International Journal of Astrobiology (2025), **24**, e17, 1–15
doi:10.1017/S1473550425100104

CAMBRIDGE
UNIVERSITY PRESS

REVIEW ARTICLE

The CRYPTOMARS project: a multi-omic approach for studying Antarctic cryptoendolithic communities as Martian-analog life-forms

Laura Selbmann[1,2,3], Carmen Del Franco[1], Gerardo Antonio Stoppiello[1], Caterina Ripa[1], Ricardo Belmonte Lopes[1], Claudio Donati[4], Pietro Franceschi[4], Mar Garcia-Aloy[4], Alessia Cemmi[5], Ilaria Di Sarcina[5], Giulia Bazzano[6], Concetta Ronsivalle[6], Paolo Nenzi[6], Daniele Murra[7], Sarah Bollanti[7], Maria Aurora Vincenti[8], Massimo Piccinini[8], Mickael Baqué[9], Maria Pedone[10] and Serena Pezzilli[10]

[1]Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy
[2]Mycological Section, Italian Antarctic National Museum (MNA), Genoa, Italy
[3]Department of Earth Systems Science and Environmental Technologies (CNR-ISP), CNR - Institute of Polar Sciences, Messina, Italy
[4]Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[5]ENEA –Nuclear Department (NUC-IRAD-GAM), Rome, Italy
[6]ENEA –Nuclear Department (NUC-TECFIS-ACP), Frascati, Rome, Italy
[7]ENEA –Nuclear Department (NUC-PLAS-FIPI), Frascati, Rome, Italy
[8]ENEA –Nuclear Department (NUC-TECFIS-MNF), Frascati, Rome, Italy
[9]Planetary Laboratories Department, German Aerospace Center (DLR), Institute of Planetary Research, Berlin, Germany
[10]ASI- Italian Space Agency, Rome, Italy
Corresponding author: Carmen Del Franco; Email: carmen.delfranco@unitus.it

Received: 29 May 2025; **Revised:** 29 May 2025; **Accepted:** 06 June 2025

Keywords: Astrobiology; Antarctica; Mars Analogue; Cryptoendolithic microbial communities; Multi-omics

Abstract

If life ever existed on Mars, it may have developed survival strategies similar to those adopted by extremophiles living in terrestrial Martian analogs, such as the cryptoendolithic communities found in the rocky substrates of the McMurdo Dry Valleys or other ice-free areas of continental Antarctica. Nearly thirty years of research on these super-adapted organisms laid the foundation for the CRYPTOMARS project, which aims to disclose the genomic and phenotypic features allowing these microbial communities to withstand specific physico-chemical stresses that may be encountered on the Red Planet. This information will provide tools to outline, in terms of diversity and competences, a putative microbial community able to survive, adapt or even perpetuate under early or present Martian conditions. The project and the background information are here presented.

Contents

Introduction

"Is there or has ever been life on Mars?" and "How can we investigate it?" are two of the most intriguing questions in modern science. From its early geological stages until now, Mars is the planet most similar to Earth and, seeing its environmental conditions, it can be considered one of the most interesting candidates to evaluate the possibility of life beyond Earth (Hansen *et al*., 2005). In recent years, research on this fascinating topic has focused on searching for extraterrestrial life by defining its limits on our planet (Cockell *et al*., 2016), by adopting a method grounded in our current knowledge of terrestrial life-forms (Yamagishi *et al*., 2019) and their different characteristics, geological settings, ecosystems and environments (Raulin-Cerceau, 2004).

The Martian environment has undergone significant changes throughout its geological history, spanning three major eras: the Noachian, the Hesperian and the Amazonian (Head *et al*., 2001). There is evidence suggesting that during the pre-Noachian period, Mars may have closely resembled early Earth and that subsequently, by the late-Noachian, environmental conditions were likely favorable for the spread of possible life-forms. However, from the Hesperian to the Amazonian period, conditions progressively deteriorated (Wordsworth, 2016; Wordsworth *et al*., 2021; Dong *et al*, 2018) reducing the habitability of the planet (Cabrol, 2018). Currently, the surface of Mars results to be uninhabitable for life, as we know it, primarily due to the loss of its magnetic field and atmosphere resulting in an increase of radiation caused by unfiltered exposure to Galactic Cosmic Rays (GCRs) and Solar Energetic Particles (SEPs) (Zeitlin *et al*., 2010), with varying doses depending on solar activity and latitude (Hassler *et al*., 2014). Additionally, it also results in a very tenuous atmosphere (around 6 mbar), extremely low oxygen levels and temperatures, with a variation from −128.15 °C during the polar night to 26.85 °C at the equator (Sebastián *et al*., 2010). Although GCRs are mostly composed of X-rays, gamma rays, protons and atomic nuclei at very high energies, the total dose due to GCRs is very small compared to that from the Sun (SEP). The latter are made up of protons, heavier atomic nuclei (significant up to iron) and electrons with energies up to about 1 GeV. In the case of the Martian surface, GCRs and SEPs are responsible for a dose rate ranging from 180 to 225 μGy/day (Hassler *et al*., 2014), with the SEPs being the major contributors to the absorbed dose. However, high energetic X-rays and gamma rays are produced as secondary radiation by the ionization processes or interaction of particles and primary radiation with matter.

Among the main factors limiting life on the Red planet, are certainly extreme values of temperature and aridity. On present Mars, although temperatures vary considerably depending on the regions of the planet and the periods of the year, there is an average atmospheric temperature of −63 °C, and a surface temperature around 20 °C, with daily variations of up to about 80 °C (Martinez *et al*., 2017). On the other hand, although there is no uniformity in the estimates of the temperatures of primordial Mars (late-Noachian/early-Hesperian period), the most recent models hypothesize a marked discontinuity in the planet's climatic conditions, which would have seen colder and drier periods alternating with more humid and warm periods, the latter with average annual temperature values ranging from about 0 to 7 °C (Palumbo *et al*., 2018; Rapin *et al*., 2021; Wordsworth *et al*., 2021). In addition to the extreme temperature values, the current Martian environment is also characterized by strong aridity. Early Mars was characterized by a strong climatic discontinuity whose conditions could have been similar to those described in the McMurdo Dry Valleys (Head and Marchant, 2014). Such arid conditions would then have been further exacerbated in the more recent history of the planet where the relative humidity near the surface can reach, depending on the season, values above 90% and the vapor pressure does not exceed values of 1.4 × 10-5 bar (Martín-Torres *et al*., 2015; Fischer *et al*., 2019). On the other hand, the low atmospheric pressure and the temperatures reached allow the presence of liquid water only in the form of transient brines in the superficial layers of the Martian regolith (Smith *et al*., 2014; Jones, 2018). Such conditions are not only not compatible with cellular processes, but can also induce oxidative stress in the microbial community (França *et al*., 2007; Gostinčar & Gunde-Cimerman, 2018).

