CHARACTERIZATION OF A SUBSURFACE BIOSPHERE IN A MASSIVE SULFIDE DEPOSIT AT RIO TINTO, SPAIN: IMPLICATIONS FOR EXTANT LIFE ON MARS.

C.R. Stoker[1], T. Stevens[2], R. Amils[3], J. Gómez-Elvira[4], N. Rodríguez[4], F. Gómez[4], E. González-Toril[4], A. Aguilera[4], D. Fernández-Remolar[5], S. Dunagan[6], L. Lemke[7], J. Zavaleta[8], J.L. Sanz[9], [1]NASA Ames Research Center, Code SS, Moffett Field, CA. 94035, (Carol.R.Stoker@nasa.gov); [2]Portland State University, Portland OR (tstevens@gorge.net); [3]Centro de Astrobiología, Madrid, Spain (ramils@trasto.cbm.uam.es); [4]Centro de Astrobiología, Madrid, Spain (gomezej@inta.es); [5]Centro de Astrobiología, Madrid, Spain (dfernandez@inta.es); [6]NASA Ames Research Center, Code SG, Moffett Field, CA. 94035, (Stephen.Dunagan@nasa.gov); [7]NASA Ames Research Center, Code P, Moffett Field, CA. 94035, (llemke@mail.arc.nasa.gov); [8]NASA Ames Research Center, Code SS, Moffett Field, CA. 94035, (jzavaleta@mail.arc.nasa.gov), [9]Universidad Autónoma de Madrid

Background: The recent discovery of abundant sulfate minerals, particularly Jarosite by the Opportunity Rover at Sinus Merdiani on Mars has been interpreted as evidence for an acidic lake or sea on ancient Mars [1,2], since the mineral Jarosite is soluble in liquid water at pH above 4. The most likely mechanism to produce sufficient protons to acidify a large body of liquid water is near surface oxidation of pyrite rich deposits [3]. The acidic waters of the Rio Tinto, and the associated deposits of Hematite, Goethite, and Jarosite have been recognized as an important chemical analog to the Sinus Merdiani site on Mars [4]. The Rio Tinto is a river in southern Spain that flows 100 km from its source in the Iberian pyrite belt, one of the Earth's largest Volcanically Hosted Massive Sulfide (VHMS) provinces, into the Atlantic ocean. The river originates in artesian springs emanating from ground water that is acidified by the interaction with subsurface pyrite ore deposits. The Mars Analog Rio Tinto Experiment (MARTE) has been investigating the hypothesis that a subsurface biosphere exists at Rio Tinto living within the VHMS deposit living on chemical energy derived from sulfur and iron minerals. Reduced iron and sulfur might provide electron donors for microbial metabolism while *in situ* oxidized iron or oxidants entrained in recharge water might provide electron acceptors.

