Advanced Materials Research Vol. 825 (2013) pp 15-18
© (2013) Trans Tech Publications, Switzerland
doi:10.4028/www.scientific.net/AMR.825.15

Iberian Pyrite Belt Subsurface Life (IPBSL), a drilling project of biohydrometallurgical interest

Ricardo Amils[1, 2, a *], David Fernández-Remolar[1, b], Victor Parro[1, c], José Antonio Rodríguez-Manfredi[1,d], Ken Timmis[3,e], Mónica Oggerin[1, f], Mónica Sánchez-Román[1, g], Francisco, J. López[1, h], José Pablo Fernández[1, i], Fernando Puente[1, j], David Gómez-Ortiz[4, k], Carlos Briones[1, l], Felipe Gómez[1, m], Enoma Omoregie[1, n], Miriam García[1, o], Nuria Rodríguez[1,p], José Luis Sanz[5, q] and the IPBSL Team

[1]Centro de Astrobiología (INTA-CSIC), 28850 Torrejón de Ardoz, Madrid, Spain.

[2]Centro de Biología Molecular Severo Ochoa (UAM-CSIC), 28049 Madrid, Spain.

[3]Technical University of Braunschweig, Germany

[4]ESCET-Area de Geología, Universidad Rey Juan Carlos, 28933 Móstoles, Madrid, Spain.

[5] Departamento de Biología Molecular, UAM, Cantoblanco 28049 Madrid, Spain.

[a]ramils@cbm.uam.es, [b]fernadezrd@cab.inta-csic.es, [c]parrogv@cab.inta-csic.es,

[d]manfredi@cab.inta-csic.es, [e]emi.mbt@gouglemail.com, [f]oggerinom@cab.inta-csic.es,

[g]msanzroman@cab.inta-csic.es, [h]lopezsfj@cab.inta-csic.es, [i]fernandezrjp@cab.inta-csic.es,

[j]puentesf@cab.inta-csic.es, [k]david.gomez@urjc.es, [l]cbriones@cab.inta-csic.es,

[m]gomezgf@cab.inta-csic.es, [n]omoregie@cab.inta-csic.es, [o]villadangosgm@cab.inta-csic.es,

[p]nrodriguez@cbm.uam.es, [q]joseluis.sanz@uam.es

Keywords: Iberian Pyrite Belt, subsurface geomicrobiology, iron cycle, sulfur cycle, methanogenesis, Sulfate Reducing Bacteria (SRB)

Abstract. The geomicrobiological characterization of Río Tinto, an extreme acidic environment, has proven the importance of the iron cycle, not only in generating the extreme conditions of the habitat (low pH, high concentration of toxic heavy metals) but also in maintaining the high level of microbial diversity detected in the water column and the sediments. The extreme conditions detected in the Tinto basin are not the product of industrial contamination but the consequence of the presence of an underground bioreactor that obtains its energy from the massive sulfide minerals of the Iberian Pyrite Belt (IPB). To test this hypothesis, a drilling project (IPBSL) to intersect ground waters interacting with the mineral ore is under way, to provide evidence of subsurface microbial activities. A dedicated geophysical characterization of the area selected two drilling sites due to the possible existence of water with high ionic content. Two wells have been drilled in Peña de Hierro, BH11 and BH10, with depths of 340 and 620 meters respectively, with recovery of cores and generation of samples in anaerobic and sterile conditions. The geological analysis of the retrieved cores showed an important alteration of mineral structures associated with the presence of water, with production of expected products from the bacterial oxidation of pyrite. Ion chromatography of water soluble compounds from uncontaminated samples showed the existence of putative electron donors, electron acceptors, as well as variable concentration of metabolic organic acids, which suggest the presence of an active subsurface ecosystem associated to the high sulfidic mineral content of the IPB. Enrichment cultures from selected samples showed evidences of an active iron and sulfur cycle, together with unexpected methanogenic, methanotrophic and acetogenic activities. The geological, geomicrobiological and molecular biology analyses which are under way, should allow the characterization of this ecosystem of biohydrometallurgical interest.

Introduction

Río Tinto is an unusual ecosystem due to its size, constant pH, high concentration of heavy metals and high level of microbial diversity [1]. Río Tinto rises in Peña de Hierro, in the core of the Iberian Pyrite Belt (IPB). The IPB is one of the largest massive sulfide deposits on Earth. One important characteristic of Río Tinto is the high concentration of ferric iron and sulfates present in its waters, products of the biooxidation of pyrite, the main mineral component of the IPB. The IPBSL project was 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).