Earth's Planetary Fields Analogs

Earth is characterized by a variety of environmental conditions and, depending on the specific physico-chemical characteristics of the various geographic locations, it is inhabited by different types of organisms (Pepper and Gentry, 2015). In some terrestrial areas, environments are so extreme that they are referred to as Planetary Fields Analogs (Cassaro *et al*., 2021), including the "Martian analogues on Earth" (Cary *et al*., 2010). These regions are mostly uninhabitable (de Los Ríos *et al*., 2014) due to their very extreme environmental parameters, that are similar to the Martian ones and well known to be limitant for the development of life (Merino *et al*., 2019). The only exception is represented by extremophilic microbial species who display a wide range of strategies and adaptations to survive otherwise lethal stresses (Horneck, 2008; de Los Ríos *et al*., 2014). Among the Martian analogs on Earth there are the ice-free areas of Continental Antarctica, the widest of which are the McMurdo Dry Valleys in Victoria Land. The geological and climatic evolution of these areas are somehow comparable to that which led to the environmental conditions on Mars. Indeed, these regions are characterized by extreme parameters, such as very low temperatures, ranging from −20 °C to −50 °C in winter, fluctuations in freeze-thaw periods, prolonged dryness stages (Scalzi *et al*., 2012), high salt concentration, high oligotrophy and high levels of ultraviolet (UV) radiation (Onofri *et al*., 2007). These conditions have been considered as incompatible with active life and these areas have been regarded as devoid of life until specialized microbial communities were discovered dwelling into rocks, finding a last refuge to avoid extinction in the endolithic niches (Friedmann and Ocampo-Friedmann, 1976).

Antarctic cryptoendolithic microbial communities

The endolithic environment, the pore and spaces inside of rocks, is a ubiquitous habitat for microorganisms on the Earth and an important target of the search for life elsewhere in the Solar System (Walker and Pace, 2007). In fact, the endolithic niche protects from direct exposure to most of the external environmental stresses, including the large spectrum of energetic radiation characterizing other planetary bodies. In the most extreme terrestrial climates, such as hot and cold deserts, endolithic microorganisms are often the main form of life hosting specialized microbial assemblages which endure the stringent conditions finding an ultimate refuge in the interstices of porous rocks (Pointing and Belnap, 2012). These communities can mediate inputs and outputs of gases, regulating rock weathering and soil formation, biomineralization and hydrological and nutrient cycles keeping biologically active otherwise sterile lands.

Endolithic microbial assemblages are the main, and often the sole, form of life colonizing the ice-free areas of Continental Antarctica where they represent the main standing biomass in the McMurdo Dry Valleys, occupying approximately 4% of sandstone boulders (Pointing *et al*., 2009), up to 30% of granite boulders (Yung *et al*., 2014), and 100% of sandstone cliffs (Friedmann, 1982). The most widespread among endolithic adaptations is known as *cryptoendolithism* (microbial life dwelling within pores of sedimentary rocks) (Friedmann and Ocampo, 1976; Nienow, 1993; de la Torre *et al*., 2003). Cryptoendoliths survive by exploiting a unique niche that offers thermal buffering, physical stability, protection from severe UV, solar radiation and, additionally, ensures water retention (Wollenzien *et al*., 1995; Wierzchos *et al*., 2012, 2018, 2020). Among them, the lichen-dominated cryptoendolithic communities are the most complex (Friedmann *et al*., 1982) characterized by a very slow growth, with an estimated turnover of 10.000 years (Friedmann *et al*., 1986, 1993); they are very widespread in Antarctic sandstone (Friedmann *et al*., 1982) and one of the most resistant life-forms on Earth (Scalzi *et al*., 2012).

Antarctic cryptoendolithic lichen-dominated communities show a high diversity in terms of abundance of both eukaryotic and prokaryotic species, which in most cases only colonize these peculiar habitats on Earth (Coleine *et al*., 2018) (Figure 1A). Regarding the eukaryotic component, they are mainly composed by non-lichenized black fungi, lichenized fungi and algae; instead, the prokaryotic counterpart is mostly represented by cyanobacteria and heterotrophic bacteria (de Los Ríos *et al*., 2014).

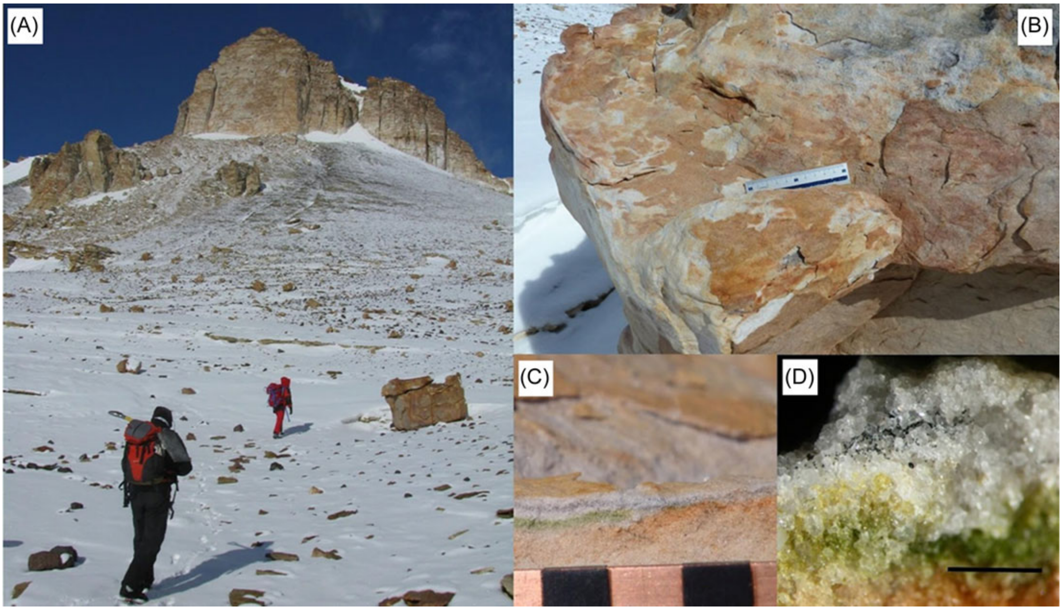

Figure 1. *(A) Sandstone cliffs at Linnaeus Terrace, McMurdo Dry Valleys, Antarctica; (B) biological weathering: patchwork colored surface of sandstone due to rock exfoliation as consequence of endolithic microbial activity; (C, D) typical stratification of cryptoendolithic lichen-dominated communities at different magnification. Scale bar (C) = 1 cm; scale bar (D) = 0.5 cm.*

These assemblages show a tiny organization with a typical stratification in different colored and biologically distinct bands, developing within the first centimeter below the rocky surface; different microbial compartments are driven at specific depth and segregated from the others, like a chromatographic separation driven by specific physiological needs and requirements.

The survival capability in such extreme conditions of the cryptoendolithic communities is due to important adaptations strategies they adopted like, for example, the development of a melanized cell-wall system improved by the microcolonial black fungi (MCF) constituting the first layer of these assemblages. MCF act as a screen against the harmful intense solar radiation which can damage the photosystems of chlorophycean algae and cyanobacteria displayed in the lower green and sometime blue/green bands, acting as primary producers sustaining the whole community, while lichenized fungi and algae sit in the white band in the middle (Friedmann, 1982) (Figure 1B, C, D).

