SULPHUR SPRING: BUSY INTERSECTION AND POSSIBLE MARTIAN ANALOGUE. A. Nankivell[1], N. Andre[2], K. Thomas-Keprta[3], C. Allen[4], D. McKay[5], [1]Cornell University (Risley Hall 446, Cornell University, Ithaca, NY 14853, an44@cornell.edu), [2]Brown University (Providence, RI), [3]Lockheed-Martin (MC C-23, NASA/JSC, Houston, TX 77058), [4]Lockheed-Martin (MC C-23, NASA/JSC, Houston, TX 77058), [5]NASA/JSC (SN Houston, TX 77058).

Introduction: Life in extreme environments exhibiting conditions similar to early Earth and Mars, such as Sulphur Spring, may harbor microbiota serving as both relics from the past as well as present day Martian analogues. Sulphur Spring, an estuary and component of the Florida limestone- aquifer network, is one such microcosm characterized by a deep, dark, sulfide-rich cave system containing saltwater vents discharging into freshwater. Using scanning electron microscopy with EDS, we analyzed biological samples on three slides placed up to 975 meters within the Sulphur Spring Cave vents for various lengths of time. The dynamic junction between light and dark, freshwater and saltwater, aerobic and microoxic, accommodates a multitude of organisms that spark three main questions: is the dark environment a graveyard or breeding ground for photosynthetic diatoms? are unusual, copper-rich bacteria being mineralized by, precipitating, or metabolically utilizing copper? how does Sulphur Spring relate to the search for microbial life on Mars?

Background: Sulphur Spring is a freshwater, marine interface that feeds into the Hillsborough River which empties into Tampa Bay. The spring is comprised of an underwater cave system containing deep vents within the cave that discharge saltwater into surrounding, fresh, aquifer water supplied by the spring. The resulting environment is an estuary.

Salinity in the spring is 1.5 ppt., while the salinity of the vents is 30 to 35 ppt., equivalent to normal marine water. The vents also contain white mats of bacteria that are thought to be sulfur oxidizers. Both the cave and vents are completely dark with the exception of a 50 foot area at the entrance to the cave. Although hydrolab readings from within the cave measured an oxygen count of 0 mg/L, the environment is realistically considered to be microoxic since accuracy is +/- 0.2 mg/L.

Sampling Methods: Dr**.** Michael Garman of the National Speleological Society headed the collection of samples from the Sulphur Spring Cave. Slides 1, 2, and 3 were placed vertically at depths of ~ 457 m, ~ 762 m, and ~ 975 m, respectively. Slide 1 was in place for 30 days, slide 2 for 24 days, and slide 3 for 97 days. The temperature at all locations was 25 degrees C. The pH levels near slides 1 and 3 were both 7.1; 7.2 at slide 2. After recovery, the slides were fixed with 8% gluteraldehyde.

Upon arrival at JSC, the slides were rinsed twice with triply distilled water and dried overnight in inverted plastic bottles. Slide 3 did not dry completely and was placed on a low-temperature hot plate to finish drying. In the final step of their preparation, the slides were sputter coated with carbon. Using a scanning electron microscope, we examined the slides. Investigation focused on slide 2.

Copper Bacteria: The most abundant forms of larger life in our sample were copper and phosphorus rich organisms or perhaps biofilm polymers that resembled "pancakes." These microorganisms range in diameter from 28 to 40 microns. Pancakes range in morphology from thin and dendritic to thick and dense with denser centers. Some pancakes appear "elevated" and plateau-like. The solid edges exhibit higher carbon peaks than the web- like edges. Perhaps living pancakes resemble their current desiccated morphology, as evidenced by images of pancakes at suspected earlier stages of growth. Bacilli, sigmoids, and filaments are often associated with pancakes. At higher kilovoltage and magnification, some pancakes reveal minute-luminescent spots, possibly areas of copper concentration. It is not clear as to whether these pancakes are microbes that store copper intracellularly or within cell membranes. If Sulphur Spring's pancakes are indeed microorganisms, they may have 1) found a way to control cellular copper influx, 2) incorporated copper as a terminal electron acceptor in metabolism, or 3) been mineralized by aqueous copper in the saltwater vents.

Diatoms: The majority of the diatoms discovered on slides from Sulphur Spring were centric diatoms, which are most abundant in the ocean. Also sighted were a few pennate diatoms—usually found in freshwater—distinguishable by bilateral symmetry along the longitudinal axis. The typically freshwater diatom species Aulacoseira was also observed. Two pristine groups were sighted on slide 2 and were morphologically similar to Aulacoseira islandica [1]. Of the two separate frustrules we examined, only one was in colonial formation and had very few filaments overlaying the frustrule, while more biofilm was associated with the single-diatom frustrule.

An unusual find from slide 1 was a colony of what appeared to be 11 large, heavily weathered diatom valves. Upon closer inspection, 5 minute valves became readily visible among the cluster.

The unexpected appearance of freshwater diatoms in dark, anoxic, saltwater vents suggests that the diatoms are contaminants which sedimented out of the water column, either arriving as skeletons or subsequently dying from the high salinity and lack of light within the vents. However, perhaps pristine diatoms enmeshed in biofilm miraculously survived the water-column 'roller-coaster' and adapted to the vent environment (of slides 1 and 2) up to 762 meters in depth.

