CONTEMPORARY AND PALEO-FILAMENTOUS BACTERIA IN IRON OXIDE DEPOSITS FROM RIO TINTO, SPAIN. L. J. Preston[1], J. Shuster[1], D. Fernández-Remolar[2], N. Banerjee[1], G. R. Osinski[1] and G. Southam[1]. [1]Centre for Planetary Science and Exploration (CPSX), University of Western Ontario, 1151 Richmond Street, London, Ontario, N6A 5B7, Canada. [2]Centro de Astrobiologíca, INTA, Ctra Ajalvir Km. 4, 28850 Torrejón de Ardoz, Spain.

Introduction: The preservation and identification of microorganisms over geological time is a key area in astrobiological research. The rock record on Earth reveals fossilized organisms preserved in a variety of ways, the most common being permineralization [1]. This process causes the organic template, in various stages of decay, to become trapped within a mineral matrix whilst preserving the biological structure. This combination of organic morphology and carbonaceous chemistry is needed to accurately and reliably identify past life on Earth and on other planetary bodies.

Rio Tinto, Spain: The Rio Tinto River Basin in southwestern Spain is a natural acid drainage system that has been active for millions of years [2,3]. It is strongly acidic with a mean pH of 2.3, a consequence of oxic weathering of massive sulfide ores. This enables ferrous and ferric iron to be transported in solution in the river in concentrations between 1.5 and 20 $g.L^{-1}$, with seasonal evaporation causing iron oxyhydroxide and sulfate precipitation [2].

The system supports a diversity of acid tolerant bacteria and microscopic eukaryotes with iron- and sulfur- oxidizing prokaryotes performing chemolithotrophy and supporting anaerobic respiration [3].

Terrace and gossan deposits formed over the past 2 Myr and 6-10 Myr, respectively, have preserved remnants of the biosphere, e.g., plant stems, pollen, insect cuticles as well as microbial filaments, which provide templates for iron sulfate (schwertmannite) and iron oxide (nanophase goethite) precipitation (see [2,3] for a detailed review). This creates morphological replicas of organisms whilst preserving them within mineral matrices (Figure 1A).

A contemporary system: Samples were collected from actively precipitating layered iron oxide deposits (Figure 1B), < 1 cm in thickness, within the river near Berrocal and from evaporites within an abandoned open-pit mine in Tharsis. Sub-samples were fixed in gluteraldehyde in the field to preserve the biological integrity of the samples for electron microscopy.

The fixed, layered samples from near Berrocal were dehydrated and embedded in epon, or were critical point dried, for examination using optical and Scanning Electron Microscopy (SEM) analysis.

