

**MARS ANALOG RIO TINTO EXPERIMENT (MARTE): 2003 DRILLING CAMPAIGN TO SEARCH FOR A SUBSURFACE BIOSPHERE AT RIO TINTO SPAIN.** Carol Stoker<sup>1</sup>, Stephen Dunagan<sup>2</sup>, Todd Stevens<sup>3</sup>, Ricardo Amils<sup>4</sup>, Javier Gómez-Elvira<sup>5</sup>, David Fernández<sup>6</sup>, James Hall<sup>7</sup>, Kennda Lynch<sup>8</sup>, Howard Cannon<sup>9</sup>, Jhony Zavaleta<sup>10</sup>, Brian Glass<sup>11</sup>, and Larry Lemke<sup>12</sup>, <sup>1</sup>NASA Ames Research Center, Code SS, Moffett Field, CA. 94035, (Carol.R.Stoker@nasa.gov); <sup>2</sup>NASA Ames Research Center, Code SG, Moffett Field, CA. 94035, (Stephen.Dunagan@nasa.gov); <sup>3</sup>Portland State University, Portland OR (tstevens@gorge.net); <sup>4</sup>Centro de Astrobiología, Madrid, Spain (ramils@trasto.cbm.uam.es); <sup>5</sup>Centro de Astrobiología, Madrid, Spain (gomezrej@inta.es); <sup>6</sup>Centro de Astrobiología, Madrid, Spain (dfernandez@inta.es); <sup>7</sup>Carnegie Institution of Washington, Washington DC. 20015 (j.hall@gl.ciw.edu); <sup>8</sup>NASA Johnson Space Center, Code SA13, Houston, TX, (kennda.l.lynch@nasa.gov); <sup>9</sup>NASA Ames Research Center, Code IC, Moffett Field, CA. 94035, (hcannon@mail.arc.nasa.gov); <sup>10</sup>NASA Ames Research Center, Code SS, Moffett Field, CA. 94035, (jzavaleta@mail.arc.nasa.gov); <sup>11</sup>NASA Ames Research Center, Code IC, Moffett Field, CA. 94035, (Brian.J.Glass@nasa.gov); <sup>12</sup>NASA Ames Research Center, Code SF, Moffett Field, CA. 94035, (llemke@mail.arc.nasa.gov)

**Introduction:** The MARTE (Mars Astrobiology Research and Technology Experiment) project, an ASTEP field experiment, is exploring for a hypothesized subsurface anaerobic chemoautotrophic biosphere in the region of the Tinto River- or Rio Tinto- in southwestern Spain. It is also demonstrating technology needed to search for a subsurface biosphere on Mars. The project has three primary objectives: (1) search for and characterize subsurface life at Rio Tinto along with the physical and chemical properties and sustaining energy sources of its environment, (2) perform a high fidelity simulation of a robotic Mars drilling mission to search for life, and (3) demonstrate the drilling, sample handling, and instrument technologies relevant to searching for life on Mars. The simulation of the robotic drilling mission is guided by the results of the aseptic drilling campaign to search for life at Rio Tinto. This paper describes results of the first phase of the aseptic drilling campaign.

A subsurface biosphere on Mars will require both the presence of liquid water and metabolic energy sources to support metabolism. Subsurface biospheres that derive energy from  $H_2$  and  $CO_2$  produced by the aqueous weathering of basalt have been identified on several locations on Earth [1,2,3]. This is the only known energy source discovered to date for subsurface life on Earth that could plausibly be found on Mars. It is important to search for other potential energy sources for chemoautotrophic ecosystems to better understand the range of environments in which life on Mars might occur. Laboratory observations suggest some organisms such as *At. ferrooxidans* can grow anaerobically and autotrophically using reduced sulfur compounds or  $H_2$  as electron donors and ferric iron as electron acceptors [4], [5], [6], but there isn't a known example of an anoxic ecosystem supported by sulfur or sulfide-oxidizing metabolism. We hypothesize that such a system exists in the subsurface of the Rio

Tinto and have performed exploratory drilling to search for it.



Figure 1. Rio Tinto geographic location.

**Geologic Setting:** The Iberian Pyritic Belt (IPB) is one of Earth's largest massive sulfide provinces and was formed by hydrothermal alteration of volcanics. Massive bodies of Fe and Cu sulfides as well as minor quantities of Pb and Zn constitute the main mineral ores. The Rio Tinto flows 100Km from Peña del Hierro, in the core of the IPB, to the Atlantic (Fig. 1) with its basin covering an area of about 1700Km<sup>2</sup>. The waters of the river are the color of dark red wine, a result of the high concentration of ferric iron maintained in solution by an acidic pH (mean 2.3). Although the acidic waters were once thought to result from environmental damage due to mining, research shows that a similar environment has existed for at least 10<sup>5</sup> years and thus predates human activity.