Hypothesizing that on Mars, during the late-Noachian, there were life-permissive environmental conditions (McKay, 2010; Westall *et al.*, 2013; Cockell, 2014), putative microorganisms living there may have adopted survival strategies similar to the ones engaged by these terrestrial extremophiles before their extinction or, theoretically, they could still endure Martian conditions in protected niches of the Red Planet. Moreover, terrestrial contamination cannot be excluded caused by human activities through contaminated spacecrafts on the ground (Baqué *et al.*, 2016) and rovers inadequately sterilized, or even through accidental impact of orbiters (Mancinelli and Klovstad, 2000).

New advances on Antarctic cryptoendolithic communities

The study of Antarctic cryptoendolithic microbial communities spans over three decades and has taken advantage of progressive advances in scientific approaches and available techniques which have improved impressively over this timeframe, especially for what concerns molecular approaches.

The geographic and genetic isolation over a timescale of evolutionary significance, coupled with the exacerbated environmental pressure, promoted speciation of highly adapted guilds; to date, phylogenetic studies led to the description of 8 and 18 fungal genera and species, respectively (Selbmann *et al*., 2005, 2008; Egidi *et al*., 2014; Muggia *et al*., 2021; Turchetti *et al*., 2015, 2018). Metabarcoding analyses confirmed that fungi are abundant guilds in these communities being composed mainly by *Lecanoromycetes, Dothideomycetes* and *Eurotiomycetes* (Coleine *et al*., 2018); for bacteria the phyla *Actinobacteria* and *alpha-Proteobacteria* largely prevail (de la Torre *et al*., 2003; Coleine *et al*., 2019).

The rock substrate has a significant role on the endolithic inhabitants: sandstone appears as the most protective substratum, allowing the communities to push toward higher altitudes and longer sea distances (Selbmann *et al*., 2017), while granites maintain higher biodiversity, particularly for the bacterial component and higher presence of cyanobacteria (Larsen *et al*., 2024; Stoppiello *et al*., 2025).

Recent metagenomic and phylogenomic studies revealed the presence of a myriad of new prokaryotic species, exceeding 2,600 new candidates, some of which with very ancient roots dating back to the Precambrian period (Albanese *et al*., 2021; Coleine *et al*., 2024). Others belong to new candidate bacterial classes and show peculiar alternative metabolic capabilities that appear fundamental for the fitness of a microbial community adapted at the edge of water stress tolerability in one of the driest and coldest environments on Earth (Williams *et al*., 2024). These metabolisms include the capacity for trace gas oxidation using high-affinity uptake hydrogenases, which could provide energy and metabolic water required for survival and persistence (Ji *et al*., 2017). Furthermore, some Metagenome Assembled Genomes encode the capacity to couple the energy generated from H_2 and CO oxidation to support carbon fixation (atmospheric chemosynthesis); differently from photosynthesis, this autotrophic metabolism does not require water, an issue of utmost importance for the success of organisms living under constant and very strong water stress. Moreover, it is becoming evident that a conspicuous presence of a largely undescribed, highly diverse and spatially structured virome, of which the final viral catalog was constituted of 14,796 viral sequences mainly associated with the endolithic bacteria and 15.2% of vOTUs only, may represent eukaryotic viruses. The predicted Auxiliary Metabolic Genes of these phages are enriched with functions indicating that they may potentially influence bacterial adaptation and biogeochemistry, playing a possible key role in the fitness of the whole community (Ettinger *et al*., 2023). Recent metabolomic studies on cryptoendolithic communities reanimated using appropriate wetting, light and temperature, indicate that several metabolic pathways are significantly differently up- or down-regulated in opposite sun-exposed communities suggesting a critical role in the adaptation to contrast intensities of environmental pressure (Fanelli *et al*., 2021). These results supplied the first insight about the process through which Antarctic endolithic communities respond to stresses, maintaining biological activities under the harshest conditions accounted for as a Martian analog on Earth and that are typically incompatible with active life. Finally, these communities, and the microorganisms isolated from them, are incredibly resistant and capable of surviving, if dehydrated, both in space, exposed outside the International Space Station, and in simulated Martian conditions over a period of 18 months (Onofri *et al*., 2012, 2015; Selbmann *et al*., 2015).

Altogether these recent advances are giving unprecedented insights on these unique microcosms about “who is there, what are they doing, and what they are potentially able to do” and supplying tools for unraveling the evolution and adaptability of these communities, representing a very new wave of research with intriguing implications of astrobiological relevance, and have been of inspiration for the *foundation of the CRYPTOMARS Project.*

The CRYPTOMARS project

CRYPTOMARS is a multi-disciplinary and interdisciplinary project aiming to unravel, applying a multi-omic approach, the metabolic responses and mechanisms of resistance of these communities after prolonged exposure to selected stresses characterizing the environment of early and present-day Mars. By integrating genomic, metabolomic and lipidomic data, the project aims to clarify how these communities have adapted to live, spread and even survive in Mars-like conditions on Earth. Data integration will unravel their structures, responses and adaptability at a level of accuracy and depth that would not have even been conceivable until very recently.

The Mycological Section of the National Museum of Antarctica (MNA), housed at the Department of Ecological and Biological Sciences (DEB) of the University of Tuscia, hosts the largest repository of rocks collected in the Antarctic desert, colonized by cryptoendolithic communities. This unique collection was built over a period of about 30 years in the frame of Antarctic expeditions funded by the Italian National Program for Research in Antarctica (PNRA). The sampling campaigns were carried out mainly in Northern and Southern Victoria Land, and covered a latitudinal gradient of 72°–77° South, an altitudinal gradient from 0 to 3,200 m above sea level, from 0 to 100 km distance from the coast and at different solar exposures (north-south), allowing to select communities, exposed and adapted to increasingly prohibitive environmental conditions up to the limit of extinction. The huge number of samples, and the related sequencing data, constitute together the KNOW HOW/HERITAGE of which this project takes advantage, allowing its notable valorization with the new experiments and analyses that will lead to a new vision and interpretation.

The innovation and originality of the project idea lies in unraveling the response to environmental challenges of microbial communities as a whole, rather than focusing on individual extremophiles, using cutting-edge experimental techniques, highlighting the adaptations coming out from emergent properties and synergies of microbial interactions. The multi-disciplinary nature of the project will provide, for the first time, a comprehensive characterization of the mechanisms and characteristics that allow microbial communities to adapt and survive in conditions similar to those that are found on Mars. Antarctic endolithic communities are perfectly suited to the proposed study: they are very stable due to the hard nature of rocks compared to soils and soil biological crusts; they display a physiologically very slow growth rate, with a calculated turnover of 10,000 years, due to the permanent sub-optimal conditions of their natural environment; their structure and functionality are tightly tailored and adapted to the features of the conditions in the locations where they have been collected, chosen along a scale of increasing environmental pressures, giving the picture of the optimal adaptation to specific environmental conditions acquired over an evolutionary timescale.