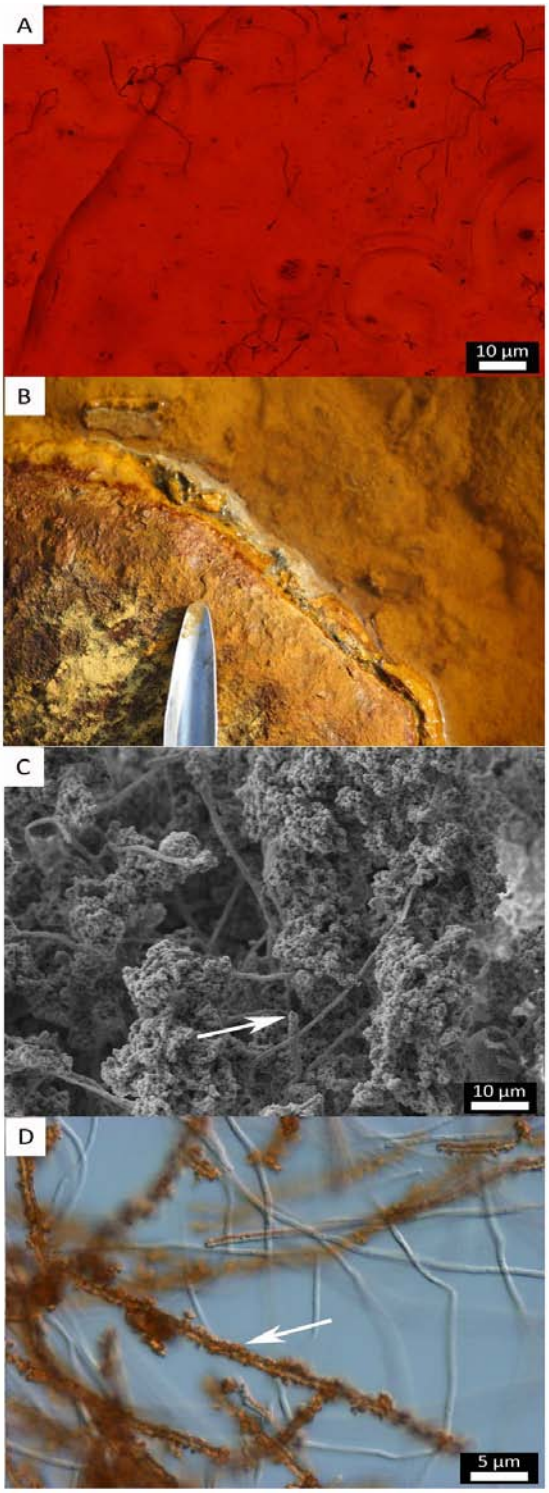

Figure 1. A. An optical image of goethite within a 2.1 Ma old terrace deposit. Dark filaments can be seen through the sample due to the stacking and compression of 25 images. **B**. Iron oxide layers forming around river cobbles within the Tinto river. **C**. SEM image of filaments preserved on the outer surface of the layered sample in B. Arrow points to a hollow mineralized filament. **D**. Culture of a (*Sphaerotilus*-like) filamentous iron oxidizing bacterium, with arrow indicating the iron oxides surrounding the filament.

Figure 1C shows a secondary electron image taken from the surface of the layered sample at the rock/water interface. Filamentous bacteria, approximately 1 µm in diameter, were observed at the outer surface of the layered oxide structure, and were partially cemented by a mixture of schwertmannite and nanophase goethite, and showing individual filament mineralization by the goethite, in some cases creating a hollow cast (arrow in Figure 1C).

An orange/yellow biofilm, collected at Tharsis, was used to enrich for iron oxidising bacteria using a 9K buffer [4] supplemented with 3.3 g/L $FeSO_4 \cdot 7H_2O$. The cultures contained 1 µm diameter filaments, some partially encrusted with iron oxides with visible cell walls, and others completely free of iron oxides (Figure 1D) that are morphologically comparable to those preserved in the rock record (Figure 1A). Examination of ultrathin sections (5) of a month-old Tharsis culture using Transmission Electron Microscopy (TEM) demonstrated that permineralization had begun, preserving the cell wall (Figure 2).

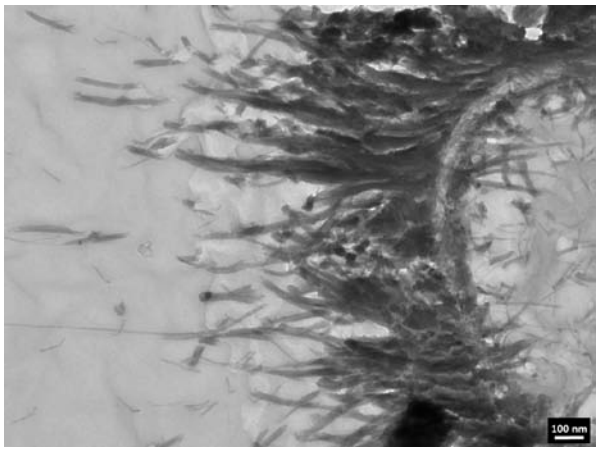

Figure 2. TEM image from the Tharsis isolate. Nanophase iron oxides appear to be growing outward and inward from the bacterial cell wall, preserving the wall inbetween.

Organic compounds from the Tharsis culture and a sample from a 2.1 Myr old terrace at Rio Tinto were detected using IR spectroscopy and analysed via Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) enabling assessment of the preservation of organics in this extreme environment over time, and the ability to correlate them with a contemporary culture.

Rio Tinto is a natural laboratory allowing living cells to be studied and correlated to morphological and biomolecular fossils in the geological record. This deposit will aid in providing predictive tools for biomarker studies. These tools could then be applied to other deposits of varying ages and possibly define strategies for searching for preserved biosignatures on other planetary bodies such as Mars.

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