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Biosignatures and Bacterial Diversity in Hydrothermal Deposits of Solfatara Crater, Italy

Mihaela Glamoclija,[1] Laurence Garrel,[1] Jonathan Berthon,[2] and Purificación López-García[2]
[1]*International Research School of Planetary Sciences, Università d'Annunzio, Viale Pindaro 42, 65127 Pescara, Italy*
[2]*Unité d'Ecologie, Systématique and Evolution, CNRS UMR 8079, Université Paris-Sud, bâtimet 360, France*

We have combined mineralogy, organic geochemistry and molecular microbiology to study hydrothermal deposits from Solfatara Crater, a geologically young volcanic formation (∼4,000 years old) displaying hot (45–95°C) and acidic (pH 1.7) mud pools and fumaroles. The search for inorganic (mineral) biosignatures revealed the presence of delicate structures, most likely mineralized extracellular polymers (EPSs), and the presence of potential biologically induced minerals: sulfides, sulfates (barite and alunite), elemental sulfur, and iron oxides. Geochemical analyses revealed a low total organic carbon content, 0.13 to 0.53%, displaying δ^{13}C values from −17.09 to −27.39‰, and total nitrogen contents from 0.03 to 0.12%, which are characteristic of hydrothermal systems and suggest the presence of autotrophic carbon fixation. Lipid biomarker analysis showed the presence of hopanoids and linear alkanes, and the absence of detectable steroids, implying the occurrence of bacteria in our samples. We constructed 16S rRNA gene libraries from the environmental samples. Most environmental sequences obtained were affiliated to the Alpha- and Betaproteobacteria (*Hydrogenophilus*-like), the Acidobacteria, and to a lesser extent, the Gammaproteobacteria and Actinobacteria. When known, the closest cultivated relatives were often thermophilic or thermotolerant bacteria oxidizing iron, hydrogen, or methane/methanol, suggesting an important microbial contribution to the formation of biominerals.

Keywords 16S rRNA, bacterial diversity, biosignatures, hydrothermal, Solfatara Crater, thermoacidophile

INTRODUCTION

Since life appeared on Earth and for most of its history, microorganisms have been the lone inhabitants of our planet. Microbes can live in a wide variety on environments, including those exhibiting the most extreme conditions (Rothschild and Mancinelli 2001). Microbial genetic diversity is huge, as has been increasingly revealed by molecular ecology surveys over the past fifteen years (Pace 1997; Hugenholtz et al. 1998), yet prokaryotic microorganisms display a restricted number of morphotypes. This, together with the fact that microorganisms are rarely preserved in fossil form, has hampered the reconstruction and timing of early evolutionary diversifications. The search for diagnostic biosignatures from past microorganisms is not only crucial to understand early evolution on our planet, but might also help to reveal traces of ancient biological activity on planets such as Mars, where physical–chemical conditions were similar to those of the Archaean Earth.

In addition to fossils discernible by their morphology, microorganisms and microbial communities influence and modify their environment during their lifetime, both at the micro and macroscale, and may thus leave traces of their existence. The most easily recognizable are stromatolites and permineralized biofilms. At a smaller scale, remnants of mineral-microbe interactions may remain but, unfortunately, surface chemistry can often yield a wide variety of mineral alterations that can be easily misinterpreted as derived from biological activities. The difficulty of finding unmistakable microbial biosignatures is evidenced by two recent controversies. The first concerns the nature

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Address correspondence to Mihaela Glamoclija, International Research School of Planetary Sciences, Università d'Annunzio, Viale Pindaro 42, 65127 Pescara, Italy. E-mail: mihaelag@irsps.unich.it