CRYPTOMARS work plan

Colonized rock samples from 5 selected ice-free sites of Victoria Land, Antarctica, and distributed at increasing environmental pressure over an altitudinal range from 834 to 3100 m asl were selected for the experiments (Figure 2).

Before the exposure to selected stresses, the rocks will be reactivated by rehydration and kept at optimal growth temperature (Selbmann *et al*., 2005, 2008) in an incubator equipped with a white light lamp (Fanelli *et al*., 2021). Viability after metabolic reactivation will be tested before proceeding with the subsequent experiments (Pacelli *et al*., 2017).

In the frame of CRYPTOMARS, Antarctic cryptoendolithic communities will be exposed to environmental factors characterizing two specific intervals of Martian geological history related to the late-Noachian/early-Hesperian period and to present-day Mars (Amazonian period). This will allow to understand how putative Martian life-forms may have been able to adapt and respond to the Martian environment i) when the environmental conditions of the planet began to become more discontinuous and hostile (late-Noachian/early-Hesperian), similarly to what happened in the geological history of Antarctica; ii) when the conditions on the surface of Mars became extremely inhospitable for life (Amazonian). To achieve our goal, a number of climatic and radiative stresses have been considered and detailed as follows.

To experimentally simulate the Martian environment irradiation for what concerns gamma radiation, the Calliope Facility (ENEA Research Centre, Casaccia Rome, Figure 3), which also allows simulating the gamma component associated with the presence of neutrons as primary radiation, will be used. The

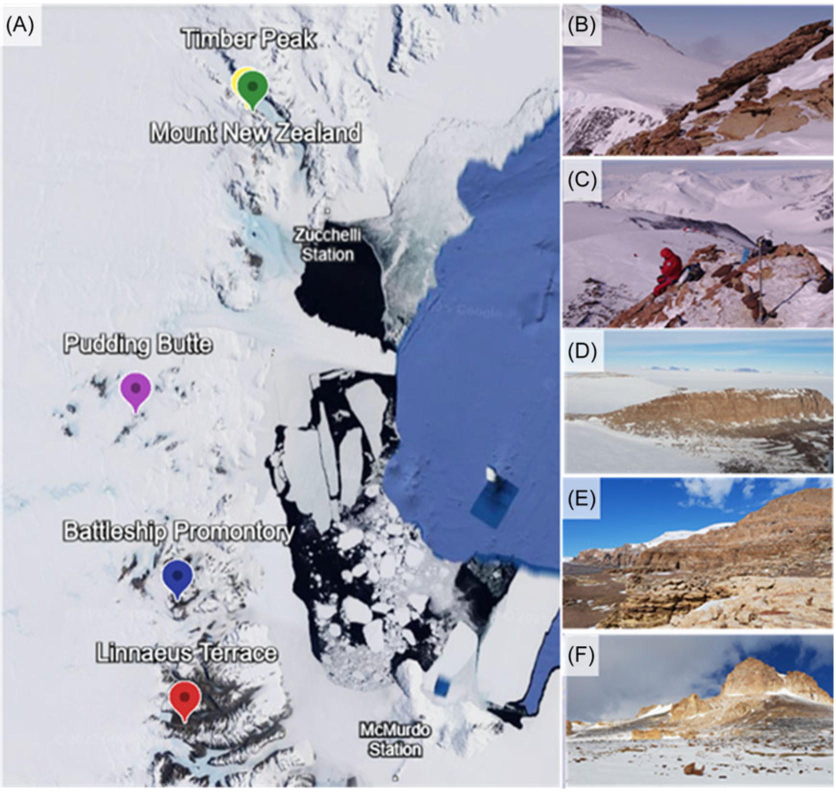

Figure 2. *Sampling map. (A) Map of Victoria Land (from Google Earth) indicating sampling locations selected for CRYPTOMARS. Landscapes of the sampling areas: (B) Timber Peak; (C) Mt New Zealand; (D) Pudding Butte; (E) Battleship Promontory; (F) Linnaeus Terrace.*

irradiation experiments will also be implemented with protons, which represent about 90% of the GCRs hitting the Martian surface (Simpson, 1983), using the TOP-IMPLART facility (ENEA Research Centre, Frascati, Rome, Figure 4). The characteristics of these facilities make it possible to modulate specific parameters (absorbed dose, dose rate). During the exposure, 1.8 cm discs of colonized Antarctic sandstone will be maintained at a temperature of 15 °C and a humidity of 65% to keep an active metabolism of the microbial communities during the experiment. At the end of the exposure, the samples will be immediately frozen in liquid nitrogen to have an unaltered picture of the metabolic responses during exposure to stress until downstream analyses.

For what concerns the spectrum of non-ionizing electromagnetic radiation (visible and UV light) on early and current Mars, as well as in the natural environment in which the communities live, it will be experimentally simulated using a UV lamp (250–400 nm) with a maximum intensity of 618 W/m^2 (61,8 mW/cm^2). The maximum UV irradiance values estimated for the three conditions (Cockell, 2000; Patel *et al.*, 2002) have been considered: chronic dose of 25 W/m^2, which corresponds to a maximum exposure value in primordial Martian conditions; chronic dose of 55 W/m^2, which corresponds to a maximum exposure value in current Martian conditions; chronic dose of 2.30 W/m^2, which corresponds to a maximum exposure value in natural conditions in Antarctica (from NASA Earth Observations [NEO]).

The ability of communities to cope with periods of extremely cold temperatures or low water availability could require the activation of *ad hoc* metabolic functions, the understanding of which is of primary importance in the study of possible life-forms on Mars. To study the effects and responses of the climatic parameters previously mentioned, we set –70 °C/+10 °C as thermal fluctuations to allow the

