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Molecular evidences of life in a poly-extreme environment in Ethiopia, the Dallol Hot Springs area, based on lipidic biomarkers.

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Abstract

The characterization of biosignatures in extreme environments on Earth with analogies to Mars is relevant to understand how hypothetical life could have developed in similar extreme conditions. The Dallol hot springs region in Ethiopia was studied for the presence and distribution of lipidic biomarkers and stable isotopes. This hydrothermal system is considered a poly-extreme environment of characteristic: high salinity, high temperature and extremely acidic waters (pH~0.2-4). A variety of lipids including *n*-alkanes, isoprenoids, *n*-carboxylic acids, *n*-alkanols, or steroids, was detected, with molecular distributions indicating the presence of (present or past) biological material from hyper-thermophilic and thermophilic bacteria.

1. Introduction

Recent new geological, chemical and computational findings rise up the hypothesis that life originated in hot springs areas on land, over the deep-sea hydrothermal vents theory (Damer, B, 2016). Organic preservation of biosignatures has been described in extreme environments of diverse typology, such as geothermal silica sinter areas from New Zealand or USA (Kaur et al. 2015; Jahnke et al. 2001), hyperarid and hypersaline environments (Sánchez-García et al., 2018), or acidic rivers developed on a Fe- and S-based chemistry (i.e. Río Tinto, SW Spain) (Amils et al., 2007). In contrast, the information is scarce on the distribution, and preservation of biosignatures in systems combining various extreme characteristics (i.e. poly- or multi-extreme environments). Located in one of the most remote, inhospitable, and poorly studied locations in the world, Dallol is a complex active hydrothermal system in the Danakil Depression of Ethiopia (Fig. 1) composed of diverse hot springs that opens into an arid dessert. In Dallol, seawater and hydrothermal fluids mix resulting into a hyper-saline environment of extremely high temperature (mean water temperature of ~90ºC) and water pH below 4 (Frenzson et al., 2015). We aim at investigating the presence and distribution of molecular and isotopic biosignatures in the remote and poly-extreme environment of Dallol hot spring region. The detection of molecular evidences of (past or present) life in the Dallol geothermal system goes with the hot spring hypothesis for life´s beginning and has implications for searching for potential evidences of life in other poly-extreme environments even beyond the Earth.

2. Sample collection

The Dallol hot springs area is situated in the Danakil Depression in northern Ethiopia (Fig 1). During the Europlanet sampling campaign of 2016 (January), a set of 5 geological samples was collected from three different hot springs from the Dallol area (-120 m below sea level) with clean stainless-steel spatula. The samples were stored in pre-cleaned polypropylene containers and maintained at cool temperature until transported to the laboratory, where they were frozen at -20ºC until analysis.

2. 1. Geolipid Extraction

About 30 g of sample were extracted with a mixture of dichloromethane/methanol (DCM/MeOH, 3:1, v/v) during 24 h in a Soxhlet apparatus. Internal standards (tetracosane-D_{50}, myristic acid-D_{27} and 2-hexadecanol) were added prior to extraction. The total lipids extracts were concentrated using rotaevaporation to 2 ml, and activated cooper added for elemental sulfur removal. The extracted sample was then separated into three fractions of different polarity (polar, non-polar, and acidic) using a Bond-elute (bond phase NH_2, 500 mg, 40 um particle size) and Al_2O_3 columns chromatography.

2.2. GC-MS Analysis

The different samples 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 ul) 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.

3. Figures

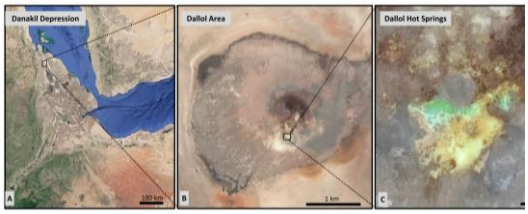

Figure 1: Map of the Danakil depression in Ethiopia (A), showing the Dallol area (B), where samples were collected (C)

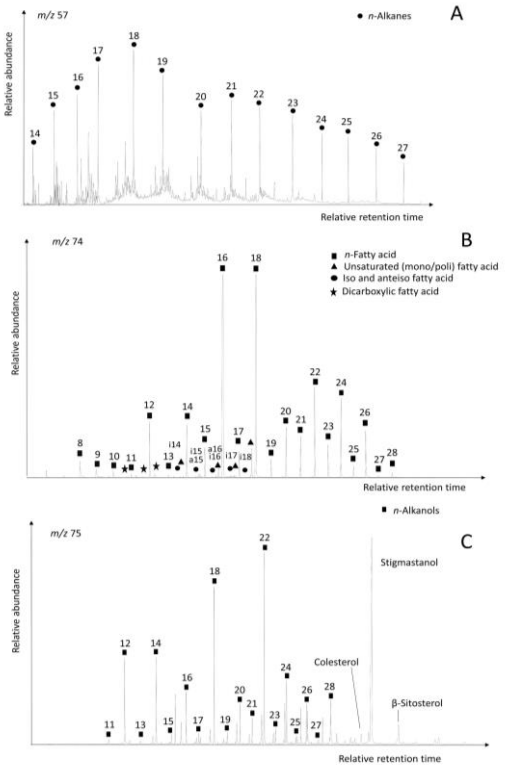

Figure 2 Mass chromatograms of the three lipidic fractions analyzed in the Dallol sample D8; *n*-alkanes (*m/z* 57) (a), *n*-carboxylic acids as methyl esters (*m/z* 74) (b), and *n*-alkanols as trimethyl-silyls (*m/z* 75) (c).

4. Summary and Conclusions

A number of lipidic families (normal and branched alkanes; normal, branched, unsaturated, and dioic fatty acids; normal and branched alkanols; wax esters and sterols) were detected in the Dallol hot spring samples, with distribution patterns revealing the presence of biological vestiges. The general preference for the even-over-odd molecular distributions illustrated the predominance of microbial signatures. Despite the inhospitability of the Dallol Hot Springs, certain microorganisms (i.e. thermophiles) are resistant enough to thrive (past or presently) in this poly-extreme environment. While a definitive distinction between presently active metabolisms or fossilized biological fingerprints cannot be accomplished, the relative abundance of functionalized (i.e. *n*-fatty acids and *n*-alkanols) over saturated hydrocarbons (i.e. *n*-alkanes) points to present or recently active metabolisms producing the typical microbial signatures. Whereas further investigation is needed to identify the microbial communities associated to the hydrothermal and evaporitic substrates, the present study constitutes the first geomicrobial approach to describe the Dallol poly-extreme environment, and has implications for interpreting GC/MS results from current and future missions in the search for life in similar extraterrestrial environments (e.g. Mars). Future work will be addressed to search for archaeal biomarkers (e.g. glycerol dialkyl glycerol tetraethers, GDGTs), metagenomics, and compound specific-isotopic composition.

Acknowledgements

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