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Instrument development to search for biomarkers on mars: Terrestrial acidophile, iron-powered chemolithoautotrophic communities as model systems

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Abstract

Recent findings by the MER rover opportunity confirming the presence of iron minerals that can only be formed in the presence of water emphasize the study of analogous environments to Mars on Earth. The study of chemolithoautotrophic communities living in acidic iron-rich habitats is highly relevant in order to identify Mars analog environment-specific biomarkers. Iron oxidizing bacteria like *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* have ways of life for which it is feasible to identify a past or present hypothetical niche on Mars. We have developed a strategy for biomarker identification based on: (i) search for biosignatures on acid and metal-rich environments; (ii) development of an immunosensor microarray; and (iii) integration into an instrument for autonomous and remote operation. The instrument that we have built, called Signs Of LIfe Detector (SOLID), is capable of processing a variety of samples for the detection of specific biomarkers. Antibodies against several bacterial strains have been developed and tested in a microarray biosensor on SOLID. Tests with field samples have been successfully performed, allowing the detection of *L. ferrooxidans*, *A. ferrooxidans* present in sediment samples.

Keywords: Mars analogs; Instrumentation; Iron-powered chemolithoautotrophic communities; Acidophiles; Antibody microarrays; Biochips

1. Introduction

The study of extreme Earth environments that are analogous to other probable extant or extinct extra-terrestrial ecosystems is of great astrobiological interest. Arguments (Fairén et al., 2004) describing scenarios for why high amounts of carbonates have not yet been detected on Mars favor the idea that carbonates were never synthesized in acidic oceans on early Mars. This possibility fits into a scenario for a warmer Mars where extensive interaction between significant masses of moderately to extremely acidic liquid water and a CO_2 atmosphere might have occurred. Remote sensing data over the extensive bedrock outcrops throughout Terra Meridiani indicating the past presence of water over a large region of Mars (Hynek, 2004), together with the observational evidences provided by MER Opportunity at Meridiani Planum (Squyres et al., 2004a–c; Moore, 2004; Morris et al., 2004), support this model.

Extreme acidic environments controlled by iron biogeochemistry (such as, e.g. the Tinto River, in southwest of Spain) produce ferric-iron-enriched sediments dominated by sulfate and oxihydroxide parageneses, resulting in goethite, hematite and jarosite, minerals analogous to those found in Meridiani

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(Fernández-Remolar et al., 2003, 2004). In the Tinto River, a chemolithotrophic bacterial community contributes to oxidize a part of the Iberian Pyritic Belt, acidifying water (pH 0.8–3.0) and allowing accumulation of high concentration of ferric iron in solution (up to 20 g L^{-1}), and other metals. In spite of these extreme conditions, broad biological diversity has been described (López-Archilla et al., 2001; Amaral Zettler et al., 2002; González-Toril et al., 2003). Chemolithoautotrophic bacteria like *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* (abundant in the Tinto River ecosystem) are very simple in their nutrient requirements (Balashova et al., 1974; Buchanan and Gibbons, 1974); they only need water, air and minerals for growth. Both are able to fix CO_2 and N_2 (Mackintosh, 1978; Norris et al., 1995; Parro and Moreno-Paz, 2003), and they obtain energy from iron (Fe^{2+}) oxidation. *A. ferrooxidans* is a very versatile bacterium that can chemolithoautotrophically grow not only on Fe^{2+}/O_2 or H_2/O_2 under aerobic conditions, but also on H_2/Fe^{3+}, H_2/S_0, or S_0/Fe^{3+} under anaerobic conditions, with Fe^{3+} and S_0 being the electron acceptors (Ohmura et al., 2002). Although *L. ferrooxidans* seems to be a strict aerobe, it can also withstand anaerobic conditions for long periods (A. García-Moyano and R. Amils, personal communication). Acidic early Mars oceans and a putative CO_2 atmosphere could have provided enough nutrients for such microorganisms: Iron as the energy source (Catling, 1999), CO_2 (Brain and Jakosky, 1998) and N_2 (Fox and Dalgarno, 1980, 1983; Hunten, 1993; Mancinelli, 1996) as the carbon and nitrogen sources, as well as other elements. It has been shown that the N_2 content of the early Mars atmosphere could have been high enough for diazotrophic growth (nitrogen fixation) of nitrogen fixing bacteria like *Rhodobacter* and *Mesorhyzobium* sp. (Klingler et al., 1989).