Moreover, since diatoms are dependent upon silica for survival and are drawn to silica-rich substrates, the glass slides may have attracted living diatoms.

Sulfur-Reducing/Oxidizing Bacteria: Filaments, Cocci, Spirilla, Bacillus: Through close study of the filamentous mats covering samples from Sulphur Spring, we have identified at least six different species of filaments, most of which fall within a width range of approximately .5 to 1.5 microns. Neither rosettes nor true-branching nor tapering was sighted in the examined areas of the samples. Thus, we could not find morphological evidence of the sulfide-oxidizing Beggiatoale *Thiothrix*, on our samples; other submarine caves within the Florida aquifer system—Peacock Springs Cave and Silver Spring Mammoth Cave—lacked Thiothrix in sampled biofilms taken from limestone and iron oxyhydroxide substrates [2].

Cocci, the most abundant of all Sulphur Spring microfauna, observed in slides 1 and 2 appear desiccated and wrinkled, averaging about 1 micron in diameter. Cocci are usually founded in pairs or groupings (perhaps sarcina-like packets), while biofilm and possibly pili or fimbriae connect spheres. These cocci are similar to Synechococcus Marine Cluster Cyanobacteria, the only strain in the Synechococcus group with a raised salt requirement for growth and capable of facultative photoheterotrophy and anaerobic nitrogenase synthesis [3].

Spirilla studied generally conform to either 1) a flattened, tight, spiral shape (some with flagella), 2) a more loose, curly-cued, filamentous morphology, 3) a "medusoid," spherical mass of filamentous spirals, or 4) spirilloids.

Tenuously coined "spiral pasta," flattened spirals are tight-coiled left hand helixes with a blunt-sphere tipped terminus, generally .5 microns in diameter and 1 to 3 microns: three were sighted in examined regions of slide 2. The diameter of medusoid mass filaments is approximately 250 nanometers. Medusoids morphologically resemble the colorless sulfur bacteria, Thiospirillopsis floridana [3]. Like the pancakes, medusoids may also simply be polymers.

Bacillus as well as ellipsoidal bacteria are visible not only in colonial form, but also on the surface of pancakes at lengths around 2 microns. Chains of bacillus are plentiful on slide 1, with bacillus links ranging from 1.25 microns by 680 nm to 1.5 microns by 780 nm. Our samples could contain the strictly anaerobic dissimilatory sulfur reducing genera Desulfuromonas and the strictly anaerobic dissimilatory *sulfate* reducing genera Desulfovibrio and Desulfobacter [3]. Although Thiobacillus ferrooxidans (pyrite oxidizer) and t. thiooxidans are extremely acidophilic while Sulphur Spring has a neutral pH, several other Thiobacillus species actively oxidize sulfur in neutral pH [4]. In Sulphur River, Kentucky, a 97% sequence identity between Sulphur River clone sequences and Candidatus Thiobacillus baregensis [5] (a sulfur oxidizer) was established—evidence that may have implications in the identification of bacillus in Sulphur Spring.

Summary: Morphologically Analogous Organisms to Sulphur Spring:

I. *Diatoms*—Cyclotella, Aulacoseira, Nitzschia
II. *Filaments*—anaerobic/ microoxic Beggiatoa
III. *Cocci*—Cyanobacteria (Synechococcus, Marine Cluster)
IV. *Spirilla*—Spirulina, Beggiatoa, Thiospirillopsis (floridana)
V. *Bacillus*—Thiobacillus (ferrooxidans, thiooxidans, novellus), Desulfuromonas, Desulfobacter, Desulfovibrio

Conclusion: If submarine cave biotas are considered natural conservatories of ancient- microfauna due to the preservation of conditions reminiscent of early Earth [6], then Sulphur Spring also serves as a sanctuary for descendants of the sulfur metabolizing bacteria that inhabited an anoxic, sulfide-rich Archean biosphere up to 3.5 billion years ago [7]. It has also been resolved that volatile elements and water, prerequisites for the emergence of life, were present on Early to Middle Noachian Mars in similar proportion to those on ancient Earth. Sulphur Spring's saltwater vents may house the essential elements for dissimilatory sulfur or sulfate reducing bacteria and sulfur oxidizers—microorganisms that may share similar features with possible Martian cousins living in dark, anoxic subsurface ice.

Sulphur Spring diatoms, dependent upon light for energy, may have acclimated to a perhaps *extreme* low-light environment via maximizing light absorption efficiency, thereby enhancing photosynthesis—as seen in benthic layer ice Nitzschia diatoms in Antarctica [8].

Without PCR and 16s RNA analysis, specifically determining the genera and species is not entirely plausible. More extensive measurements of pH would also aid in ascertaining whether or not areas of localized acidity exist in Sulphur Spring, which would shed light on the preponderance of certain species of bacillus. Dissolved carbon dioxide analysis, light measurements at various depths, organic carbon counts, and sulfide counts may also assist in further studies. Finally, the ability to grow and culture copper pancakes would not only shed light on their morphology and mechanisms of copper concentration, but also might convey the optimal conditions for growth and whether the high levels of copper are linked to the environment.

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