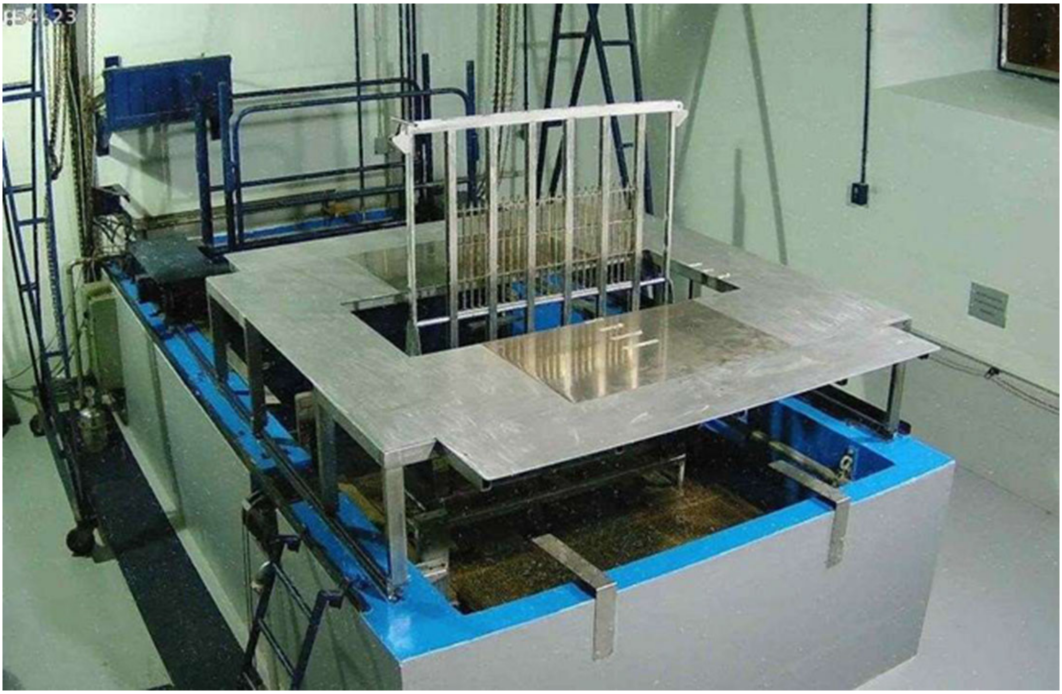

Figure 3. *Calliope facility.*

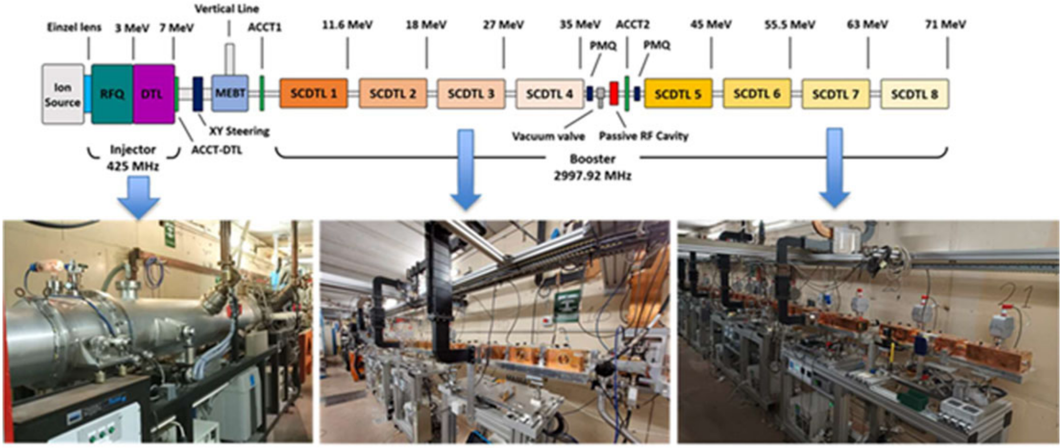

Figure 4. *TOP-IMPLART Accelerator.*

rocks to reach temperatures like those observed on Mars at present. For early Mars, conditions of thermal fluctuations were set as −5 °C/+15 °C and, for simulating Antarctic natural conditions, we referred to what was reported as the average thermal fluctuations in the month of December which is the mildest (−18°C/+6°C) (Friedmann *et al.*, 1987). Water stress (in terms of dehydration and rehydration cycles characterizing both early and present Mars conditions), will also be tested. UV exposure and climatic tests will be implemented in the Climatic Chamber (ENEA Research Centre, Casaccia Rome, ACS Angelantoni DM-340-C) (Figure 5).

Selected testing conditions and number of samples to be treated are displayed in Table 1 and include: (i) exposure to simulated natural Antarctic conditions; (ii) the spectrum of electromagnetic radiation

Table 1. *Selected stress conditions and replicates of treated colonized rocks*

	Gamma rays irradiation (nr)				Protons irradiation (nr)				UV-vis irradiation (nr)				Thermal cycles (nr)				Hydration-dehydration cycles (nr)				Mars simulation chamber (nr)			Untreatable samples (nr)
Samples sites	EM	PM	A	D	EM	PM	A	D	EM	PM	A	D	EM	PM	A	D	EM	PM	A	D	Light	Dark	D	
Linnaeus Terrace	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Battleship Promontory (Northern sun-exposition)	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Battleship Promontory (Southern sun-exposition)	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Pudding Butte (Northern sun-exposition)	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Pudding Butte (Southern sun-exposition)	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Timber Peak	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Mt New Zealand	–	10	–	2	–	10	–	2	10	10	10	6	10	10	10	6		10		2	5	5	2	30
Sub-tot	84				84				252				252				84				84			210
Tot	1050																							

EM: Early Mars; PM: Present Mars; A: Antarctica; D: Dosimetry.

Figure 5. *Climatic Chamber.*

(visible and UV light) present in early and current Martian environments; (iii) part of the spectrum of ionizing radiation that characterizes the Martian radioactive environment, represented by gamma rays and accelerated protons, which represent 90% of the cosmic rays (Galactic Cosmic Rays [GCR] and Solar Energetic Particles [SEP]) that strike the Martian surface; (iv) the temperature variation during the Martian day (sol); (v) water stress (dehydration and rehydration cycles); (vi) the effect of concomitant exposure to the atmosphere, temperature and spectrum of electromagnetic radiation (visible and UV light) of early Mars and present-day Mars in Mars simulation chambers (Figure 6).

Viability pre- and after-treatments will be tested with culturomic, colorimetric and molecular approaches that will be implemented as part of the experimental work plan of the project (Del Franco *et al.*, 2025, this issue).

A metagenomic study will display the genetic and functional characteristics of the analyzed communities, and how they vary according to the environment in which they have been collected to display their diversity, the functional genes and the potential metabolic pathways, tailored to the environmental pressure in which they are found and potentially involved in the stress response. Additionally, alternative metabolic pathways (i.e. atmospheric chemosynthesis, far-red photosynthesis) will also be identified and related to specific skills that can promote the ability to live and perpetuate in these terrestrial and possible extraterrestrial limit environments helping to define the possible genetic and predictive functional properties of hypothetical Martian microbial communities. These first data will also provide the necessary information for the scheduled metabolomic and lipidomic analyses.

The complete characterization of the metabolome of cryptoendolithic communities, both in optimal and stressed conditions, represents an essential contribution for understanding the genetic and molecular bases of their adaptability. Indeed, it is reasonable to hypothesize that the study of its composition represents an ideal proxy to evaluate and compare the mechanisms of response/resilience/adaptation of

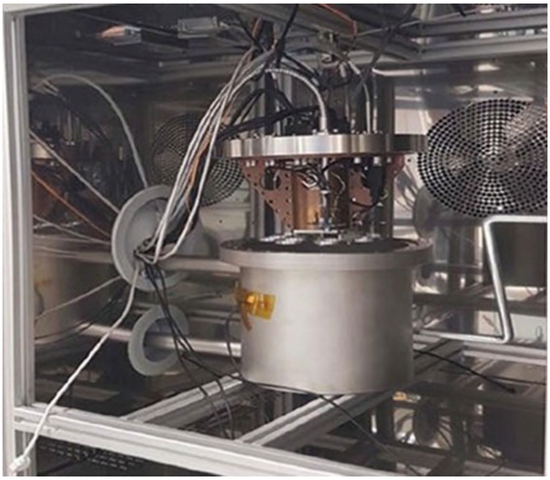

Figure 6. *Mars simulation facility in PASLAB at Berlin DLR (Lorenz* et al.*, 2023).*

each community, allowing, at the same time, the identification of sets of metabolites that may be integrated into metabolic functions or associated with specific metabolic pathways that could constitute the basis for the survival of potential communities in extraterrestrial environments. For these reasons, the activities focus on the direct measurement of the metabolic/lipid profile of microbial communities by applying untargeted metabolomics and lipidomics techniques. These analyses will be performed using high-resolution mass spectrometry techniques coupled to liquid chromatography: (U) High-Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry [HPLC-HRMS] is one of the best choices for untargeted analysis techniques, given the versatility in metabolite coverage and the sensitivity of these instruments (Letertre, 2021), by detecting and profiling hundreds to thousands of metabolites with different chemical properties in a biological sample. All the experimental procedures for the metabolomic and lipidomic analyses will be implemented and optimized as part of the experimental work plan of the project, adapting the methods previously developed at the Metabolomics Research Unit of the Fondazione E. Mach (Garcia-Aloy *et al*., 2020, 2023).