Methodology

Boreholes were continuously cored by rotary diamond-bit drilling using a Boart-Longyear HQ wireline system producing 3 meters of 60 mm diameter cores. Well water was used as drilling fluid to lubricate the bit and return cuttings to the surface. Fluids were re-circulated. To detect potential contamination of the samples, sodium bromide (200 ppm) was added to the drilling fluid as a marker. Upon retrieval from the drilling rig, cores were divided into 60 cm length pieces, inspected for signs of alteration and stored in boxes for its permanent storage and curation in the Instituto Geológico Minero de España (IGME) lithoteque in Peñaroya. Selected cores were deposited in plastic bags, oxygen was displaced with N_2, sealed and transported to a field laboratory within 60 minutes. After drilling, boreholes were cased with PVC tubes with holes at different depths to allow water movement. Upon arrival at the field laboratory cores were placed in an anaerobic chamber (5% H_2, 95% N_2), logged and photographed. Aseptic subsamples were obtained by splitting cores with an hydraulic core splitter and drilling out the central untouched portion with a rotary hammer with sterile bits, with strict temperature control (40^{o}C maximum). Rock leachates were produced by adding 5 ml sterile water to 0.5 g of powdered core subsamples and allowing them to stand overnight before filtration through pre-rinsed nylon 0.2 μm filters and analysed in a Advanced Compact Ion Chromatographer IC (Metrohm AG). XRD analysis was done with a Seifert 3003 T-T X-Ray difractometer. Elemental analysis was done by ICP-MS using a ELAN-6000 PE-Sciex instrument. Gases were analysed by gas chromatography using a Shirmadzu GC-8A equipped with a 2 m glass column packed with Poroàck Q. Samples for DNA extraction were kept at 4^{o}C. Samples for RNA were mixed with 2.5 volumes of LifeGuard to preserve and increase the stability of the RNA molecules. Previous test in our lab have demonstrated that the addition of EDTA increases nucleic acids stability in this type of samples. It has been described that pyrite can induce RNA degradation in few hours. Since pyrite is the main component present in the IPB subsurface, 100 mM EDTA was added to the LifeGuard reagent. We also avoid freeze the samples to prevent cell lyses. Environmental DNA was extracted using the commercial MoBio DNA extraction kit from soil. Cloning into plasmid vectors, sequencing and phylogenetic studies were performed as previously described [2, 3]. Powder and chips from different core samples were directly analysed by Sandwich Microarray Immunoassay (SMI) as described elsewhere [3]. Culture independent detection of microorganisms was done by epifluorescent microscopy after staining samples with DAPI and hybridization with universal probes for Bacteria (EUB388) and Archaea (Arch915) using CARD-FISHS. Samples were fixed with 4% formaldehyde and stored at -20^{o}C in ethanol:PBS (1:1) until further processing. Fluorescence in situ hybridizations were done following the protocols described in [2]. Chemolithotrophic enrichment cultures were performed in a minimal Mackintosh medium [4], with the addition of ferrous iron or a sterile pyrite sample as electron donors. Anaerobic enrichments for denitrifying microorganisms were performed as described by Stevens and McKinley [5], for sulfate reducers according to González.-Toril et al. [2], and for methanogens according to Sanz et al. [6].

Results

Two distinctive geological activities were performed concerning the selection of the drilling sites. Firstly, a careful mapping and surface sampling for solid rocks and springs in the Peña de Hierro area was performed to evaluate all available geological information. Secondly, different geophysical procedures were applied to detect the most probable subsurface areas hosting microbial activity in deep regions of the basement (Transient Electromagnetic sounding and 1D resistivity logging). These measurements were followed by emplacing two different resistivity lines that allowed determining the structure and lithological distribution of the sites with high interest for drilling. From the analysis of the geophysical information it was decided to drill two wells, BH10 and BH11, with depths of 620 and 340 meters respectively.

In addition to the geological core login in the drilling site, selected samples were obtained for mineralogical (XRD), elemental analysis (ICP-MS), and stable isotopic and petrographic analysis. The mineralogical results showed the presence of pyrite and alteration products like hematite and magnesite in both boreholes. The elemental analysis of leachates from the solid samples showed the presence of iron and other metals at different depths in both boreholes, indicating an alteration of the metal sufides at specific positions along the boreholes. The stable isotopic analysis of pyrites showed important sulfur fractionation at different depths in samples from both boreholes, which is a clear indication of activity of sulphate reducing microorganisms at these depths, which have been corroborated by enrichment cultures (see below). Also carbon fractionation signals were obtained in samples from both boreholes, being also a clear biosignature of microbial activity at these depths.

