BIOMARKERS IN CARBONATE THERMAL SPRING DEPOSITS: IMPLICATIONS FOR MARS.
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Introduction: We are engaged in a study of the signatures produced by contemporary biogenic activity (biomarkers) in carbonate thermal springs. We are examining deposits from these environments to determine the preservation of microbes, biofilms, trace biochemical markers and petrographic fabrics indicative of life.

Evidence of possible relict biogenic activity has been reported in carbonate inclusions within martian meteorite ALH84001 [1]. The initial evidence included ovoid and elongated forms, 50 to 500 nm in length, that are morphologically similar to but significantly smaller than many terrestrial microbes. More recently, structures resembling the remains of organic biofilms have been reported in the same meteorite [2].

Carbonates have also been discussed in the context of Mars sample return missions. Thermal spring deposits have often been cited as prime locations for exobiological exploration [3]. By analogy to Earth, specialized microbes may have existed in the heated, mineralized waters, and precipitates of carbonate and/or silica from these waters may have trapped and preserved evidence of life. Since the geological interactions which produce thermal springs can be recognized in orbital imagery, directed searches for microfossils in such deposits are deemed possible [4].

Samples and Methods: We are currently studying samples from four active thermal springs: Le Zitelle in the Viterbo region of Italy [5], Narrow Gauge in the Mammoth complex of Yellowstone National Park, WY [6], Hot Springs National Park, AR [7] and springs at Jemez on the slopes of the Valles caldera, NM [8]. In each case, water reaches the surface at 60 to 72°C and near-neutral pH (6.3 to 7.5), nearly saturated with respect to $CaCO_3$, and rapidly precipitates large amounts of aragonite and calcite.

The Italian, Yellowstone and Jemez springs were sampled at the surface, while the Hot Springs National Park sampling was conducted in a subterranean tunnel. Samples were fixed in glutaraldehyde, alcohol dehydrated, air dried or critical point dried, etched in 1% HCl and examined in a high resolution SEM.

Microbes: The near-vent environments of thermal springs support a variety of microorganisms. *Thermothrix thiopara*, a prolific sulfur-oxidizing bacterium [9], is the highest-temperature species identified at our sites in Yellowstone and Jemez. Pentecost [10] identified a species of the photosynthetic, filamentous bacteria *Chloroflexus* as the dominant form immediately downstream from the vent of the Italian spring. Spherical microbes as large as 15 μm in diameter populate the waters at Hot Springs National Park. Rod-shaped microbes several micrometers in length are found in samples from all four sites.

The thermal spring deposits also contain objects, significantly smaller than conventional bacteria, some of which appear to be biological. Large numbers of 100-200 nm spheres, the first "nanobacteria" described by Folk [11], are common in portions of the Italian and Yellowstone carbonates. The spheres are composed of C, O, F, P and Ca with minor Si and S, and are often found enmeshed in organic mucus. Numerous 300-500 nm rods and spheres, some apparently dividing, populate the Hot Springs National Park samples.

Many of the microbes die and their remains are rapidly destroyed in carbonate thermal spring environments. Organic matter is generally rare in carbonate sinters deposited at >30°C, suggesting that decomposition rates in such thermal environments are very high [12]. Detailed SEM examination of samples from all four localities consistently reveal mineralized cell remains but only scattered intact cells.

While thermal spring microbes are apparently poorly preserved in carbonates, fossilization by silica can provide enduring evidence of life. At Jemez, amorphous silica spheres 50 to 300 nm in diameter coat and preserve the forms of dissolved 1 to 2 μm cells [8]. A wide variety of thermophilic bacteria has been found well preserved in the silica sinter deposited by many Yellowstone thermal features [13]. Similar fossil assemblages have been described in siliceous thermal spring deposits as old as 400 Ma [14].

Biofilms: The three-dimensional network of polysaccharide matrix, living cells and cell remains which constitutes a biofilm is a distinctive macroscopic biomarker [15]. Samples from all four of our sites contain biofilms in various states of preservation.

Biofilms in our samples are stable in water at least as hot as 72°C and retain their three dimensional nature as the water cools. Upon drying, however, the polysaccharide matrix shrinks and deforms, becoming strand-like but preserving its intercellular binding structure. Carbonate samples display extensive biofilm remains after years of desiccation.

Morphologic evidence of biofilms can be preserved by mineralization. Biofilms in the Jemez samples are extensively coated with the 50 to 300 nm diameter silica spheres [8]. Cady and Farmer [13] have demonstrated that silica in thermal springs preferentially nucleates on and preserves organic mucus. Westall [16,17] provides evidence of silicified biofilm in Late Miocene (~10 Ma) deep-sea sediments and Archean (3.4 Ga) rocks.

Trace Biochemical Markers: Aragonite fragments from the Yellowstone site were found to contain microbial cell wall constituents as assayed with the limulus amebocyte lysate (LAL) test [18]. The LAL technique is capable of detecting 0.05 picograms of lipopolysaccharide (LPS) or beta glucan per mg of solid (0.5 ppb). Preliminary tests of a Yellowstone aragonite needle, shown by SEM analysis to contain only scattered microbial cells and mucus, were able to detect 200 pg/mg of LPS in the carbonate interior. This is equivalent to ~200 cells per mg of aragonite.

Petrographic Fabrics: The importance of microbes in promoting the deposition of calcium carbonate, particularly aragonite, in thermal springs is a matter of ongoing debate. Pentecost [10] argues against significant microbial influence in the case of Yellowstone deposits. He accepts microbially-induced precipitation only in cases where aragonite crystals clearly copy the structures of filamentary bacteria. Other investigators, however, provide evidence for a range of bacterially induced lithification styles [19].

Thermothrix thiopara, found near the vents of our Yellowstone sampling sites, forms filaments up to 10 cm long. Immediately downstream, the filaments become overgrown by aragonite. The result is a mass of parallel aragonite needles, each millimeters in diameter and centimeters long, which closely mimic the *Thermothrix* filaments. In this case, bacterially-induced precipitation yields a distinctive petrographic fabric in the carbonate rocks which is specifically attributable to life. This fabric has been recognized in deposits as old as ~360 Ma [12].

Biological action can also promote the selective dissolution of minerals. Pitting of calcite crystals is a common feature of the Jemez samples [8]. Pristine crystals show no evidence of microbes, while pitted areas are invariably associated with biofilms.

Implications for Mars: Previous studies of microbes in thermal springs, coupled with these results, have implications in the search for martian life:

- Specialized microbes are common in carbonate-precipitating environments as hot as 72°C.
- Several types of microorganisms in such environments are significantly smaller than 1µm.
- Biofilms are characteristic of thermal spring microbial communities.
- Microbes are poorly preserved in carbonates.
- Silicified microbes and biofilms can be well preserved over geologic time.
- Cellular remains, while constituting <1 ppm of some high-temperature thermal spring carbonate deposits, are readily identifiable.
- Some petrographic fabrics, recognizable in the geologic record, are specifically attributable to thermal springs microorganisms.

The submicrometer organisms approach the size range of possible microfossils described in ALH84001. Mineralized biofilms resemble structures found in that meteorite. The preservation of morphological and chemical biomarkers at all of our field sites supports the sample return strategy of searching for biomarkers in thermal spring deposits on Mars.

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