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The iron-sulfur world in a bottle: FeS_2 formation in microbial enrichment cultures

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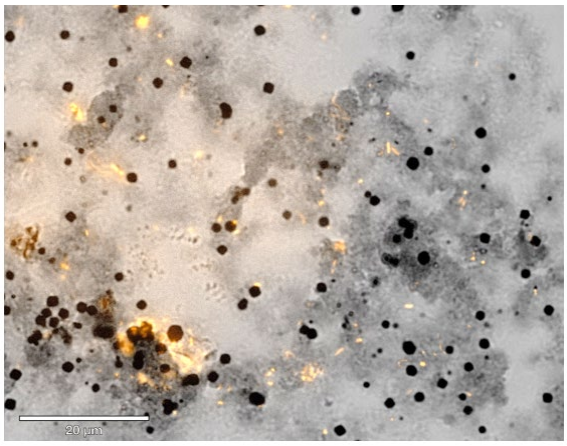

Figure 1. Fluorescence microscopy image overlaid onto a transmitted light image of the same field of an enrichment culture after one year of inoculation. PFA-fixed and DAPI-stained cells are shown in orange while the FeS_2 mineral grains appear black.

The Frasassi cave system in central Italy hosts a permanently stratified sulfidic aquifer with a shallow microoxic zone and a deeper anoxic zone. The deep aquifer lacks most of the oxidants used for respiration by surface life, and has sulfate concentrations of approximately one-eighth of marine, making it a promising analog for Proterozoic oceans. Biofilms in the deep aquifer contain abundant pyrite (FeS2) mineral grains and a treasure trove of microbial "dark matter," microorganisms with only distant relatives in public databases. There are multiple formation pathways for FeS2 [1], and the role that microbes play in iron sulfide mineral nucleation and crystal growth is the topic of ongoing debate.

In February 2023, Frasassi deep aquifer biofilms were retrieved to inoculate enrichment cultures targeting a "missing" metabolism–Wächtershäuser's proposed pyrite- and hydrogen-producing metabolism that reacts iron sulfide (FeS) with hydrogen sulfide [1]. After a year of incubation, clusters of cells and framboid-like mineral grains were observed with transmitted light and epifluorescence microscopy (Fig. 1). Further imaging and mineral analyses were performed using Raman spectroscopy and SEM-EDS. DNA extracted from the biofilm (inoculum) and the enrichment cultures were analyzed using 16S rRNA amplicon sequencing to determine the taxonomy of the major populations. Raman and SEM-EDS data indicate the presence of FeS2 in the enrichment culture bottles but not in the abiotic controls. Mineral grains appear pseudo-framboidal but do not look like microbially-mediated pyrite produced in the literature. The enrichment communities show changes in structure from the native biofilm and are much less diverse. Known sulfur cyclers are abundant in the bottles, yielding clues to the potential role of the enrichment microbes in FeS2 formation.

[1] Rickard & Luther (2007), *Chemical Reviews* 107, 514–562.

[2] Wächtershäuser (1988), *Systematic and Applied Microbiology* 10, 207–210.