

**MICROBES IN CARBONATE THERMAL SPRINGS: HOT SPRINGS NATIONAL PARK.** Carlton C. Allen<sup>1</sup>, Anne E. Taunton<sup>1</sup>, Michael R. Taylor<sup>2</sup> and David S. McKay<sup>3</sup> <sup>1</sup>Lockheed Martin, Houston, TX 77258 carlton.c.allen1@jsc.nasa.gov <sup>2</sup>Henderson State University, Arkadelphia, AR 71923 <sup>3</sup>NASA Johnson Space Center, Houston, TX 77058.

Evidence of possible relict biogenic activity has been reported in carbonate inclusions within martian meteorite ALH84001 [1]. We are studying evidence of contemporary biogenic activity in a possible terrestrial analog, high temperature thermal springs which precipitate calcium carbonate minerals [2,3]. We are examining thermophilic organisms which live in such environments and their relationship to the deposition of carbonates and other minerals.

**Samples** Hot Springs National Park, Arkansas, includes approximately 47 thermal springs. Water reaches the surface at a mean temperature of 60 to 70°C [4]. The spring waters are mildly basic (pH 7.1 to 7.5) and average 162 ppm bicarbonate, 45 ppm calcium, 45 ppm silica and 7.8 ppm sulfate [4].

The springs have experienced extensive commercial development, prior to and following their incorporation into the Park. All have been excavated, lined with masonry and their water channeled to a series of bathhouses. Water at all sampling locations either flowed through or in the vicinity of iron pipes.

We sampled carbonates and water from several underground springs in the Park. We also placed sterilized glass slides in a number of springs to assess both inorganic and organic deposition. None of the sampling locations were exposed to sunlight, and most remained dark. Samples were fixed in glutaraldehyde, air dried or critical point dried, coated with ~17 nm of Au-Pd and examined in a high resolution SEM.

The carbonate deposits consist of various mixtures of acicular aragonite with rhombohedral and massive calcite. Carbonates are deposited at the water-air interface, either in active splash zones or immediately above the surface of flowing springs. The precipitation of aragonite versus calcite is favored by high saturation states, water warmer than 40 to 45°C and Mg/Ca molar ratios greater than unity [5].

**Microbes** Bacteria have long been recognized in Hot Springs flows exposed to sunlight, but no survey of microorganisms in the underground springs has been undertaken. All of our samples contain small numbers of microbes which span a range of sizes and morphologies. High temperatures in the sampled springs are strongly selective for thermophiles, while the lack of light selects against photosynthetic

organisms. We are currently engaged in culture and DNA studies designed to identify these microbes.

The carbonates contain microbes with several distinctive morphologies. Small numbers of simple 1-2  $\mu\text{m}$  rods are found on the surfaces, along with more numerous elongated forms 4-6  $\mu\text{m}$  in length and <100 nm in width (Fig. 1).

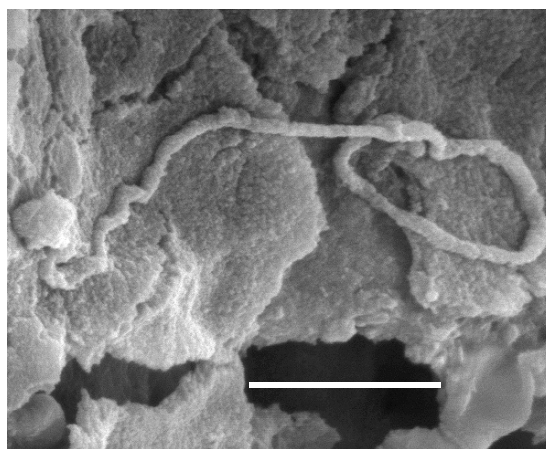


Figure 1. Microbial form on calcite; 1  $\mu\text{m}$  scale bar

The elongated forms are typically attached to spherical bodies approximately 200 nm in size. Some microbial forms have characteristic spiral shapes (Fig. 2). Whether these are independent organisms or microbial appendages remains to be determined.

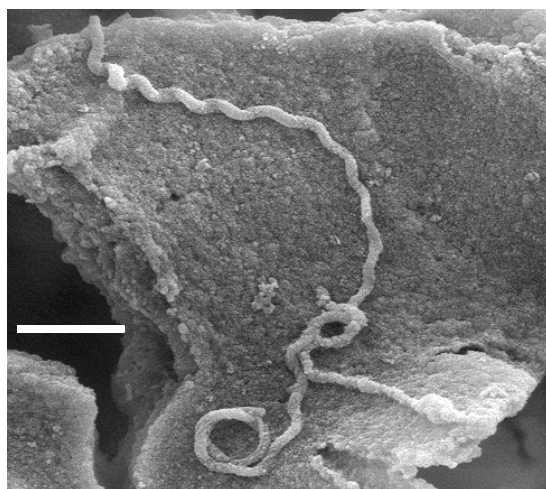


Figure 2. Spiral form on calcite; 1  $\mu\text{m}$  scale bar

Glass slides submerged completely below the water level became coated with translucent orange films in experiments lasting 3-14 days. The films are composed of Fe, Si and O in roughly constant ratios and are x-ray and optically amorphous. Surfaces exhibit patches of carbon-rich organic mucus, some of which incorporates microbes in localized biofilms.

