Microscopic and Spectroscopic Analysis of Microbes Colonizing Mineral Coupons Incubated at ~750 m Depth in a Borehole near Death Valley, CA, USA.
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Introduction: The study of 'intraterrestrials' has been facilitated by recent unprecedented access to the deep subsurface via mines, like the Sanford Underground Research Facility, and boreholes. This abstract details one approach involving *in situ* microbial colonization experiments, conducted within a ~750-m-deep monitoring well (BLM-1). This well was drilled in 2005 into an anoxic, thermal aquifer beneath the Mojave Desert near Death Valley National Park in California. The borehole was originally created for sampling water from the Lower Carbonate Aquifer of the "Death Valley Regional Flow System" discharge zone. The hole is, thus, continuously cased (steel) to 730 m; with an open hole completion to hole bottom at about 750 m. Although the hole bisects a variety of lithologies (e.g. volcanic tuff, lake sediments and freshwater carbonates), the uncased segment is confined to the Hidden Valley Dolomite geological unit. To better understand the microbiology throughout the BLM-1 water column, a line was suspended for several months, with sponges attached roughly every 30 meters and flow-through columns containing various mineral samples in the open hole portion. Enrichments and microbial isolations from sponges, formation waters, and mineral samples were used to explore the culturable fraction of the microbial assemblage and their physiological characteristics. DNA-based characterizations of microbial community structure (16S and 18S rRNA gene libraries) are also in progress. In tandem with the community analysis, microscopic and spectroscopic techniques (scanning electron microscopy (SEM), are being applied to quantify and characterize the distribution of the microorganisms on the different substrates.

Results/Discussion: Substrates for the colonization experiment were chosen to represent both simple tractable surfaces and some of the native rocks collected during the drilling of the borehole itself (e.g. calcite, dolomite, glass and steel). DNA from the sponges and minerals was recovered and sequenced to determine bacterial and archaeal community structure. Microscopic analysis revealed that both minerals and sponges were colonized within 3 month incubation period. Colonization of the sponges, as examined microscopically, was highest for samples in the open hole near the bottom and sparse within the steel-encased regions at shallower depths. Neither dense biofilms nor extensive coverage by polysaccharides were observed on the mineral substrates; rather, relatively diffuse colonization was the rule. This may be due to the relatively short deployment or may be more representative of the density of surface-associated cells in the deep subsurface [1]. On the dolomite samples, microorganisms were observed to be closely associated with small (i.e. <5 µm) pyrite grains. Pyrite is present in the uninoculated dolomite samples however these grains are larger and incorporated in the substrate matrix. Thus, the pyrites observed on the surface of the mineral coupons appear to have formed during the incubation period. It is not known whether the formation of these pyrite grains are catalyzed biogenically, but the close association with individual microbial cells is suggestive of a correlative relationship (Fig 1).

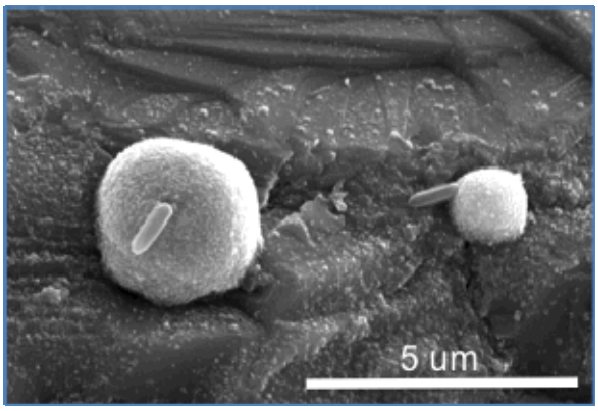

Figure 1: Scanning electron micrograph of a mineral chip (Hidden Valley Dolomite from BLM-1 at 750m depth) showing cells associated with small iron sulfides, presumably pyrite.

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References: [1] Wanger. G et al. (2006), Geomicro 23: 443-452.