**Site Selection:** Exploring the hypothesis of a subsurface microbial ecosystem based on the metabolism of iron and sulfur minerals requires locating a site where these minerals interact with groundwater. Geologic mapping was used to identify locations where the host rock of the sulfide deposits, highly localized and formed from hydrothermal activity, occurred [7]. Field work further identified areas where mining activity in Peña del Hierro had exposed sulfide deposits. A mine pit on Peña del Hierro has a sulfide ore deposit exposed on the wall of a 200m deep crater that is filled with water at 90m below the surface, indicating the depth of the groundwater table. The Rio Tinto is sourced from a network of artesian springs that out-

crop south of (downhill from) the mine pit crater. The association of these streams with faulting and the elevations at which they outcrop suggest an underlying groundwater system in the area. Acidic artesian springs have a pH at their source ranging from 0 to 3, but are surrounded by other artesian springs with normal pH suggesting that the low pH is produced by interaction of groundwater with a subsurface sulfide orebody or possibly with sulfide tailings remaining from mine operations. Drill sites were chosen in a location near the northeast edge of the mine pit crater where sulfide was observed in the crater wall, and where two faults intersected. We expected that the faulting might control water flow paths.

**Drilling Campaign:** The MARTE drilling expedition, from Sept. 15 - Oct. 15, 2003, was accomplished using a commercial coring drill rig that used water as lubricating fluid. Because one of the major issues in subsurface microbial ecology is surface contamination, the fluid was "spiked" with contamination tracers. Three types of tracers were used: Sodium Bromide, microbium *Lactobacillus*, and fluorescent microspheres. At the borehole, 78mm cores were extracted encased in plastic liners, placed in plastic bags filled with N<sub>2</sub> gas, heat sealed, and then rapidly transported to a nearby laboratory. At the laboratory, the cores were placed inside an anaerobic chamber and broken apart using a sterile core cutting press. The cores' surfaces were expected to be contaminated by the drilling fluid so powdered samples were collected from the center of the cores using sterile drill bits. The intent was to yield sterile samples but, in the case of porous rock, the fluid may still have contaminated the core interiors. The tracers in the drilling fluid allow the degree of contamination to be determined. Immediately after cutting open the bags, the core face was rubbed with a sterile cotton swab subjected to analysis by an ATP luminometer. This provided a quick triage measurement to search for presence of bacteria. Powdered samples were extracted and placed in sterile sealed containers for further processing in a variety of analyses for chemical and biological characterization. The analyses include ion chromatography, to identify metabolic resources (anions and cations); Fluorescent In Situ Hybridization (FISH), to identify where bacteria are present; PCR, used for a tracer of contamination based on the *Lactobacillus* tracer, and identification of organisms located using FISH.

**Results:** The borehole was drilled to a depth of 166.3m. Core recovery of 98% was achieved in all but the top 20m where highly porous and fractured rock led to poor core recovery. The near subsurface environment was highly porous down to 30m where the pyrite stockwork began. Geological logging was performed

on site and the results of the geologic analyses are discussed in [7]. Figure 2 shows the stratigraphic column of the borehole plotted along with the ATP luminometer results and the locations where preliminary FISH analysis using DAPI staining revealed the presence of bacteria. Further work is underway to determine the identity of microbes found in the subsurface, to characterize potential contamination from the drilling fluid, and to characterize potential metabolic energy sources. The results suggest that water was interacting with pyrite ores in a confined area near 90m, and then in a more extensive area below 135m. A number of cores were identified with measurable bacteria populations (red bars in Fig. 2). The two zones where biological activity was identified were mineralogically distinct, with the lower region appearing more porous and oxidized than the upper region. The borehole was completed and packers set at 120m to separate these aquifers.

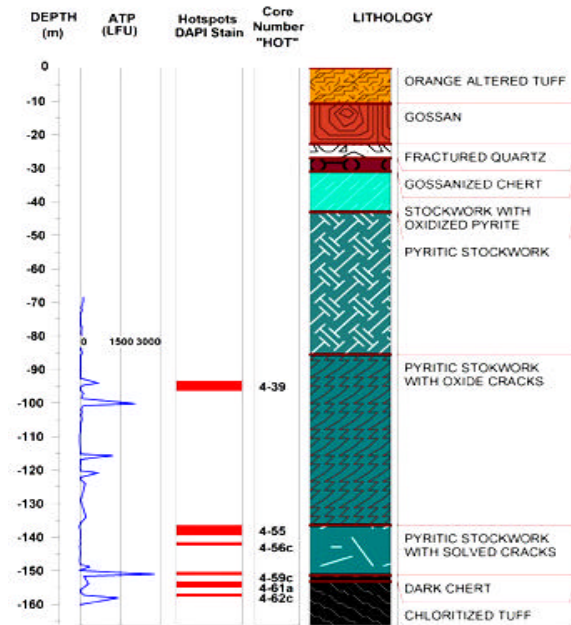


Figure 2. Borehole data with stratigraphic column. Red bars: DAPI staining reveals presence of microbes. Blue line: ATP luminometry results from fresh core faces.

**References:** [1] Stevens T. O. and McKinley J. P. (1995) *Science* 270:450-454. [2] Chappelle F. H. *et al.* (2002) *Nature* 415:312-315. [3] Stevens T. O. (2002) In: *Biodiversity of Microbial Life* pp. 439-474. Wiley-Liss, New York. [4] Brock, T.D., and J. Gustafson. (1976). *Appl. Environ. Microbiol.* 32:567-571. [5] Pronk, J.T., J.C. De Bruyn, P. Bos, and J.G. Kuenen. (1992). *Appl. Environ. Microbiol.* 58:2227-2230. [6] Ohmura, N., K. Sasaki, N. Matsumoto, and H. Saiki. (2002). *J. Bact.* 184:2081-2087. [7] Fernández-Remolar *et al.*, LPSC 35, 2004.