The orange films contain a significantly different biota from those on the carbonate deposits. Films display large spherical bacteria 5-15  $\mu\text{m}$  in diameter. All films also contain low concentrations of rod shaped microbes ranging in length from 0.5-1  $\mu\text{m}$ , some with polar flagellae (Fig. 3).

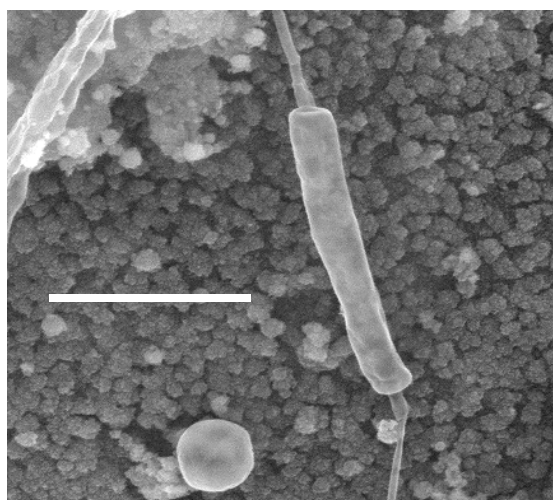


Figure 3. Rod in orange film; 1  $\mu\text{m}$  scale bar

The orange films also contain sub-micrometer spheres which may be microbes. In one case a sphere of this size shows evidence of division (Fig. 4).

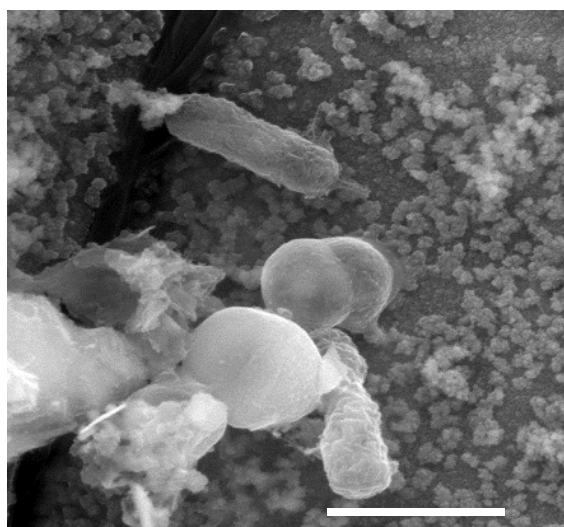


Figure 4. Rods and dividing sphere; 1  $\mu\text{m}$  scale bar

**Effects of Microbes on Deposition** The importance of microbes in promoting calcium carbonate deposition in thermal springs is a matter of ongoing debate. Pentecost [6] argued against significant microbial influence in the case of Yellowstone deposits. He accepted microbially-induced precipitation only where aragonite crystals clearly copied the structures of filamentary bacteria. Chafetz and Buczynski [7], however, provided evidence for a range of bacterially induced lithification styles in microbial mats.

The areal extent of organisms on our carbonate samples is extremely low ( $\ll 1\%$ ). In no case do carbonates copy the morphologies of individual microbes. Evidence to date indicates that precipitation of aragonite and calcite in these thermal springs apparently occurs abiotically, although mediation by dissolved organic molecules cannot be discounted.

We conducted an experiment to determine the effects of water flow and microorganisms on formation of the orange films. A sterilized glass slide was submerged in a flowing 60°C spring, other slides were sealed in a jar containing spring water and placed in the spring, and additional slides in spring water which had been passed through a 0.45  $\mu\text{m}$  filter were sealed in a jar and placed in the spring. The filter pore size excluded most of the microbes in our samples. Slides were left in place for 3 days.

The slide placed directly into the spring developed an orange film similar to those on previous samples. The slides sealed in spring water, both filtered and unfiltered, showed very slight film development in qualitatively similar amounts. Apparently film development is much more strongly controlled by the active flow of significant volumes of hot water than by the presence or absence of microbes.

**Conclusions** A range of microorganisms exists in underground thermal springs. These microbes are found on the surfaces of carbonates and iron-silica films, but apparently do not control their deposition. The biota is strongly selected by temperature and chemical conditions and lives independent of sunlight.

**References.** [1] McKay, D.S. et al. (1996) *Science* **273**, 924. [2] Allen, C.C. et al. (1997) *LPS XXVIII*, 29. [3] Allen, C.C. et al (1998) *LPS XXIX*. [4] Norsworthy, S.F., PhD dissertation, UCLA, 1970. [5] Folk, R.L. (1994) *Geograph. Phys. et Quat.* **48**, 233. [6] Pentecost, A. (1990) *Geol. Mag.* **127**, 159. [7] Chafetz, H. S. and Buczynski, C. (1992) *Palaios* **7**, 277.