BIOMARKER DETECTION IN FOSSILIZATED ANAEROBIC BACTERIAL STRAINS

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Abstract

The origin of Life in terms of how it appeared and what conditions made it possible, as well as the search for life beyond Earth is a central objective for the Astrobiology community. Its emergence and its evolution need to be well-constrained by the set of conditions that allows the appearance of life and successful establishment and survival of microorganisms, in any location, which is known as habitability [1, 2]. Key to this is also acknowledgement of the fact that microbial life is remarkably resilient and capable of flourishing under extreme conditions if the necessary requirements are available [3, 4]. In the last decade several studies have focused especially on the study of changes in the biosignatures and their detectability in an "artificial ageing" context. To better understand how organic matter and cells interact with minerals and how this affects the detection of biomarkers would be a powerful tool to enhance searching for these biosignatures beyond the Earth.
This study concerned the artificial mineralization of two microbial species under hydrothermal conditions and further analysis of the changes in biomarker preservation during the process. Life appeared and flourished on an anaerobic volcanic planet whose crust was predominantly composed of basalt and komatiite lavas and strongly influenced by hydrothermal fluids [5, 6]. For this reason these two substrates as mineralization sources were used to carry out our experiments at hydrothermal temperatures. Two models of mesophiles, isolated from environmental MASE European project samples were used with the named substrates: *Buttiauxella* sp. MASE-IM-9 and *Halanaerobium* sp. MASE-BB-1. Vials containing 50 ml of samples were stored both at 60°C and 120°C to simulate a hydrothermal context (hot, anaerobic and basalt-rich conditions). Vials with cells in MASE medium [7] and without minerals were also stored at each temperature and vials of each model were kept at room temperature to be used as reference. Three different approaches were stablish to (1) Biomarker detection of fractionated analytes from samples by competitive sandwich immunoassay in microarrays, (2) comparison of electrophoretic protein profiles of cultures by SDS-PAGE and (3) monitoring of the state of the samples by Scanning Electron Microscope of immobilized and desiccated cultures on polycarbonate filters. One vial at 60°C of each bacteria was also placed into silica gel in acidic conditions to check how the detectability of biosignatures differ from non-silicified samples.

Immunograms of the extracted samples from *Butiauxella* sp. MASE-IM-9 shows a very similar pattern in signals detected as positives both at 60°C and 120°C as well as at room temperature (RT) and in the silicified vial. On the other hand, although immunoprofiles of

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samples from *Halanaerobium* sp. MASE-BB-1 show similarities in signals detected at both temperatures, there was a clear decrease in number of biomarkers detected at 120°C compared to at 60°C or even at RT and to the silicified vial. The pattern in proteins extracted and separated by electrophoresis in acrylamide gel in denaturing conditions (SDS-PAGE) reveals some differences within each bacterial model tested between the several culture conditions regarding temperature and time.

It is concluded that, although the process of fossilization has harsh effects on the biomarkers and, although longer times to complete fossilization, some biomarker traces are still detectable in the samples. This brief study highlights a promising field of research to be followed in the following years that will help us in understanding how life copes with environmental events in both short and long terms, as well as providing a very interesting tool for the search of life traces here and beyond Earth.

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