In situ mineral incubation experiments reveal patterns in microbial colonization within a warm deep subsurface Carbonate Aquifer

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Introduction

The terrestrial deep biosphere represents an important analog environment for astrobiology, as these environments are almost universally anoxic, hot, and characterized by low carbon fluxes, highly reducing conditions, and little to no direct solar energy input—all conditions expected to be common among habitable extraterrestrial bodies. These environments have been traditionally understudied and our understanding of colonization and microbial life strategies, selection pressures, and detectable biosignatures is extremely limited. Here we describe *in situ* microbial colonization experiments conducted in a ~750 m-deep borehole (BLM-1) within a hot carbonate aquifer in the Mojave Desert, CA. The borehole is cased in unscreened steel to a depth of 730m and open to virgin rock and warm (57ºC) reducing fluids at the bottom of the hole. This hole passes through several rock types, however open hole section is limited to the Hidden Valley Dolomite geological unit. Six sets of *in situ* incubations were conducted over a period of two years. Flow-through columns filled with various locally-sourced and laboratory-standard sterilized minerals were incubated in the open hole portion of the aquifer for several months before recovery and geochemical characterization of the aquifer waters was conducted. Colonizing microbial assemblages were characterized using iTAG sequencing and rock surface colonization patterns were analyzed by both microscopic and spectroscopic techniques, including deep-UV fluorescence microscopy, Raman spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX).

Results & Discussion

These analyses revealed a large population of candidate division organisms (KB-1) as well as the consistent presence of putative nitrifying and sulfate-reducing bacteria. Secondary framboidal pyrite was also observed on some of the incubated dolomite coupons, associated mainly with two main bacterial OTU's in the incubation—one putative sulfide-oxidizing (*Sulfurovum)* and one sulfate-reducing (*Desulfotomaculum*) organism. Enrichments of hydrogen-oxidizing bacteria associated with the low-carbon steel incubation implicate anaerobic corrosion of the steel casing as important consideration for any deep biosphere studies of cased boreholes. The influence of carbon and nitrogen amendment *in situ* was tested used sterile collagen-rich sponges as a nutrient source and the effect on the mineral-colonizing microbes was tracked by iTAG sequencing. These time course analyses revealed the major microorganisms responsible for initial labile carbon degradation through methanogenesis and, finally, a reset of the *in situ* diversity to the original, persistent, slow-growing community. These results also provide an estimate for the carbon retention time in the subsurface system of approximately nine months. Taken in sum, they not only demonstrate the efficacy of several tools in detecting subsurface life, but afford a rich portrait of the conditions and niches in one subsurface ecosystem, the dominant organisms and their metabolic byproducts, and an estimate for these organisms' activity.

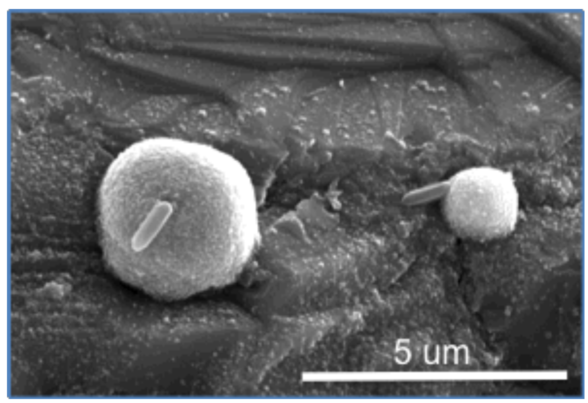

Figure 1: Scanning electron micrograph of microorganisms colonizing secondary framboidal pyrite on dolomite. Iron sulfides (round features) were identified with EDX.

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