MULTI-OMIC CHARACTERIZATION OF ANTARCTIC CRYPTOENDOLITHIC COMMUNITIES AS PROXY FOR SEARCHING LIFE ON MARS - CRYPTOMARS.

L. Selbmann[1,2] C. Coleine[1], C. Del Franco[1], C. Ripa[1], M. Garcia-Aloy[3], P. Franceschi[3], C. Donati[3], M. Pedone[4], S. Pezzilli[4], B. Negri[4]

[1]Università degli Studi della Tuscia,

[2]Sezione di Micologia del Museo Nazionale dell'Antartide

[3]Centro di Ricerca e Innovazione, Fondazione Edmund Mach, Via Edmund Mach, 1, 38098 San Michele all'Adige, Trento, Italy.

[4]Agenzia Spaziale Italiana, ASI, via del Politecnico snc, 00133 Rome, Italy.

selbmann@unitus.it

Introduction: Studying the possibility of life outside our planet is a central theme in space research; from this perspective, the planet Mars certainly represents one of the most interesting targets.

The present project fits into this area with the main aim of answering the question "which set of characteristics, in terms of genetic and functional diversity, would allow a microbial community to resist, adapt, spread and perpetuate itself on Mars or on Mars-like planets?"

To this end, we are focusing on unique microbial communities, known as cryptoendolithic that live in ice-free areas in continental Antarctica (e.g., the McMurdo Dry Valleys), universally considered as the best terrestrial analogue for the Martian environment [1]. The innovation and originality of the project idea, along with the most updated experimental approaches proposed, lies in the fact that the entire study is focused on microbial communities as a whole. The success, resilience and functionality of ecosystems depend, in fact, on microbial assemblages that act in synergy, rather than on individual microorganisms, even if they have particular capabilities. In the environment in which these communities live, due to the low temperatures, very strong aridity, oligotrophy and strong solar radiation during the Austral summer, the limiting conditions for supporting life are reached and exceeded [2]. For this reason, these environments were considered sterile until these particular microbial communities were discovered [3], capable of exploiting the endolithic niche by developing inside the pores of the sedimentary rocks, finding a last refuge during the process of cooling and aridification of the continent. Due to the analogy of the geological history of the planet Mars with that of these areas, these niches are optimal models for understanding the characteristics and adaptations that may allow life settlement in Mars-like environments, offering a new perspective on the possibility of life, past or present, on the Red Planet. These communities, and the microorganisms isolated from them, are incredibly resistant and capable of surviving, if dehydrated, both in space exposure outside the International Space Station and in simulated Martian conditions for as long as 18 months [4,5,6]. However, studies on their metabolic responses [6] and their genomic characteristics [7] are in their infancy and nothing is known about the real possibilities of active life in simulated extraterrestrial conditions.

To fill this gap and clarify what are the characteristics that allow these communities to live in such extreme conditions, we propose here a multidisciplinary project (CRYPTOMARS), where biological, chemical and physical skills interact synergistically, in order to:

1) predict the genetic and functional characteristics allowing these microbial communities to perpetuate in the Martian analogue on Earth ;

2) assess the vitality of these communities after chronically exposing them to different selected stresses characterizing the past and present Martian environment (UV and ionizing agents, water stress, extreme temperatures);

3) test the responses through a metabolomic and lipidomic approaches after the selected treatments; integrate all previous results related to each stress and the genomic bases of the response in terms of both the genes responsible for resistance and specific metabolic pathways.

The previous twenty years of studies on the crypto-endolithic communities of the Antarctic desert by the proponent (Laura Selbmann), who participated and coordinated several sampling campaigns in Antarctica, have laid the foundations for the realization of this project, which can benefit from a wide selection of colonized rock samples. The sampling covers a large area of Victoria Land on an altitudinal gradient (from 800 to 3,100 m above sea level), and different solar exposures. The proposed microbial communities have been collected in a notable range of environmental conditions, which allow a comparative analysis in terms of biodiversity and responses along an increasing pressure, up to the limit of extinction. These communities are very stable due to the nature of the rock matrix and present a very slow growth rate, further slowed down in the permanent stress conditions in their natural environment: their turnover is estimated at 10,000 years; therefore, their structure and functionality are the result of a long evolution and is the result of optimal adaptation to specific environmental conditions.

Studies on all these samples have been conducted, over the years, applying increasingly innovative molecular techniques leading to a wide bulk of metagenomics data obtained from numerous projects

funded by the National Research Program in Antarctica (PNRA), the Joint Genome Institute (JGI) and the Molecular and Environmental Sciences Laboratory (EMSL), Department of Energy, USA. This unique and precious wealth of samples and immense amount of data constitutes the existing Know-How/Heritage of the proponent team. Based on this, CRYPTOMARS proposes a complex, multidisciplinary analysis, combining different expertise and in-kind facilities, to understand the complexity of resistance of microbial communities *in toto.* The project will return a scenario of habitability in which the ecosystem including the abiotic component has a role in terms of perpetuation of life in our Solar System.

Acknowledgements: The authors thank the Italian Space Agency (ASI) for funding CRYPTOMARS. Endolithically colonized rocks used in this project have been collected in the frame of Antarctic Expeditions funded by the Italian National Program for Antarctic Researches (PNRA). The Italian National Antarctic Museum (MNA) is acknowledged for supporting the Mycological Section of MNA where rocks are maintained.

References: [1] Preston, L. J. and Dartnell, L. R. (2014) *Int. J Astrobiol 13,* 81–98. [2] Friedmann, E. I. and Weed, R. (1987) *Science, 236,* 703–705. [3] Friedmann, E. I. (1982) *Science, 215,* 1045–1053. [4] Onofri S., et al. (2012) *Astrobiol, 12,* 508–516. [5] Onofri S., et al. (2015) *Astrobiol, 15,* 1052–1059. [6] Selbmann L., et al. (2015) *Curr Genet, 61,* 335–345. [7] Fanelli G., et al. (2021) *Life, 11,* 96. [8] Albanese, D., et al. (2021) *Microbiome, 9,* 1–15.