

Cyanobacteria from Extreme Deserts to Space*

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ABSTRACT

The development of space technology makes the exposure of organisms and molecules to the space environment possible by using the ESA Biopan and Expose facilities and NASA nanosatellites; the aim is to decipher the origin, evolution and distribution of life on Earth and in the Universe. The study of microbial communities thriving in lithic habitats in cold and hot deserts is gathering appreciation when dealing with the limits of life as we know it, the identification of biosignatures for searching life beyond Earth and the validation of the (litho)-Panspermia theory. Cyanobacteria of the genus *Chroococcidiopsis* dominate rock-dwelling communities in extreme deserts that are considered terrestrial analogues of Mars, like the Dry Valleys in Antarctica, the Atacama Desert in Chile or the Mojave Desert in California. The extraordinary tolerance of these cyanobacteria towards desiccation, ionizing and UV radiation makes them suitable experimental strains which have been already used in astrobiological experiments and already selected for future space missions. Evidence gained so far supports the use of desert cyanobacteria to develop life support systems and *in-situ* resource utilization for the human space exploration and settlement on the Moon or Mars.

Keywords: Astrobiology; Cyanobacteria; Extremophiles; Space Exploration; Mars Analogues

1. Desert Cyanobacteria and Astrobiology

Astrobiology is a rather young field of research gathering scientists from different backgrounds around the questions of the origin, evolution, distribution and future of life on Earth and in the Universe. The term “exobiology” was coined by Nobel Laureate Joshua Lederberg to mean the study of life beyond the Earth [1]. However the failure of the Viking missions in 1976 in finding evidences for life on Mars surface switched off the exobiologists’ hopes. Only around 1995-1996, the announcement of the first extrasolar planet around 51 Pegasi, the Galileo spacecraft’s evidence for an ocean beneath the ice on Europe and the finding of the Martian meteorite ALH-84001 triggered a new interest for space exploration. Thus the term “astrobiology” was used to extend the field of exobiology to the search for evidence of prebiotic chemistry, signs of past or present life on Mars, the search for habitable environments in our solar system and habitable planets outside our solar system [2].

So far the astounding exploration missions to our neighboring planets in the Solar System and the ground-

based simulations of planetary conditions, provided new tools to address ancient humanity’s questions: are we alone? How and where did life appear and evolve? What is the future of life on Earth and beyond?

To begin to answer to these astrobiological questions without leaving our planet or its orbit, platforms placed in Low Earth Orbit (LEO) and ground based simulators have been developed to expose organisms and molecules to the space environment. Five short-duration missions (two weeks) were performed using the Russian Foton spacecraft carrying the European Space Agency (ESA) BIOPAN facility [3]. More recently another ESA facility, called EXPOSE, has provided a long-duration exposure platform (1.5 years) on the International Space Station (ISS), which provides a complete laboratory orbiting Earth at about 400 km [4,5]. New generation astrobiological experiments are being carried out by using nanosatellites, named cubesats [6] that orbit where the radiation dose is significantly higher (at least one order of magnitude) than on the ISS [7].

The need to perform experiments in space is driven by the impossibility to completely reproduce on the ground the full-spectrum of solar irradiation or the combined effects of all the space constraints (vacuum, radiations

*This paper is dedicated to Prof. Maria Grilli Caiola in occasion of her 85th birthday.

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and temperature cycles). Such stressors make the space a hostile environment for terrestrial organisms; nevertheless a small group of organisms, the so called anhydrobiotes, (from *Greek* “life without water”), adapted to cope with extreme desiccation, have the potential to survive in space. The survival to 16 days in space has been reported for desiccation-tolerant lichens and tardigrades during BIOPAN missions [8,9], while 1.5 year endurance was documented for lichens [10]. The record of survival in space remains that of 6 years of *Bacillus subtilis* spores [11].