The effect of each stress applied to the communities will be studied individually first and then by integrating all the responses together with other applied stresses and with the vitality and diversity data, in order to define in detail what the possible genetic and functional properties of hypothetical Martian microbial communities are.

An integrated approach, based on multivariate analysis and machine learning models, will be implemented to combine the relative abundance of genes and metabolic pathways predicted with metabolomic/lipidomic data and vitality tests to outline specific characteristics of communities that exhibit different responses to the applied stresses. Finally, the results obtained will allow the construction of ecological models predictive of the habitability of extraterrestrial environments, such as those of early and present-day Mars.

Expected results

The integrated application of -omic approaches and advanced bioinformatics strategies will enable the identification of metabolites, potentially linked to specific pathways, that allow a community to remain active, both in the limiting conditions of their natural environment and under selected Martian stresses to which they will be exposed, and to identify the genetic bases of such responses and adaptations. This information will also deepen our level of knowledge of the evolutionary mechanisms that have led to the establishment of microbial communities capable of being successful in Mars-like terrestrial environments. All together these results will provide new awareness of the genetic and functional characteristics that determine the "resilience" and "resistance" of cryptoendolithic communities, both at the level of single organisms and of the whole community and to outline the feature of a putative microbial community, in terms of diversity and competences, theoretically able to have colonized Mars or Mars-like bodies.

Acknowledgments. The authors wish to thank the Italian Space Agency (ASI) for coordination and financial support of the CRYPTOMARS project, under the contract n. 2023-12-U.0. The Italian National Program for Antarctic Research (PNRA) is kindly acknowledged for funding sampling campaigns and research activities in Italy in the frame of PNRA projects. The Italian Antarctic National Museum (MNA) is rewarded for financial support to the Mycological Section of the MNA and for providing rock samples used in this study and stored at the University of Tuscia, DEB, Italy.

References

Albanese D, Coleine C, Rota-Stabelli O, Onofri S, Tringe SG, Stajich JE, Selbmann L and Donati C (2021) Pre-Cambrian roots of novel Antarctic cryptoendolithic bacterial lineages. *Microbiome* **9**(1), 63.

Baqué M, Verseux C, Böttger U, Rabbow E, de Vera JPP and Billi D (2016) Preservation of biomarkers from cyanobacteria mixed with Marslike regolith under simulated Martian atmosphere and UV flux. *Origins of Life and Evolution of Biospheres* **46**, 289–310.

Cabrol NA (2018) The coevolution of life and environment on Mars: an ecosystem perspective on the robotic exploration of biosignatures.

Cary SC, McDonald IR, Barrett JE and Cowan DA (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. *Nature Reviews Microbiology* **8**(2), 129–138.

Cassaro A, Pacelli C, Aureli L, Catanzaro I, Leo P and Onofri S (2021) Antarctica as a reservoir of planetary analogue environments. *Extremophiles*, 1–22.

Cockell CS (2000) The ultraviolet history of the terrestrial planets—implications for biological evolution. *Planetary and Space Science* **48**(2-3), 203–214.

Cockell CS (2014) 11. The subsurface habitability of terrestrial rocky planets: Mars. In *Microbial Life of the Deep Biosphere*, pp. 225–260.

Cockell CS, Bush T, Bryce C, Direito S, Fox-Powell M, Harrison JP, Lammer H, Landenmark H, Martin-Torres J, Nicholson N, Noack L, O'Malley-James J, Payler SJ, Rushby A, Samuels T, Schwendner P, Wadsworth J and Zorzano MP (2016) Habitability: a review. *Astrobiology* **16**(1), 89–117.

Coleine C, Albanese D, Ray AE, Delgado-Baquerizo M, Stajich JE, Williams TJ, Larsen S, Tringe S, Pennacchio C, Ferrari BC, Donati C and Selbmann L (2024) Metagenomics untangles potential adaptations of Antarctic endolithic bacteria at the fringe of habitability. *Science of the Total Environment* **917**, 170290.

Coleine C, Stajich JE, Pombubpa N, Zucconi L, Onofri S, Canini F and Selbmann L (2019) Altitude and fungal diversity influence the structure of Antarctic cryptoendolithic Bacteria communities. *Environmental Microbiology Reports* **11**(5), 718–726.

Coleine C, Stajich JE, Zucconi L, Onofri S, Pombubpa N, Egidi E, Franks A, Buzzini P and Selbmann L (2018) Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Frontiers in Microbiology* **9**, 1392.

de la Torre JR, Goebel BM, Friedmann EI and Pace NR (2003) Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. *Applied and Environmental Microbiology* **69**(7), 3858–3867.

de Los Ríos A, Wierzchos J and Ascaso C (2014) The lithic microbial ecosystems of Antarctica's McMurdo Dry Valleys. *Antarctic Science* **26**(5), 459–477.

Dong C, Lee Y, Ma Y, Lingam M, Bougher S, Luhmann J, Curry S, Toth G, Nagy A, Tenishev V, Fang X, Mitchell D, Brain D and Jakosky B (2018) Modeling Martian atmospheric losses over time: implications for exoplanetary climate evolution and habitability. *The Astrophysical Journal Letters* **859**(1), L14.

Egidi E, de Hoog GS, Isola D, Onofri S, Quaedvlieg W, De Vries M, Verkley GJM, Stielow JB, Zucconi L and Selbmann L (2014) Phylogeny and taxonomy of meristematic rock-inhabiting black fungi in the Dothideomycetes based on multi-locus phylogenies. *Fungal Diversity* **65**, 127–165.

Ettinger CL, Saunders M, Selbmann L, Delgado-Baquerizo M, Donati C, Albanese D, Roux S, Tringe S, Pennacchio C, del Rio TG, Stajich JE and Coleine C (2023) Highly diverse and unknown viruses may enhance Antarctic endoliths' adaptability. *Microbiome* **11**(1), 103.

Fanelli G, Coleine C, Gevi F, Onofri S, Selbmann L and Timperio AM (2021) Metabolomics of dry versus reanimated antarctic lichen-dominated endolithic communities. *Life* **11**(2), 96.

