MICROBIAL PRODUCTION OF H_2, CH_4 AND CO_2 IN THE SUBSURFACE OF RÍO TINTO, A GEOCHEMICAL MARS ANALOG. C. Escudero[1,2], N. Rodriguez[1,2], R.Amils[1,2], D. A. Carrizo[1], E. R. Uceda[2], A. G. Fairén[1,3], F. Gómez[1] and J. L. Sanz[4]. [1]Departamento de Planetología and Habitabilidad, Centro de Astrobiología (INTA-CSIC) Ctra. Ajalvir km 4 Torrejón de Ardoz, Madrid, Spain, [2]Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain, [3]Department of Astronomy, Cornell University, Ithaca, NY, USA. [4]Departamento de Biologia Molecular, Universidad Autónoma de Madrid, Madrid, Spain. (cescudero@cab.inta-csic.es)

Introduction: Because the extremely inhospitable conditions on the surface of Mars, the subsurface of the planet has been regarded as a potential habitable environment. The possibility of existing life in the subsurface of Mars has been increasingly debated since the presence of brines was confirmed [1]. Thus, studies on how life thrives in Earth's subsurface are critical to understand how life could have been (or is) thriving in the subsurface of Mars.

Life in Earth's subsurface is known to reach down to several kilometers within the continental crust. In fact, recent studies estimated that almost 90% of the Earth's prokaryotic biomass is within the deep subsurface [2].How all this biomass is sustained over time is still in discussion.

Most of the terrestrial deep subsurface locations are oligotrophic environments in which some gases, mainly H_2, CH_4, and CO_2, are thought to play an important role as energy and/or carbon sources. These molecules have been detected in most drilling operations, but it is not always clear their source as they can be generated by both biotic and abiotic processes. Generally, in subsurface environments, while CH_4 is considered a biological product, it is assumed that H_2 and CO_2 are of abiotic origin. Different studies have shown that deep subsurface life is supported by chemolithoautotrophic primary producers, mostly methanogenic archaea, which take advantage of the H_2 and CO_2 generated by geological processes to produce methane [3]. The same scenario has been suggested to occur in Mars [4]. On the other hand, in the deep continental subsurface, alternative energy sources can be found, for example organic matter. Organic matter can be used by heterotrophic microorganisms as their energy source, and its utilization may lead to the generation of H_2 or CO_2, which can sustain chemolithoautotrophic metabolisms [5].

The Iberian Pyrite Belt Subsurface Life Detection (IPBSL) project was a drilling project carried out to study the subsurface ecosystem responsible for the peculiarities of the Río Tinto basin, a geochemical Mars analogue. The IPBSL project revealed a subsurface ecosystem with active iron and sulfur cycles [6], and variable concentrations of H_2, CH_4, and CO_2 were detected along the entire length of the boreholes. Here we assess the biotic and abiotic origin of these gases obtained from subsurface hard-rock cores of the Iberian Pyrite Belt at three different depths.

Methods: Subsurface rock samples from 414, 497, and 520 mbsl were incubated in anaerobic conditions in the dark. For each sample two experimental conditions were implemented: one in which the rocks were sterilized, and other in which the rocks were left untreated (biotic production). After maintaining the bottles in the above-mentioned conditions for several months, 0.5 ml of sterile MilliQ water was added to the anaerobic reactors. The H_2, CH_4, and CO_2 content in the headspace was measured periodically by gas chromatography. In addition, CARD-FISH analyses to identify the microorganisms present in the rock samples, and analysis of stable isotopes of methane, were carried out.

Results: In natural samples with low or no humidity, the abiotic and/or biological production of H_2, CH_4, and CO_2 was, in general, low. The addition of water promoted the release of these gases in the non-sterilized samples. At the analyzed depths, we identified microorganisms with genes responsible for different metabolic activities producing the detected biogases. Carbon isotopic signatures, $\delta^{13}C_{CH4}$- values, revealed the biological origin of methane.

Conclusions: Our results suggest that an important proportion of the H_2, CH_4, and CO_2 detected in the IPB's subsurface has a biological origin. We propose here that this is not a particularity of the studied ecosystem, but that the biological production of gases in the deep subsurface should be considered and incorporated into the model systems describing the dark biosphere of Earth and other planets such as Mars.

Acknowledgments: This research was supported by ERC project "MarsFirstWater" consolidator Grant 818602 and MICINN project PID2019-10481266GB-I00. C. E is a "María de Maeztu" postdoctoral research, AEI project MDM-2017-0737.

References:

[1] S. E. Lauro et al. (2021) *Nat Astron*, 5(1), 63-70. [2] C. Magnabosco et al. (2018) *Nat Geosci*, 11(10), 707-717. [3] T. O. Stevens and J. P. McKinley (1995) *Science*, 270(5235), 450-455. [4] V. A. Krasnopolsky (2004) *Icarus*, 172(2), 537-547. [5] L. Purkamo et al., (2015). *Microb Ecol*, 69(2), 319-332. [6] R. Amils et al., (2014) *Life*, 4(3), 511-534.