

# ANTARCTIC CRYPTOENDOLITHIC COMMUNITIES: A NATURAL BENCHMARK FOR SEARCHING LIFE ON THE RED PLANET.

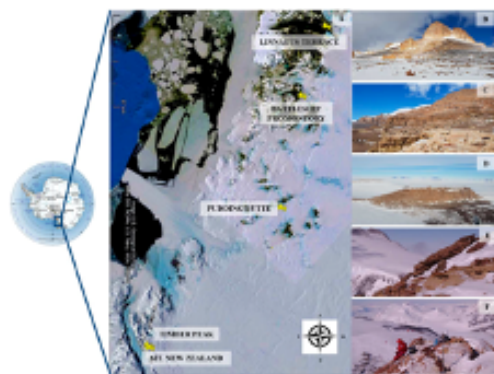
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**Introduction:** The ice-free areas of Antarctica, such as the McMurdo Dry Valleys, constitute some of the oldest, coldest, driest and most oligotrophic areas on Earth [1]; there, environmental parameters are very similar to the ones described on Mars [2], which is why these regions are accounted as “Martian analogues on earth” [1]. Here, life is not possible except for specialized microbial species colonizing the interstices of porous rocks, adopting cryptoendolithism as a survival strategy [3]. Recent studies conducted in the Victoria Land allowed a detailed screening of taxonomic, metabolic and functional diversity of antarctic cryptoendolithic communities, by using next generation sequencing (NGS) techniques [4].

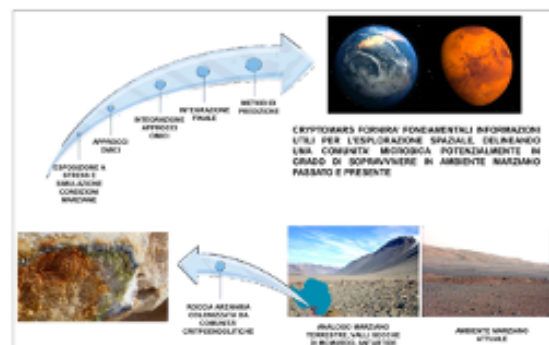
These communities, perpetuating in the long run under Mars-like conditions, are optimal models to investigate the possibility of life on the Red Planet, suggesting that if Mars has never been colonized, the species living there may have adopted survival strategies similar to the one adopted by cryptoendolithic species living on Earth before their extinction [5].

In this contest, we here propose to focus on cryptoendolithic antarctic communities as a proxy to evaluate and understand *if* and *how* microbial species may have lived on Mars or Mars-like planets. The aim of CRYPTOMARS is to outline the characteristics of a possible microbial community that could potentially have lived on Mars, in its early geological stages or under current conditions, based on the responses and survival strategies adopted by terrestrial antarctic species.



**Figure 1:** A) Map of the locations in Victoria Land, Antarctica, where samples of sandstone rocks, colonized by cryptoendolithic communities object of this project, were collected; B) Linnaeus Terrace; C) Battleship Promontory; D) Budding Butte; E) Timber Peak; F) Mt New Zealand.

**Work plan:** the very first step is to set a selection of colonized rocks samples from the collection of the “Mycological section of National Museum of Antarctica (MNA)” collected in specific locations (figure 1) characterized by increasing environmental pressure due to altitude and sun exposure; microbial communities will be reactivated by rehydration and incubation at 15°C. Samples will be exposed to selected conditions, terrestrial and early and present martian environments, into a simulation chamber for 21 days. After that, vitality will be tested by colorimetric analysis, by detection of active metabolism by, for example, fluorescein diacetate and Thiazolyl blue tetrazolium bromide assay (MTT), Griess reaction assay to analyze nitrites reduction, blue methylene assay to detect sulfides production and LCK320 assay for Fe<sup>3+</sup> reduction. Vitality will be also tested by culturomic approach counting (Colony-Forming Units); samples will be seeded in plates with MEA (Malt Extract Agar) and PCA (Plate Count Agar) to detect growth of fungi, algae and bacteria. Where applicable, vitality will be also verified with molecular approach by propidium monoazide assay (PMA) which allows to amplify DNA from viable cells only (figure 2). The results on vitality tests will give a clear picture of the survival capability of each community under specific stress before the metabolomic/metagenomic subsequent analyses.



**Figure 2:** CRYPTOMARS workflow. On the arrow, by left to right: exposition to stresses and martian simulated conditions; -omic approaches; -omic approaches integrations; final integration; prediction methods. Cryptomars would give useful information for space exploration, outlining a microbial community potentially capable of surviving in the early and present Martian environmental conditions. On the bottom of the figure, we can see an example of the present Mars and terrestrial Mars-like environment (McMurdo Dry Valleys, Antarctica), focusing on sandstone rocks colonized by cryptoendolithic communities (image on the left).

**References:**

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