Multianalytical detection of biomarkers on a 1m-subsurface drill of an acidic environment (Rio Tinto) with analogies to Mars. Simulating a future drilling and bioanalysis on Mars polar latitudes. L. Sánchez-García[1], D. Carrizo[1], M.A. Fernández-Martínez[1], M. García-Villadangos[1], J.M. Manchado[1], Y. Blanco[1], M. Moreno[1], C. Stoker[2], B. Glass[2], and V. Parro[1]; [1]Centro de Astrobiología (CSIC-INTA), 28850, Torrejón de Ardoz, Spain (lsanchez@cab.inta-csic.es); [2]NASA Ames Research Center, Moffett Field, CA 94305, USA.

Introduction: Looking for organics, biomarkers and signs of past or extant life in Mars is one of the key objectives for present and future planetary exploration. Findings on the existence of water (liquid in the past, frozen in present) on Mars raised the probabilities of finding signs of life on the planet. The "Icebreaker", one of the mission concepts with astrobiological interest, was conceived to return to the Mars polar latitudes first visited by Phoenix lander in 2008 [1], to drill and sample the martian permafrost. The plan is to go through the hard ice layers that Phoenix encountered, with the aim of acquiring material to search for organic biosignatures on the ideally radiation-preserved 1 m deep subsurface.

Given the excessive costs and risks associated with full Mars missions, maturing the technology, procedures and the analytical techniques in terrestrial analogues is mandatory. Simulation campaigns enable to address troubleshooting and tune the whole process of sampling, material delivery and distribution, and *in-situ* analysis. Extreme terrestrial environments with analogies to Mars offer great potential for making progress in understanding how life may have adapted, spread, and left its fingertprints in the apparently inhospitable martian conditions.

Fig. 1. Picture of the Icebreaker 1m-class planetary prototype drill, during the LMAP 2017 Rio Tinto field campaign.

Río Tinto is an extreme environment in SW Spain considered a geochemical and mineralogical analogue of Mars due to the similarities of its sulfide bioleaching products and the vast sulfate and iron oxide deposits detected on the martian Meridiani Planum rocks [2]. The constant acidic waters and high concentration of heavy metals in Rio Tinto are direct consequence of the active metabolism of chemolithotrophic microorganisms thriving in the rich polymetallic sulfides present in the massive Iberian Pyritic Belt. Previous drilling projects MARTE (Mars Astrobiology Research and Technology Experiments, 2003-2006) and IPBSL (Iberian Pyrite Belt Subsurface Life Detection, 2011-2015) provided evidence of subsurface microbial activity based on the presence of groundwater and reduced substrates and oxidants resulting from the matrix bioleaching [3]. The extremophiles inhabiting the Rio Tinto environment generate biosignatures imparted to sedimentary rocks (e.g. goethite, jarosite and hematite) that facilitate their preservation, with astrobiological implications for Mars.

In the framework of the NASA funded Life-Detection Mars Analog Project (LMAP), we simulated the future search for signs of life on the martian subsurface by searching for molecular evidences of life in an acidic environment developed on a Fe- and S-based chemistry with analogies to the martian Meridiani Planum. As part of the Icebreaker start-up phase, a complete "groundtruth" bioanalytical test (lipidic biomarkers, metagenomics, immunoassay) is being carried out on the drill samples collected from an evaporitic-like site. This work aims at serve as a first approximation for the field work and *in-situ* analyses to accomplish on an ideally and successfully-funded Icebreaker mission.

Fig. 2. Surficial section (30 cm) of the vibro-core collected as groundtruth of the Icebreaker drill.

Field work: In June 2017, the Icebreaker 1m-class planetary prototype drill was tested with a full-scale Phoenix-like lander mockup at Rio Tinto (Fig. 1), together with a robotic scoop and arm (for sample collection and transfer), and the SOLID (Signs of Life

Detector) instrument for *in-situ* immunodetection of polymeric microbial markers [4]. As grountruth, complete metagenomic and lipidic analysis was performed back in the lab with the collected samples. Drilling was conducted adjacent to an acidic evaporitic site, rich in sulfur-iron material, with expected stickness properties as those forseen for water-rich Martian permafrost. Cutting from different depths (surface, 20 cm, 40 cm, 80 cm, and 100 cm) were acquired and transferred to the SOLID instrument for *in-situ* immunoassay (to be report elvewhere). A second sampling was conducted by vibro-coring to ca. 2 m deep core as analytical groundtruth for deeper characterization.

