PROKARYOTIC LIFE IN HYPERSALINE BRINES FROM CRYOSYSTEMS OF THE NORTHERN VICTORIA LAND.

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Introduction: Continental Antarctica hosts a number of cryosystems (e.g., ice covered and subglacial lakes, permafrost, glaciers) which conceal liquid hypersaline brine lenses [1-6]. Their unfrozen conditions, even several degrees below 0 °C, are maintained by their high salt content. The genesis and mobilization of brines within Antarctic cryosystems remain poorly understood [7]. However, geophysical observations demonstrated that brines are dynamic systems, with subglacial streams that can connect lakes and ponds with their movements [4, 8]. Besides their geological relevance, brines are particularly interesting for better delineating and elucidating the functioning of briny ecosystems by the description of inhabiting lifeforms, mainly microscopic. This latter aspect is particularly attractive also from a planetary geo-biological perspective and becomes fundamental in the assessment of the habitability of other worlds within our Solar system [9].

The analogies between terrestrial sites and their extraterrestrial targets can rely on mineralogical, geochemical and geomorphological features, as well as on physico-chemical environmental parameters and conditions. Among them, the principal habitability criteria defined by NASA are addressed to extended regions of liquid water, intrinsically supporting life as on Earth [10]. At this regard, liquid brines have been discovered below the ice of the South Polar layered deposits on Mars [11], in the subsurface of Pluto [12], on Jupiter's moon Europa, and Saturn's moons Enceladus and Titan [13, 14].

The exploration of terrestrial analogues represents a unique opportunity to gain a "critical ground-truthing for astrobiological studies" also in terms of detectability of biosignatures and potential for the long-term preservation of signs of past life [9]. Terrestrial brines are "second-order analogues" as their classification is based on indirect or highly suggestive evidence that have the potential to be proven false. In turn, extremophiles are "analogues of third order" as no direct or indirect evidence that life exists anywhere other than on Earth is available [9]. As stated by Lyons et al. [7], "*Whether these sub-cryospheric oceans can sustain life today is a major question in astrobiology and planetary exploration*". As potential astrobiological targets, the exploration of brines, and their psychrophilic microbial inhabitants, on Earth becomes incredibly sounding for our comprehension of the boundaries of life on Earth and the development of scenarios for planetary habitability

Some authors have speculated on the possible existence of Northern Victoria Land environments with similar chemical and physical features on the Martian surface [15, 16], while subsurface origin of brines resembles physical conditions on the icy moons Enceladus and Europa, both characterized by a high habitability potential [17]. Thus, endoglacial and subglacial brines could represent a second-order terrestrial analogue environment giving us the opportunity to investigate planetary habitability by the exploration of diversity and activity of extremophilic microorganims.

This contribution is aimed at showcasing our current knowledge on the genomic diversity of prokaryotes (Bacteria and Archaea) in brines from the Northern Victoria Land.

Methods: Brines were collected from three perennially ice-covered lakes in the Boulder Clay and Tarn Flat areas of the Northern Victoria Land [18-21], along with a englacial brine from the Boulder Clay Glacier [22].

Sampling activities. Brine samples (BC1, BC2 and BC3) were collected near the ice blisters of Lake 16 (L16; i.e., BC1 and BC2) and Lake L-2 (i.e. BC3) in the Bouder Clay (BC) area. In particular, brines BC1 and BC2 were collected from two different sampling points and depths (2.5 and 0.9 m, respectively) of Lake L16, whereas the brine BC3 derived from 2.3 m-depth of Lake L-2. In a lake of the Tarn Flat (TF) area, two brine samples, namely TF4 (between 3.78 and 3.98 m depth) and TF5 (between 4.10 and 4.94 m depth), were collected. Brines from the perennially ice-covered lakes in BC and TF were collected by a sterilized peristaltic pump and tubing. After collection, the samples were kept at −20°C until processing in Italy. Salinity values were as follows: BC1, 6333±15 mg l^{-1}; BC2 37.6±2.1 mg l^{-1}; BC3, 1583±6.81 mg l^{-1}; TF4, 90 psu; TF5, 75 psu.

The englacial brine was was collected at 9.1 m depth in a borehole on the Boulder Clay Glacier (BCG). Il was hypersaline with a salinity of >70 psu.

