. As expected, amplicon libraries also show that enrichments have lower taxonomic richness than the biofilm inoculum. Despite a lack of added sulfate in the medium, second-generation enrichments are dominated almost exclusively by amplicons in the phylum Desulfobacterota (Figure 1). The most abundant genera are *Desulfomicrobium*, *Desulfocapsa*,*Geotalea*, and MSBL7. The known physiologies of these taxa (aside from MSBL7) are consistent with low sulfate and organic carbon availability and suggest that electrons from the FeS/$H_2S$ system flow into microbial metabolism either in the form of $H_2$ released by pyrite formation, or by direct electron transfer from the FeS surface. Given our observations to date, possible fates of these reducing equivalents include $CO_2$ reduction to biomass and/or acetate. Some DNA sequences amplified from the enrichments cannot be assigned to order, class, or phylum, and taxonomic novelty at and below the family level is especially high (up to 55% of sequences). Metagenomic sequencing of the enrichments in progress will confirm or expand our knowledge of metabolic capabilities in the dominant enrichment populations and further constrain hypotheses about the mechanisms of microbial conversion of FeS to $FeS_2$.