Once in the lab, both drill and groundtruth core samples were processed for extraction and analysis of DNA (16S rRNA gene sequencing), and lipidic biomarkers (DCM:MeOH, 3:1 Soxhlet extraction and GC-MS). The preliminary SOLID *in-situ* immunoassay was tested with a second bench-analysis with the Life Detector Chip (LDChip). The phylogenetic characterization would be combined with the detection of lipidic signatures to obtain a detailed characterization of biosources that may be contrasted with the SOLID-LDChip immunodetections. Comparing the biosignatures at different depths on the samples obtained with the Icebreaker drill versus the vibro-corer will inform on the analytical functionality of the Icebreaker sampler.

Results: Preliminary results suggest the occurrence of a variety of lipid biosignatures of prokaryotic and eukaryotic origin. The distribution patterns of functionalized lipids (e.g. *n*-alkanes and isoprenoids) showed mixed inputs of organic matter dominated by microbial sources (bacteria, archaea) and higher plants (Fig. 3), with variable trend with depth depending on the congener or ratio. Certain diagnosis ratios inform on the environmental oxicity (pristine/phytane), or on the dominance of eukaryotic versus prokaryotic sources (high molecular over low molecular weight alkanes). Preliminar values of carbon stable isotopic analysis ($\delta^{13}C$) support the presence of mixed sources, ranging from more depleted (from -27 to -27.5 ‰), characteristic of vascular plants, to more enriched (from -25 to -25.5 ‰) values, potentially deriving from diverse sources (e.g. algae, microbial). The phylogenetic analysis of the prokaryotic 16S rRNA gene is in agreement with the lipids and immunological (SOLID) results.

References:

[1] McKay et al., (2013) Astrobiology 13: 334-53; [2] Fernández-Remolar et al., (2005) *Earth Planet Sci Lett* 240: 149-167; [3] Amils, R. et al. (2014) Life 4: 511-534; [4] Parro, V. et al, (2011) Astrobiology 11: 15-28.

Acknowledgments:

This work has been possible thanks to the funding of the *Life-Detection Mars Analog Project* (LMAP; NASASolicitation No. 13-MMAMA13-0007). The authors L. Sánchez-García, D. Carrizo and V. Parro are also thankful to the Spanish Ministry of Economy and Competitiveness (MINECO) for funding their respective grants (CGL2015-74254-JIN; RYC-2014-19446; and ESP2015-69540-R) .

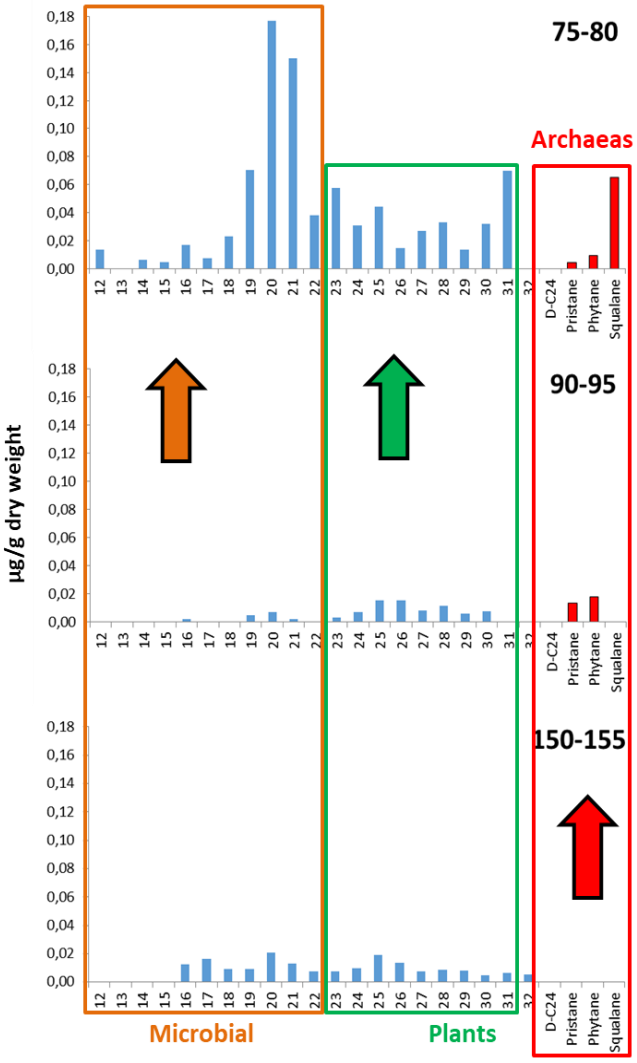

Fig. 3. Distribution of diagnostic alkanes at three different depths (right upper corners) on the groundtruth core, showing decreasing microbial and vegetal signals with depth.