Fischer E, Martínez GM, Rennó NO, Tamppari LK and Zent AP (2019) Relative humidity on Mars: new results from the Phoenix TECP sensor. *Journal of Geophysical Research: Planets* **124**(11), 2780–2792.

França MB, Panek AD and Eleutherio ECA (2007) Oxidative stress and its effects during dehydration. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **146**(4), 621–631.

Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. *Science* **215**(4536), 1045–1053.

Friedmann EI (1986) The Antarctic cold desert and the search for traces of life on Mars. *Advances in Space Research* **6**(12), 265–268.

Friedmann EI and Ocampo R (1976) Endolithic blue-green algae in the dry valleys: primary producers in the Antarctic desert ecosystem. *Science* **193**(4259), 1247–1249.

Friedmann EI, Kappen L, Meyer MA and Nienow JA (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. *Microbial Ecology* **25**, 51–69.

Friedmann EI, McKay CP and Nienow JA (1987) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: satellite-transmitted continuous nanoclimate data, 1984 to 1986. *Polar Biology* **7**, 273–287.

Garcia-Aloy M, Masuero D, Chitarrini G, Škrab D, Sivilotti P, Guella G, Vrhovsek U and Franceschi P (2023) Untargeted lipidomic profiling of grapes highlights the importance of modified lipid species beyond the traditional compound classes. *Food Chemistry* **410**, 135360.

Garcia-Aloy M, Ulaszewska M, Franceschi P, Estruel-Amades S, Weinert C.H, Tor-Roca A, Urpi-Sarda M, Mattivi F and Andres-Lacueva C (2020) Discovery of intake biomarkers of lentils, chickpeas, and white beans by untargeted LC-MS metabolomics in serum and urine. *Molecular Nutrition & Food Research* **64**(13), e1901137.

Gostinčar C and Gunde-Cimerman N (2018) Overview of oxidative stress response genes in selected halophilic fungi. *Genes* **9**(3), 143.

Hansen AA, Merrison J, Nørnberg P, Lomstein BA and Finster K (2005) Activity and stability of a complex bacterial soil community under simulated Martian conditions. *International Journal of Astrobiology* **4**(2), 135–144.

Hassler DM, Zeitlin C, Wimmer-Schweingruber RF, … (2014) Mars' surface radiation environment measured with the Mars Science Laboratory's Curiosity rover. *Science* **343**(6169), 1244797.

Head JW, Greeley R, Golombek MP, Hartmann WK, Hauber E, Jaumann R, Masson P, Neukum G, Nyquist LE and Carr MH (2001) Geological processes and evolution. *Space Science Reviews* **96**, 263–292.

Head JW and Marchant DR (2014) The climate history of early Mars: insights from the Antarctic McMurdo Dry Valleys hydrologic system. *Antarctic Science* **26**(6), 774–800.

Horneck G (2008). The microbial case for Mars and its implication for human expeditions to Mars. *Acta Astronautica* **63**(7-10), 1015–1024.

Ji M, Greening C, Vanwonterghem I, Carere CR, Bay SK, Steen JA, Montgomery K, Lines T, Beardall J, van Dorst J, Snape I, Stott MB, Hugenholtz P and Ferrari BC (2017) Atmospheric trace gases support primary production in Antarctic desert surface soil. *Nature* **552**(7685), 400–403.

Jones EG (2018) Shallow transient liquid water environments on present-day mars, and their implications for life. *Acta Astronautica* **146**, 144–150.

Larsen S, Coleine C, Albanese D, Stegen J. C, Selbmann L and Donati C (2024) Geology and elevation shape bacterial assembly in Antarctic endolithic communities. *Science of the Total Environment* **907**, 168050.

Letertre MP, Giraudeau P and De Tullio P (2021) Nuclear magnetic resonance spectroscopy in clinical metabolomics and personalized medicine: current challenges and perspectives. *Frontiers in Molecular Biosciences* **8**, 698337.

Lorenz C, Bianchi E, Poggiali G, Alemanno G, Benesperi R, Brucato JR, Garland S, Helbert J, Loppi S, Lorek A, Maturilli A, Papini A, de Vera JP and Baqué M (2023) Survivability of the lichen Xanthoria parietina in simulated Martian environmental conditions. *Scientific Reports* **13**(1), 4893.

Mancinelli RL and Klovstad M (2000) Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. *Planetary and Space Science* **48**(11), 1093–1097.

Martinez GM, Newman CN, De Vicente-Retortillo A, Fischer E, Rennó NO, Richardson MI, Fairén G, Genzer M, Guzewich SD, Haberle RM, Harri AM, Kemppinen O, Lemmon MT, Smith MD, de la Torre-Juárez M and Vasavada AR (2017) The modern near-surface Martian climate: A review of in-situ meteorological data from Viking to Curiosity. *Space Science Reviews* **212**, 295–338.

Martín-Torres FJ, Zorzano MP, Valentín-Serrano P, Harri AM, Genzer M, Kemppinen O, Rivera-Valentin EG, Jun I, Wray J, Madsen MB, Goetz W, McEwen AS, Hardgrove C, Renno N, Chevrier VF, Mischna M, Navarro-González R, Martínez-Frías J, Conrad P, McConnochie T, Cockell C, Berger G, Vasavada AR, Sumner D and Vaniman D (2015). Transient liquid water and water activity at Gale crater on Mars. *Nature Geoscience* **8**(5), 357–361.

McKay CP (2010) An origin of life on Mars. *Cold Spring Harbor Perspectives in Biology* **2**(4), a003509.

Merino N, Aronson HS, Bojanova DP, Feyhl-Buska J, Wong ML, Zhang S and Giovannelli D (2019) Living at the extremes: extremophiles and the limits of life in a planetary context. *Frontiers in Microbiology* **10**, 780.

Muggia L, Coleine C, De Carolis R, Cometto A and Selbmann L (2021) Antarctolichenia onofrii gen. nov. sp. nov. from Antarctic endolithic communities untangles the evolution of rock-inhabiting and lichenized fungi in Arthoniomycetes. *Journal of Fungi* **7**(11), 935.

Nienow JA (1993) Terrestrial lithophytic (rock) communities. *Antarctic Microbiology*, 343–412.

Onofri S, de la Torre R, de Vera JP, Ott S, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, Sánchez Iñigo FJ and Horneck G (2012) Survival of rock-colonizing organisms after 1.5 years in outer space. *Astrobiology* **12**(5), 508–516.

Onofri S, de Vera JP, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, de la Torre R and Horneck G (2015) Survival of Antarctic cryptoendolithic fungi in simulated Martian conditions on board the International Space Station. *Astrobiology* **15**(12), 1052–1059.

Onofri S, Selbmann L, de Hoog GS, Grube M, Barreca D, Ruisi S and Zucconi L (2007) Evolution and adaptation of fungi at boundaries of life. *Advances in Space Research* **40**(11), 1657–1664.

Pacelli C, Bryan RA, Onofri S, Selbmann L, Shuryak I and Dadachova E (2017) Melanin is effective in protecting fast and slow growing fungi from various types of ionizing radiation. *Environmental Microbiology* **19**(4), 1612–1624.

