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The Iron oxidizing bacterium *Leptospirillum ferrooxidans* as a model for biomarker search on Mars

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The study of extreme Earth environments as analogous to other possible extraterrestrial ecosystems, is of great astrobiological interest. The finding of hematitic strata on Mars, make relevant the search for similar terrestrial places where we can identify unequivocal biological signatures. One of such analogous is the Tinto river (South-western Spain), where a chemolithotrophic bacterial community oxidizes the Iberian Pyritic Belt, acidifying water (pH 0.8 to 3.0) and allowing accumulation of high concentration of ferric iron in solution (up to 20 gL⁻¹), as well as other metals [1]. In spite of these conditions, high biological diversity has been described [2,3,4]. One of the most abundant bacteria is the strict iron oxidizer *Leptospirillum ferrooxidans*, whose nutrient requirements are very simple: air and minerals. It takes carbon and nitrogen [5] from air by fixing CO₂ and N₂ respectively, energy (from pyrite or free Fe²⁺) and other elements from minerals.

Previous work [6] indicated that biological nitrogen fixation might have occurred on promordial Mars (pN₂= 18 mb). Microorganisms having metabolisms similar to *L. ferrooxidans* could have been crucial for early evolution of life on the Red Planet. We have recently identified most of the genes of this bacterium involved in nitrogen fixation (4), among them all codifying the nitrogenase system, some metal binding and transport as well as energy systems, or other involved in sulphur assimilation. Because nitrogenase and related enzymes, like those involved in Fe-S cluster formation, are very ancient and well conserved, we developed specific antibodies against synthetic peptides from some of these proteins. Protein microarrays with these and other antibodies have been constructed to search for recent biomarkers indicative of extant life forms.

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Amino and carboxylic acids in a Mars soil analogue from the Atacama desert

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Our laboratories are collaborating to develop an experiment suite SAM (Sample Analysis at Mars) for in situ organic analysis. In order to investigate whether organic compounds, especially those that might be associated with life, are present in Martian surface soils, we have developed a new technique for the *in situ* analysis of amino and carboxylic acids using gas chromatography mass spectrometry (GCMS). These compounds would have likely been missed in the analysis of the Martian surface soil by the Viking GCMS instruments [1]. Soil samples from the Atacama desert in Chile were used as a Martian analogue material in this study because these samples are known to contain very low levels of organic compounds and were collected from one of the driest places on Earth [2].

Amino and carboxylic acids were isolated from the soil sample using ultrasonic extraction with water or 2-propanol. After solvent evaporation, these targeted organic compounds were derivatised with MTBSTFA and analysed by GCMS. Our results indicate that low levels of the amino acids alanine, valine and glycine are present in some Atacama samples. Carboxylic acids were detected in all samples with concentrations ranging from 1 to 100 ppb.

We found that even in the driest part of the Atacama desert, some organic compounds could still be detected.

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