Methods: Coring operations were conducted on Peña de Hierro, above the source of the Rio Tinto in September - October 2003 at two locations designated wells 4 and 1. In September 2004, an additional core was obtained at well 8 near well 4. Wells 4 and 8 sampled the subsurface pyrite orebody where it interacted with groundwater. Drilling extended from the surface to 165 m and was performed using a commercial coring rig. Well 4 sampled down stream (in a ground water flow sense) from the orebody. Drilling was 60 m into shale. Bromide salt was added to the drilling fluid as a tracer of potential contamination in samples extracted aseptically from the cores. Sterilized cores were used as controls for core processing. At the borehole, cores were extracted encased in plastic liners, cut into 1 m sections that were flushed with N_2 gas, sealed, and then rapidly transported to a nearby laboratory. At the laboratory, the cores were placed inside an anaerobic chamber, the liners were cut open, and the cores were photographed. The cores were examined for evidence of mineral alteration potentially associated with bacterial processing and this drove location selection for core subsampling. Aseptically acquired core samples were sealed in anaerobic bags for further processing. The remainder of cores were removed from the anaerobic chamber and then subjected to periodic microsopic imaging and spectroscopic observations in the 0.4 to 1 micrometer spectral range. Samples collected in Well 8 were additionally scanned with a hyperspectral imager with 100 micron/pixel spatial resolution and spectral response in the 0.4 to 1 micrometer spectral range. These measurements form a core log that permits examination for mineralogical clues that may be biomarkers of life (See related LPSC abstract from Battler and Stoker, 2005[5]). Powdered samples extracted from cores were first analyzed with DAPI staining to screen for the presence of microorganisms. Promising samples selected based on DAPI stain results or field observations were incubated under aerobic conditions using chemoautotrophic enrichment media both with and without iron added. A separate set of powdered samples were incubated in anaerobic chemoautotrophic enrichment media containing thiosulfate and hydrogen. Finally, a limited set of samples from near the bottom of well 4 were incubated in anaerobic enrichment cultures with the addition of hydrogen to search for the presence of strict anaerobic methanogens. This procedure was repeated throughout wells 1 and 8. In addition, aliquots of powdered samples from all cores were analyzed using ion chromatography for the presence of soluble ions. Samples showing the presence of Bromide were assumed to be contaminated with surface derived organisms and were not included in our biological analysis. After drilling, the holes were completed with PVC casings including perforated sections installed in depth intervals where biological activity was suspected based on field data. Multilevel diffusion samplers were installed in the perforated sections to characterize the groundwaters. Water samples collected using these devices were analyzed using ion and gas chromatography for dissolved anions, metals, and gases.

Results: Analysis of results from well 8 are still ongoing, so the talk will focus on biological characterization of well 4 that sampled water-rock interactions in the pyrite orebody. Data analyzed to date support detection of viable microbes in uncontaminated samples and demonstrates a subsurface ecosystem at Rio Tinto [6]. Samples were obtained from 156 cores using sterile and anoxic technique. 75 samples were free from contamination as determined by tracer analysis. The water table occurred at 90 m below the surface. Microbes have been positively identified in contamination free cores both above and below the water table. Some rock leachates, but not groundwater, contained unexpected concentrations of nitrite, suggesting an *in situ* source. Dissolved hydrogen was detected in fairly large quantity in borehole fluids in a region near the top of the water table (85-105 m), and in biologically useful concentrations throughout the sampled intervals (135-150m), suggesting *in situ* production from water/rock reactions. Considered with the massive ferrous iron and sulfide content of the ore, these results indicate that geochemical resources are available in the Rio Tinto subsurface to support several kinds of anaerobic chemolithotrophic metabolism. Small quantities of methane were observed in groundwaters, indicating the presence of methanogenic microbes. Microbial growth occurred in aerobic cultures on chemoautotrophic media in 10 samples that included the addition of iron, and 11 samples without the addition of iron. Indications of growth were found in 37 samples cultured anaerobically in chemoautotrophic thiosulfate media. Enrichment cultures using hydrogen as an electron donor allowed detection of methanogenic activity in 4 samples from the bottom of the hole. Though small amounts of oxygen appears to be delivered to the top of the ore body by groundwater transport, the results suggest that at least part of the ecosystem must be anaerobic.

Implications: This is the first report of a subsurface biosphere found to utilize sulfide minerals as an energy source, and perhaps the second type of chemical system supporting a subsurface anaerobic chemoautrophic biosphere ever observed. The results are important since a similar type of environment may have existed on early Mars at the surface at Sinus Merdiani. Sulfide minerals could feasibly be found in the Martian subsurface today, left as a relict of ancient hydrothermalism or even actively produced by current hydrothermal activity. Borehole organisms and chemical processes involved with the interaction of groundwater and pyrite ores are producing hydrogen and methane in significant quantities. A similar subsurface chemoautotrophic biosphere could be living on Mars today and producing methane that, when released to the atmosphere, could potentially be a source for methane that has been observed in the Martian atmosphere by the Mars Express mission [7].

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