Palumbo AM, Head JW and Wordsworth RD (2018) Late Noachian Icy Highlands climate model: Exploring the possibility of transient melting and fluvial/lacustrine activity through peak annual and seasonal temperatures. *Icarus* **300**, 261–286.

Patel MR, Zarnecki JC and Catling DC (2002) Ultraviolet radiation on the surface of Mars and the Beagle 2 UV sensor. *Planetary and Space Science* **50**(9), 915–927.

Pepper IL and Gentry TJ (2015). Earth environments. In *Environmental Microbiology.* Academic Press, pp. 59–88.

Pointing SB and Belnap J (2012) Microbial colonization and controls in dryland systems. *Nature Reviews Microbiology* **10**(8), 551–562.

Pointing SB, Chan Y, Lacap DC, Lau MC, Jurgens JA and Farrell RL (2009) Highly specialized microbial diversity in hyper-arid polar desert. *Proceedings of the National Academy of Sciences* **106**(47), 19964–19969.

Rapin W, Dromart G, Rubin D, Le Deit L, Mangold N, Edgar LA, Gasnault O, Herkenhoff K, Le Mouélic S, Anderson RB, Maurice S, Fox V, Ehlmann BL, Dickson JL and Wiens RC (2021) Alternating wet and dry depositional environments recorded in the stratigraphy of Mount Sharp at Gale crater, Mars. *Geology* **49**(7), 842–846.

Raulin-Cerceau F (2004) Historical review of the origin of life and astrobiology. In *Origins: Genesis, Evolution and Diversity of Life*. Dordrecht: Springer Netherlands, pp. 15–33.

Scalzi G, Selbmann L, Zucconi L, Rabbow E, Horneck G, Albertano P and Onofri S (2012) LIFE experiment: isolation of cryptoendolithic organisms from Antarctic colonized sandstone exposed to space and simulated Mars conditions on the International Space Station. *Origins of Life and Evolution of Biospheres* **42**, 253–262.

Sebastián E, Armiens C, Gómez-Elvira J, Zorzano MP, Martinez-Frias J, Esteban B and Ramos M (2010) The rover environmental monitoring station ground temperature sensor: A pyrometer for measuring ground temperature on Mars. *Sensors* **10**(10), 9211–9231.

Selbmann L, De Hoog GS, Mazzaglia A, Friedmann EI and Onofri S (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology* **51**(1), 1–32.

Selbmann L, De Hoog GS, Zucconi L, Isola D, Ruisi S, Van Den Ende AHG, Ruibal C, De Leo F, Urzì C and Onofri S (2008) Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. *Studies in Mycology* **61**(1), 1–20.

Selbmann L, Onofri S, Coleine C, Buzzini P, Canini F and Zucconi L (2017) Effect of environmental parameters on biodiversity of the fungal component in lithic Antarctic communities. *Extremophiles* **21**, 1069–1080.

Selbmann L, Zucconi L, Isola D and Onofri S (2015) Rock black fungi: excellence in the extremes, from the Antarctic to space. *Current Genetics* **61**, 335–345.

Simpson JA (1983) Elemental and isotopic composition of the galactic cosmic rays. *Annual Review of Nuclear and Particle Science* **33**.

Smith ML, Claire MW, Catling DC and Zahnle KJ (2014) The formation of sulfate, nitrate and perchlorate salts in the martian atmosphere. *Icarus* **231**, 51–64.

Stoppiello GA, Muggia L, De Carolis R, Coleine C and Selbmann L (2025) Ecological niche drives fungal and bacterial diversity in endolithic and epilithic communities inhabiting granites in Victoria Land, Antarctica. *Polar Biology* **48**(1), 16.

Turchetti B, Selbmann L, Blanchette RA, Di Mauro S, Marchegiani E, Zucconi L, Arenz BE and Buzzini P (2015) *Cryptococcus vaughanmartiniae* sp. nov. and *Cryptococcus onofrii* sp. nov.: two new species isolated from worldwide cold environments. *Extremophiles* **19**, 149–159.

Turchetti B, Selbmann L, Gunde-Cimerman N, Buzzini P, Sampaio JP and Zalar P (2018) Cystobasidium alpinum sp. nov. and Rhodosporidiobolus oreadorum sp. nov. from European Cold Environments and Arctic Region. *Life* **8**(2), 9.

Walker JJ and Pace NR (2007) Endolithic microbial ecosystems. *Annual Review of Microbiology* **61**(1), 331–347.

Westall F, Loizeau D, Foucher F, Bost N, Betrand M, Vago J and Kminek G (2013) Habitability on Mars from a microbial point of view. *Astrobiology* **13**(9), 887–897.

Wierzchos J, Ascaso C, Artieda O and Casero MC (2020) The desert polyextreme environment and endolithic habitats. In *Microbial Ecosystems in Central Andes Extreme Environments: Biofilms, Microbial Mats, Microbialites and Endoevaporites*, pp. 37–49.

Wierzchos J, Casero MC, Artieda O and Ascaso C (2018) Endolithic microbial habitats as refuges for life in polyextreme environment of the Atacama Desert. *Current Opinion in Microbiology* **43**, 124–131.

Wierzchos J, Ríos ADL and Ascaso C (2012) Microorganisms in desert rocks: the edge of life on Earth.

Williams TJ, Allen MA, Ray AE, Benaud N, Chelliah DS, Albanese D, Donati C, Selbmann L, Coleine C and Ferrari BC (2024) Novel endolithic bacteria of phylum Chloroflexota reveal a myriad of potential survival strategies in the Antarctic desert. *Applied and Environmental Microbiology* **90**(3), e02264–23.

Wollenzien U, De Hoog GS, Krumbein WE and Urzi C (1995) On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. *Science of the Total Environment* **167**(1-3), 287–294.

Wordsworth RD (2016). The climate of early Mars. *Annual Review of Earth and Planetary Sciences* **44**, 381–408.

Wordsworth R, Knoll AH, Hurowitz J, Baum M, Ehlmann BL, Head JW and Steakley K (2021) A coupled model of episodic warming, oxidation and geochemical transitions on early Mars. *Nature Geoscience* **14**(3), 127–132.

Yamagishi A (2019) *What Is Astrobiology?*. Singapore: Springer, pp. 3–7.

Yung CC, Chan Y, Lacap DC, Pérez-Ortega S, de Los Rios-Murillo A, Lee CK, Craig Cary S and Pointing SB (2014) Characterization of chasmoendolithic community in Miers Valley, McMurdo dry valleys, Antarctica. *Microbial Ecology* **68**, 351–359.

Zeitlin C, Boynton W, Mitrofanov I, Hassler D, Atwell W, Cleghorn TF, Cucinotta FA, Dayeh M, Desai M, Guetersloh SB, Kozarev K, Lee KT, Pinsky L, Saganti P, Schwadron NA and Turner R (2010) Mars Odyssey measurements of galactic cosmic rays and solar particles in Mars orbit, 2002–2008. *Space Weather* **8**(11).