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# Iberian Pyrite Belt Subsurface Life (IPBSL): searching for life in the Rio Tinto subsurface

R. Amils (1, 2), F. Gómez (1), O. Prieto-Ballesteros (1), D. Fernández-Remolar (1), V. Parro (1), J.A. Rodríguez-Manfredi (1), F. Tornos-Arroyo (1), K. Timmis (3), M. Oggerin (1), M. Sánchez-Román (1), F. J. López (1), J. P. Fernández (1), E. Omoregie (1), F. Puente-Sánchez(1), D. Gómez-Ortiz (4). C. Briones (1), M. García (1), N. Rodríguez (1), J.I. Velasco-Acebes (1), J.L. Sanz (5) and the IPBSL Team
(1) Centro de Astrobiología (CSIC-INTA), Torrejón de Ardoz, Spain, (2) Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Cantoblanco, Spain, (3) Technical University of Braunschweig, Germany, (4) Universidad Rey Juan Carlos, Madrid, Spain, (5) Departamento de Biología Molecular, Universidad Autónoma de Madrid, Cantoblanco, Spain (ramils@cbm.uam.es)

## Abstract

The geomicrobiological characterization of Río Tinto (Iberian Pyrite Belt), has proven the importance of the iron and sulfur cycles in generating the extreme conditions of acidity and high concentration of heavy metals of the habitat. It has been hypothesized that the extreme conditions found in the Tinto basin are the product of the subsurface chemolithotrophic metabolism of microorganisms thriving on the high concentration of metal sulfides of the IPB. To test this hypothesis, a drilling project (IPBSL) is currently under development to provide evidence of subsurface microbial activities and the potential resources to support them.

## 1. Introduction

Río Tinto is an unusual ecosystem due to its size, constant acidic pH, high concentration of heavy metals and a high level of microbial diversity [1]. Río Tinto raises in Peña de Hierro, in the core of the Iberian Pyrite Belt (IPB), one of the largest massive sulfide deposits on Earth. Rio Tinto is considered a good geochemical terrestrial analogue of Mars as a consequence of its mineral associations that are formed under acidic conditions prevailing in Early Mars [2, 3]. The Iberian Pyrite Belt Subsurface Life (IPBSL) is a drilling project specifically designed to answer basic questions related with the subsurface geomicrobiology responsible of the extreme conditions detected in the Tinto basin (http://auditore.cab.inta-csic.es/ipbsl).

## 2. Methodology and Results

A dedicated geophysical characterization of the area selected two drilling sites due to the possible existence of both water with high ionic content and iron sulfides, which provide a signal of low resistivity sounding run under different geophysical techniques. As a result two different subsurface areas showed a high potential to be inhabited by microbial communities.

Based on these resistivity results, two wells have been drilled, BH10 and BH11, to depths of 620 and 340 meters respectively, with recovery of cores and generation of samples in anaerobic and sterile conditions. The results obtained so far showed an important alteration of mineral structures associated with the presence of water, with production of expected products from the microbial oxidation of pyrite (sulfates and ferric iron). Complementary analytical techniques from uncontaminated samples showed the existence of putative electron donors (metal sulfides, ferrous iron, hydrogen, methane, nitrite), electron acceptors (sulfate, nitrate, ferric iron, $CO_2$) as well as variable concentration of organic acids (mainly acetate), which are strong signals of the presence of active subsurface ecosystems associated to the high sulfidic mineral content of the IPB. Fluorescence in situ hybridization techniques (CARD-FISH) allowed the detection of colonies of bacteria and archaea associated to the mineral structures at different depths DNA and RNA extracted from these samples are being use for the identification of the microorganisms and for functional studies (metagenomics, retrottranscriptomics and proteomics). Enrichment cultures allowed the identification of different metabolic activities, like iron and sulfur oxidizers and reducers, methanogens, methanotrophs, acetogens and denitrifyers. These observations confirmed the hypothesis that microorganisms are active in the subsurface of the IPB. The astrobiological interest of these observations will be presented and discussed.


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