Biosensor development is now at a very exciting stage and is growing very fast. Environmental monitoring as well as the risk of bioterrorism attacks need new, more specific, faster and efficient tools (Ziegler and Wolfgang, 1998; Rodriguez-Mozaz et al., 2004a). Among the most specific and sensitive bioaffinity-based biosensors are those using antibodies (immunosensors) as the recognizing molecule (Mallat et al., 2001; Estévez-Alberola and Marco, 2004). A number of two-dimensional array-based multianalyte biosensors using fluoro-immunoassay as their detecting system have been described for rapid analysis of complex samples (Wadkins et al., 1997; Anderson et al., 2000; Huang et al., 2001; Wiese et al., 2001), and portable instrumentation is being developed (Taitt et al., 2004; Rodriguez-Mozaz et al., 2004b; Gómez-Elvira et al., 2004). Protein microarrays (or protein chips) permit us to fix up to several thousand of different capturing antibodies on a few square centimeters (MacBeath and Schreiber, 2000; Kusnezow et al., 2003; Glökler and Angenendt, 2003; Weller, 2005). This class of immunosensor can be used for the detection of multiple different compounds in complex mixtures like environmental samples or soil extracts. A positive signal indicates the presence of molecules with an identical or highly similar structure to the ones used to produce the capturing antibodies. One extraordinary advantage of using antibodies is that they recognize structural features rather than mass, allowing the inference of the level of complexity in the detected compound.

Here we describe an integrated approach for the detection and identification of specific biomarkers from Mars analog environments, the development of immunological tools (antibodies and microarray biosensors), as well as the implementation of the instrument Signs Of LIfe Detector (SOLID) for robotic and remote field analysis.

2. Materials and methods

2.1. Strain and culture conditions

L. ferrooxidans L3.2, *A. ferrooxidans A201*, *Acidithiobacillus* sp. and *Acidiphilium* sp., bacterial environmental isolates from the Tinto River, were kindly provided by professor R. Amils (Universidad Autónoma de Madrid and Centro de Astrobiología). All strains were cultivated on McKintosh medium (Mackintosh, 1978). Environmental samples were collected from Tinto River either from water or sediments in sterile screw cap tubes.

2.2. Antibody production, purification and fluorescent labeling

Polyclonal rabbit antibodies against all strains listed above were produced as follows: late exponential or stationary growth phase cultures were harvested by filtration through 0.22 μm, resuspended into 1× PBS (phosphate buffer saline buffer), and injected to rabbits using complete Freund adjuvant. Three further boosts were done before the total serum was obtained. For the best performance of the protein microarray, antibodies must be purified either as an IgG fraction or by affinity using the immobilized antigen. We purified the IgG fraction of all antibodies by protein A columns (Sigma). Purified antibodies were fluorescently labeled with Alexa 647 fluorochrome at a concentration of 2 mg ml^{-1}, as recommended by the provider (Molecular Probes, Invitrogen). Commercial polyclonal rabbit antibodies were purchased from Sigma-Aldrich: Anti-GroEL (G6532), anti-Thioredoxin (T0803), anti-gluthatione S-transferase (G7781), anti-Streptavidin (S6390), anti-Glutamate (G6642), anti-aspartate (A9684), anti-cortisol (C8409). The rabbit anti-*Salmonella* sp. antibody (B65701R) was obtained from Biodesign International.