Prokaryotic diversity. The prokaryotic community diversity and composition were evaluated by a next generation approach. Total DNA was extracted from all brine samples, and the 16S rRNA genes were amplified and analyzed as previously reported [18, 20-22].

Results: The analyzed cryo-environments were different in terms of prokaryotic diversity. Bacteria and Archaea assemblages in brines BC1, BC2, and BC3 from two adjacent and perennially frozen Antarctic lakes (L16 and L-2) in the Boulder Clay (BC) area were similar at phylum level, but differences were encountered when considering the distribution in species. Overall, the total bacterial communities were dominated by *Bacteroidota*. The BC lakes hosted sequences of the most thermally tolerant archaea, also related to well-known hyperthermophiles. Interestingly, RNA sequences *(data not shown*) of the hyperthermophilic genus *Ferroglobus* were retrieved in all brine samples.

Although similar diversity indices were computed for both Bacteria and Archaea, distinct bacterial and archaeal assemblages were also observed in TF4 and TF5. *Bacteroidota* and *Gammaproteobacteria* were more abundant in the shallowest brine pocket, TF4, and *Deltaproteobacteria*, mainly represented by versatile sulphate-reducing bacteria, dominated in the deepest, TF5. The detection of sulphate-reducing bacteria and methanogenic Archaea likely reflects the presence of a distinct synthrophic consortium in TF5. Surprisingly, members assigned to hyperthermophilic *Crenarchaeota* and *Euryarchaeota* were common to both brines, indicating that these cold habitats host the most thermally tolerant Archaea.

The prokaryotic community inhabiting BCG presented some peculiarities, such as the occurrence of sequences of *Patescibacteriota* (which can thrive in nutrient-limited water environments) or few *Spirochaetota*, and the presence of archaeal sequences of *Methanomicrobiota* closely related to *Methanoculleus*, a methanogen commonly detected in marine and estuarine environments.

Overall, our findings indicate hypersaline brines from the Northern Vicrotia Land (Antarctica as plausible terrestrial candidates for the study of the potential for extant life on different bodies of our solar system.

References: [1] Murray A. E. et al. (2012) *PNAS, 2012,* 20626-20631. [2] Dickson J. et al. (2013) *Sci. Rep., 3,* 1166. [3] Toner J. D. et al. (2013) *Geochim. Cosmochim. Acta, 110,* 84-105. [4] Mikucki J. et al. (2015) *Nat. Comm., 6,* 6831. [5] Forte E. et al. (2016) *Sci- Rep., 6,* 33158. [6] Badgeley J. A. et al. (2017) *J. Glaciol., 63,* 387-400. [7] Lyons W. B. (2019) *J. Geophys. Res. Biogeosci., 124,* 633– 648. [8] Siegert, M.J., Ross, N., and Le Brocq, A.M. (2016) *Philosoph.l Trans. Royal Soc. A: Math., Phys. Engin. Sci., 374*. [9] Preston L. J. and Dartnell L. R. (2014) *Int. J. Astrobiol., 13,* 81–98. [10] Hendrix A. R. (2019) *Astrobiology, 19,* 1-27. [11] Orosei R. et al. (2018) *Science, 3,* 490-493. [12] Nimmo F. et al. (2016) *Nature, 540,* 94–96. [13] Postberg F. (2009) *Nature, 459,* 1098–1101. [14] Mitri G. et al. (2014) *Planet. Space Sci., 104,* 78-92. [15] Burr D. M. et al. (2009) *Geophys. Res. Lett., 36,* L22203- L22207. [16] Porcino N. et al. (2020) *Chemosphere, 251,* 126229. [17] Garcia-Lopez E. and Cid C. (2017) *Front. Microbiol., 8,* 1407. [18] Papale M. et al. (2019) *Microorganisms, 7,* 333. [19] Rizzo C. et al. (2020) *Microorganisms, 8,* 819. [20] Papale M. et al. (2021) *Diversity, 13,* 323. doi: 10.3390/d13070323. [21] Lo Giudice A. et al. (2021) *Astrobiology, 21,* 551-565. [22] Guglielmin M. (2023) *Sci. Rep., 13,* 177.

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