

## Subsurface methanogenic activity in the Iberian Pyrite Belt detected by fluorescence in situ hybridization

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### Abstract

The IPBSL is a drilling project designed to investigate the subsurface geomicrobiology of the Iberian Pyrite Belt (Southwestern, Spain), a geological and mineralogical terrestrial analogue of Mars. Fluorescence in situ hybridization (CARD-FISH) has been used to detect the presence of methanogenic archaea in samples from uncontaminated cores of the IPBSL.

### 1. Introduction

Subsurface is an extreme environment that is characterized by anaerobic, oligotrophic conditions and also by absence of sunlight. Subsurface microorganisms should live in pores and rocks fractures just because that's where water is more abundant. And they must be able to use any thermodynamically available source of energy. Hydrogen is one of the most abundant gases in the subsurface, so microorganisms developing there can obtain energy by coupling the oxidation of hydrogen using different electron acceptors ( $\text{NO}_3^-$ ,  $\text{Fe}^{3+}$ ,  $\text{SO}_4^{2-}$  or  $\text{CO}_2$ ), being the latter one of the most important subterranean sources of carbon ([1], [2]). Therefore, Pedersen [3] and Stevens & McKinley [4] suggested a model in which autotrophic methanogens and homoacetogenics microorganisms would form the ecological basis for consumption of hydrogen and  $\text{CO}_2$ . Several authors suggested the possibility that hydrogen is the main source of energy for primary producers. So far this is one of the most widely accepted models for explaining subsurface life independent from radiation ([2], [5]).

### 2. Methodology and Results.

Based on a through geophysical study [6], two wells have been drilled in Peña de Hierro area of the IPB, BH10 and BH11, at depths of 620 and 340 meters

respectively, with recovery of cores and generation of samples in anaerobic and sterile conditions. Analytical techniques showed the presence of  $\text{H}_2$ ,  $\text{CO}_2$  and  $\text{CH}_4$  associated to samples from different depths and enrichment cultures the correspondent generation of  $\text{CH}_4$  by methanogenic archaea. We report the use of fluorescence in situ hybridization techniques (CARD-FISH) to detect active methanogens at several depths in the IPBSL core samples by using the MSSH859 (Methanosarcinales) probe [7].

The combination of these results clearly probes the presence of metabolically active methanogens in the subsurface of the IPB in nonmethanogenic conditions. A functional activity of astrobiological interest due to the recent detection of methane on the Martian atmosphere and the discussion about its possible origin. These results are consistent with the model proposed by Pedersen [3] and Steven & Mckinley [4].

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