Rock leachates were analysed by ion chromatography to determine the concentration of water soluble anions. The obtained results indicate the presence of reduced organic anions like acetate, formate and propionate and oxidized inorganic anions such as nitrate, nitrite and sulfate. The appearance of these compounds at different depths is a strong indication of biological activity along the boreholes. Total protein and sugar content was also detected at different depths, indicating the presence of extant or recent microbiological activity. Samples along the entire length of borehole BH10 were analysed with the immunosensor LDChip450, an antibody microarray containing 450 antibodies against microbial cells, environmental extracts, proteins, exopolysaccharides, etc. More than 40 core samples were analysed and plotted to compare the immunorpofiles at different depths. It was observed high biomarker detection at -392 m and around the interval between -500 and -550 m in borehole BH10. Positive Ag-Ab reactions were detected with specific antibodies against methanogenic archaea and SRB which agree with the results obtained with enrichment cultures (see below). H_2, CO_2 and CH_4 have been detected in mineral samples from both boreholes.

DNA and RNA have been successfully extracted from different BH10 and BH11 samples. Most of them rendered positive PCR amplifications of the bacterial 16S rRNA gene.

During the drilling campaign samples from the two boreholes were collected (47 samples for BH10 and 21 for BH11) for fluorescence in situ hybridization. The results obtained so far showed positive hybridizations at different depths (-207, -352, -497 and -608 meters) for borehole BH10. The detected microorganisms were grouped in colonies attached to mineral particles, thus is unlikely that they might correspond to contamination, especially because all the selected samples had the background Br concentration (less than 0.5%). Further hybridization with specific probes selected or designed after identification of putative organisms using 16S rRNA gene sequences of the same samples is under development.

Anaerobic enrichment cultures have been prepared in the anaerobic chamber using mineral salts medium with the addition of different electron donors (ferrous iron, pyrite, thiosulfate, H_2, acetate, mixture of organic acids) and electron acceptors (ferric iron, sulfate, nitrate, CO_2, arsenate). The

following activities have been detected unambiguously after more than 10 months of incubation: methanogens, methanotrphs, sulfur reducers, iron oxidizers, acetogens and denitrifiers, using samples from both boreholes. From all the available data two hot spots have been detected in BH10, one at -352 m and at -497 m, and two in BH11, one at –236 m and at -311 m. The identification of hotspots is required for the selection of samples for metagenomic and retrotranscriptomic analysis, which is under way.

Conclusions

The results obtained so far allowed to reach the following conclusions. As groundwater enters the Volcanogenic-hosted Massive Sulfide (VHMS) system, biological and abiotc processes are activated. Electron acceptors available for microbial metabolism include transient oxygen, nitrate, sulfate, ferric iron and inorganic carbon. Electron donors include ferrous iron, sulfide, H_2 generated by water/rock interaction, supporting the generation of methane and organic acids, like acetate. This supports a community of different microbial metabolisms. As the fluid becomes more reduced, methanogenesis and sulfate reduction, using hydrogen, become the dominant microbial processes. Oxidants to drive the system appear to be supplied by the rock matrix, in contrast to conventional ARD models. Only mobilization of these sources by ground water appears to be necessary to allow microbial metabolism. These observations confirmed the hypothesis that microorganisms are active in the subsurface of the IPB. The characterization of these activities is extremely important to gain inside on the microbial ecology that might be operating in heap leaching processes, in some cases affecting negatively their efficiencies.

References

[1] E. Gonzalez-Toril, E. Llobet-Brossa, EO. Casamayor, R. Amann, R. Amils, (2003) Microbial ecology of an extreme acidic environment, the Tinto River. Appl Environ Microbiol 69: 4853-4865.

[2] E. Gonzáklez-Toril, F. Gómez, M. Malki, R. (2006) Isolation and study of acidophilic microorganisms. In “Methods in Microbiology”, F. Rainey and A. Oren (eds.), Elsevier, Oxford, Vol. 35, pp. 463-502.

[3] V. Parro (2010) Antibody microarrays for environmental monitoring. In “Handbook of Hydrocarbon and Lipid Microbiology”, K.N. Timmis (ed.), Springer-Verlag, Berlin, pp. 2699-2710.

[4] M.E. Mackintosh (1978). Nitrogen fixation by Thiobacillus ferrooxidans. J. Gen. Microbiol., 105: 215-218.

[5] T. Stevens, J.P. McKinley (1995), Lithoautotrophic microbial ecosystems in deep basalt aquifers, Science, 250: 450-454..

[6] J.L. Sanz, N. Rodriguez, R. Amils (1997). “Effect of chlorinated aliphatic hydrocarbons of the acetoclastic methanogenic activity of granular sludge”, Appl. Microbiol. Biotechnol., 47, 324-328.