

BACTERIAL AND ARCHEAL COMMUNITIES IN AN ANTARCTIC SALT EFFLORESCENCES FROM THE BOULDER CLAY GLACIER (NORTHERN VICTORIA LAND). M. Azzaro¹, M. Papale¹, C. Rizzo^{1,2}, M. Guglielmin³, A. Lo Giudice^{1*}, ¹Institute of Polar Sciences (CNR-ISP), National Research Council, Spianata S. Raineri 86, 98122 Messina, Italy, angelina.logiudice@cnr.it, ²Zoological Station “Anton Dohrn” Department of Ecosustainable Marine Biotechnology, Villa Pace, Contrada Porticattello, 98168 Messina, Italy, ³Department of Theoretical and Applied Sciences, Insubria University, Via Dunant, 3, 21100 Varese, Italy

Introduction: The interest in extreme environments has grown in recent years to understand what the real limits of the survival of species are and to what extent life goes to persist. Hyper-saline environments are present on our planet in all continents, including coastal lagoons, salt and soda lakes, briny pools, brine channels in sea-ice and subsurface brine deposits. Antarctica hosts several subsurface hyper-saline liquid cryobrine that reach the eu-tectic point (i.e., the point at which all compounds, including water, pass to the solid state) at temperatures below 0 °C. This makes such Antarctic saline cryoenvironments possible terrestrial analogs of Mars [1]. In fact, geomorphic and geochemical observations suggest the occurrence of liquid water or brines in the subsurface of the red planet [2], possibly representing a protected environment for potential actual or past life. More interestingly, evaporite deposits, probably deriving by eruptions of subsurface fluids (mounds), were discovered on Mars [3,4].

The presence of salt deposits is generally related to warm and arid environmental conditions. However, some salts form by cooling and a concentration mechanism based on cooling and/or freezing. In fact, the freezing process acts similarly to the evaporation in the concentration of saline liquids (called frigid and evaporative concentration, respectively), as water is removed from the solution by the formation of ice, leading to a concentrated residue and the progressive precipitation of saline minerals [5]. The minerals formed under these conditions are known as cryogenic [6,7].

These kinds of habitats are considered extreme due to the stress imposed on cells through the strong salinity gradient between intra- and extracellular environments. Microbial communities, at the basis of the functioning of all ecosystems, have been widely proven to have great adaptability and resistance capacities even in the presence of very adverse conditions. However, the adverse conditions of such environments affect the microbial community diversity, by favoring the only few taxonomic groups that can survive adapting to such peculiar conditions. Among extremophilic microorganisms, halophiles are known to be able to tolerate salty habitats thanks to special mechanisms of adaptation. Indeed, they accumulate or produce nontoxic solutes to maintain the internal environment isosmotic with the external environment and use special proteins to avoid denaturation at high salt concentration. Halophiles dominate the

microbial communities and despite in many cases they are mainly represented by several groups of archaeal microorganisms, in some hypersaline environments the predominance is given by Bacteria. They are also of special interest for astrobiological perspectives, due to the strong similarities of hypersaline environments with Martian surfaces, i.e., high salt concentration and strong UV levels.

The present research was aimed at studying salt efflorescences from the Boulder Clay Glacier (Antarctica) debris-cone from the perspectives of microbial diversity, as a hot spot of microbial life, in extreme environmental conditions, comparable to extraterrestrial life. Mineralogical analyses were, moreover, performed to investigate the minerals occurring in the salt efflorescences.

Methods: Salt efflorescence (mirabilite and thenardite) samples were collected on the Boulder Clay Glacier (Northern Victoria Land, Antarctica)

Sampling activities. The salt cone was about 3.5 m high. Its stratigraphy, from the surface to the bottom, encompasses moraine materials (5-15 cm thick) with some blocks and boulders, altered and fresh semi-transparent crystals, compact ice with vertical bubbles and sediments. Close to the surface the materials were altered into a white powder, while in contact with ice the crystals seemed to be stable [8].

The collection of salt samples was performed after removing the external layers above the salt cone. Samples for DNA extraction were aseptically collected from both fresh crystals, from about 5 cm below the cone surface, and in the proximity of sediment, resulting in two colors, i.e., white (WS) and dark (DS) salt, respectively. The sampling was conducted taking care to collect the two types of salt separately. All samples were stored at -20 °C until analysis.

Prokaryotic diversity. The prokaryotic community diversity and composition were evaluated by a next generation approach (Illumina MiSeq platforms). Total DNA was extracted and the 16S rRNA genes were amplified and analyzed as previously reported [8].

Results: Overall, the phylogenetic analysis allowed the identification of *Bacteroidota*, *Actinobacteriota*, *Firmicutes*, and *Gammaproteobacteria* as the main bacterial lineages, in addition to Archaea in the phylum *Halobacterota*. The genera *Arthrobacter*,

Rhodoglobus, *Gillisia*, *Marinobacter* and *Psychrobacter* were particularly abundant. Interestingly, several bacterial and archaeal sequences were related to halotolerant and halophilic genera, previously reported in a variety of marine environments and saline habitats, also in Antarctica. The analyzed salt community also included members that are believed to play a major role in the sulfur cycle.

This study has contributed to deepening the current knowledge on the microbial ecology of salt efflorescences from a Boulder Clay Glacier debris-cone in the Northern Victoria Valley (Antarctica). The cryogenic ecosystem studied could be an oasis of life, with differences between the WS and DS layers, where four main bacterial lineages live. Part of the sequences found could represent part of the ancestral microbial communities living in the salt efflorescences or in the briny system of origin. The microorganisms present in the salt efflorescence on Earth could be candidates for future implants on distant planets with extreme conditions such as those found in Antarctica.

References: [1] Peterson R. C. et al. (2007) *Am. Mineral.*, 92, 1756–1759. [2] Gulick V. C. (2001) *Geomorphology*, 37, 241–268. [3] Oehler D. Z. and Allen C. C. (2010) *Icarus*, 208, 636–657. [4] Burr D. M. et al. (2009) *Icarus*, 200, 52–76. [5] Stark S. C. et al. (2003) *Australian J. Chem.*, 56, 181–186. [6] Babel M. and Schreiber B. C. (2014) In *Treatise on Geochemistry*, 2nd ed.; Turekian, K., Holland, H., Eds.; Elsevier: Oxford, UK. [7] Liu T. et al. (2015) *Antarc. Sci.*, 27, 73–84. [8] Arraro M. (2022) *Microorganisms*, 10, 1753.

Funding: Research activities were funded by the Programma Nazionale di Ricerche in Antartide (grants PNRA 2013/AZ1.05, PNRA 2016_00194-A1; PNRA2018_00186-E).