Lipid biomarkers and geochemical characterization of Icelandic hydrothermal zones with analogy to early Mars

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Abstract: The characterization of lipid biomarkers and their relation with substrate mineralogy in Mars analogs on Earth is relevant to understand how hypothetical life could have developed in similar conditions on the Red Planet. Biogeochemical samples from sulfur-rich environments in Iceland, that is active/inactive fumaroles, mud pots and hydrothermal springs, were studied for the presence and distribution of lipid biomarkers, C and N stable isotopes, and mineralogical composition. A variety of lipids including *n*-alkanes, isoprenoids, *n*-carboxylic acids, *n*-alkanols, and steroids was detected, with molecular distributions indicating the presence of biological material from thermophilic and acidophilic bacteria.

Introduction: Icelandic geothermal systems have been proposed as Mars analogs for its ancient hydrothermal activity. In modern terrestrial hot springs and fumaroles, sulfates and clays precipitate under similar conditions as in early Mars. Thermal springs are extreme environments hosting life, especially extremophiles, with astrobiological interest for understanding the origin of life. Learning about habitability in other planets requires of a deep knowledge of adaptability and life boundaries on Earth. Remote and inhospitable environments provide excellent settings for assessing the capability of the most resistant forms of life (extremophiles) to endure and thrive in the harshest conditions. Extremophile communities in volcanic hydrothermal systems use energy from redox reactions to synthesize ATP for their metabolic processes. On Earth, redox couples include iron, sulfur, nitrogen, and carbon as electron acceptors/donors [1]. On Mars, the abundant iron and sulfur would likely support potential redox metabolisms. Studying the microbial forms thriving on sulfur-rich geothermal environments on Earth will contribute to understanding how hypothetical life could have developed on the early geothermal Mars.

Sample collection: Biogeochemical samples were collected from three geothermal areas (from E to W; Seltun, Hengill and Námafjall) in Iceland (Fig 1). A total set of 11 samples was collected from two active and inactive fumaroles (6 substrate samples, temperature range 20 to 90ºC), two mud pots (2 mud samples, temp. range 74 to 87ºC), and a thermal spring (3 microbial mats, with a range of temperature from 64 to 88 ºC), with a solvent-clean stainless steel spatula. The biogeochemical samples were stored in polypropylene containers and maintained cool (~4 °C) until transported to the laboratory, where they were frozen at -20ºC until analysis.

Geolipid Extraction: About 30 g of the substrate and mud samples were extracted for 24 h with a mixture of dichloromethane/methanol (DCM:MeOH, 3:1, v/v) in a Soxhlet apparatus, while 1-3 g (dry weight) of the microbial mats were extracted by ultrasound extraction (three 35-min cycles at room temperature) with DCM:MeOH (5:1, v/v) [2]. Internal standards (tetracosane-D_{50}, myristic acid-D_{27} and 2-hexadecanol) were added prior to extraction. The total lipid extract (TLE) was concentrated to 2 ml using rotary evaporation, and activated cooper was added for removing any elemental sulfur. The TLE was then separated into three fractions of different polarity (polar, non-polar, and acidic) using a Bond-elute and Al_2O_3 chromatography columns according to the method described by Sánchez-García [3].

GC-MS Analysis: The three lipid fractions were analyzed by gas chromatography mass spectrometry using a 6850 GC system coupled to a 5975 VL MSD with a triple axis detector (Agilent Technologies), operating with electron ionization at 70 eV and scanning from *m/z* 50 to 650. The analytes were injected (1 µl) and separated on a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 um film thickness) using He as a carrier gas at 1.1 ml·min^{-1}. The temperature details for the oven, injection, transfer line, and MS source are described elsewhere [4]. Compounds identification was based on the comparison of mass spectra with reference materials, and their quantification on the use of external calibration curves of *n*-alkanes, *n*-carboxylic acids, and *n*-alkanols.

Figure 1. Map of the three geothermal areas on Iceland: Seltun (SE), Hveragerdi (Hdi in Henghill) and Hverir (HV in Námafjall).
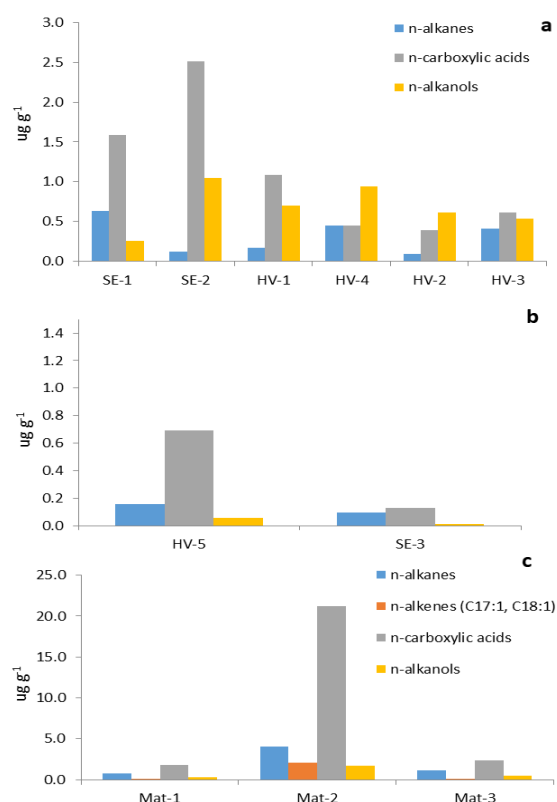

Figure 2. Concentration of lipid biomarkers in a) fumaroles (active, SE; inactive, HV), b) mud pots (HV and SE), and c) microbial mats from Hdi (Mat-1, Mat-2 and Mat-3).

Summary and Conclusions: A variety of lipid families (normal, branched, and unsaturated alkanes; normal, branched, unsaturated, dioic and oxo carboxylic acids; normal and branched alkanols; PAHs; and sterols) were detected in the Icelandic geothermal samples (Figure 2). Total organic carbon ranged from 0.01 to 5.00 % dw and $\delta 13C$ from -8 to -21‰). The mineralo-

gy of the fumaroles and mud pot samples was dominated by silica, sulphur-rich forms (elemental sulfur and pyrite), as well as hematite and clays (montmorillonite and kaolinite). The presence of certain lipid biomarkers revealed different organic sources: a) fossil material (PAHs), b) modern plant debris (long chain n-alkanes), and c) extremophile microbial biomass (functionalized mid/short chain n-acids, n-alkanols, and isoprenoids). Overall, there was a predominance of the microbial biosignatures in all samples. Different lipid abundance and distribution patterns were found as a function of the sample type (i.e. fumarole, mud pot or microbial mat), and of the temperature and mineralogy

References: [1] Krebs, J.E. et al. Astrobiology. 2014. 3:229-240.; [2] Martrat B, et al. Science. 2004. 306:1762–1765; [3] Sánchez-García L., et al. Frontiers in Microbiology 2019. 9, doi: 10.3389/fmicb.2018.03350; [4] Sánchez-García L., et al Biogeochemistry. 2018. 1:31-52.