In this context, desiccation-, radiation-resistant desert strains of *Chroococcidiopsis* have been employed in several experimentations in LEO and ground-based simulations of space and Martian conditions in order to investigate the tenacity of life as we know it, to detect biosignatures to search for life on Mars and test the (litho)-Panspermia theory [4,5]. Since cyanobacteria started to introduce oxygen into the Earth atmosphere 2.5 billion years ago, the spectroscopic detection of oxygen in a planet’s atmosphere has been suggested as an indicator of the presence of life, exploiting its star as its primary energy source [12].

2. Desert Cyanobacteria and the Terrestrial Analogues of Mars

The study of organisms thriving in extreme environments on Earth is of particular interest in Astrobiology; the persistence of active biota in almost any environment containing transient liquid water, an energy source and nutrients, even under extreme physico-chemical conditions, extends the possibilities for life on other planets of our Solar System and beyond [13]. Environments comparable to certain conditions encountered on other planets are used as terrestrial analogues. For example, Lake Vida (Antarctica) is an ice-bound system that was presumably isolated with solar-derived organic carbon and coincident microbial life, that has survived for millennia since isolation; such an aphotic and anoxic ecosystem provides a potential analog for habitats on other icy worlds where water-rock reactions may co-occur with saline deposits and subsurface oceans [14].

Obviously, the design of new missions searching for past or present life on Mars, Europa, Enceladus and other planetary bodies will benefit from our understanding of life resistance on Earth. When it comes to the assessment of the potential for the presence and preservation of evidence of extinct or extant life on Mars, the identification of terrestrial analogues paralleling Martian climatic evolution has been of paramount relevance [15].

In extremely dry hot and cold deserts, where life is pushed to its dry limit, organisms find refuge in lithic niches, colonizing microscopic fissures (chasmoendoliths) and structural cavities (cryptoendoliths) of rocks, or

forming biofilms at the stone-soil interface under pebbles of desert pavements (hypoliths, for terminology see ref. [16]). When living under, or in, a rock, microbial communities have a wetter habitat than the surrounding, in addition translucent rocks provides a selective absorption of UV [17] along with adequate light for photosynthetic activities; for a half-buried quartz rock (opacity of 0.2) it was reported that the transmission at the bottom is 64% for sunlight at a solar zenith and 82% for skylight [18].

In the McMurdo Dry Valleys in Antarctica and in the hyper-arid core of the Atacama Desert in Chile rock-inhabiting communities are dominated by cyanobacteria, mostly belonging to the genus *Chroococcidiopsis*; the study of these communities is gathering appreciation in astrobiology since they are considered the closest terrestrial analogous of two Mars environmental extremes: cold and aridity [19]. Field expeditions have also taught us that life is not obvious at large scales but rather rare in isolated islands amidst a microbially depauperated bare soil, thus suggesting that if life ever existed or exists on Mars, microhabitats would probably be widely dispersed among virtually lifeless surroundings [19].

Hence, the choice of targets to detect life, called biosignatures, and the understanding of their degradation under different extraterrestrial conditions is a key feature for future missions to recognize life when encountered [20]. Putative biosignatures have been identified by Raman spectroscopy in photoprotective and antioxidant molecules, UV screening compounds in rock-inhabiting communities from hot and cold deserts which are perceived as critically important for the forthcoming ExoMars mission for the robotic search of life on Mars [21-23].

Microbial communities dominated by cyanobacteria of the genus *Chroococcidiopsis* inhabit halite deposits in the hyper-aridity core of the Atacama Desert [24,25]. The colonization of the evaporites interior (3 - 7 mm beneath the halite crust) deserves interest as it might represent a way for microbial colonization in salt lake environments that have been described in different Mars areas [25]. Whereas a terrestrial analogue for carbonates on Mars has been identified in the Mojave Desert, Southern California [26]. It is noteworthy that in this desert cyanobacteria of the genus *Chroococcidiopsis* dominate the microbial communities in red-coated carbonate rocks (**Figure 1**), thus supporting the role of this cyanobacterium as pioneer phototroph in extreme desert niches and its relevance in searching for biosignatures on Mars.

3. Desert Cyanobacteria Have the Prerequisite to Survive in Space

Outer space is a harsh and inhospitable environment for terrestrial organisms due to lethal effects of vacuum, solar and galactic cosmic radiations and temperature ex-

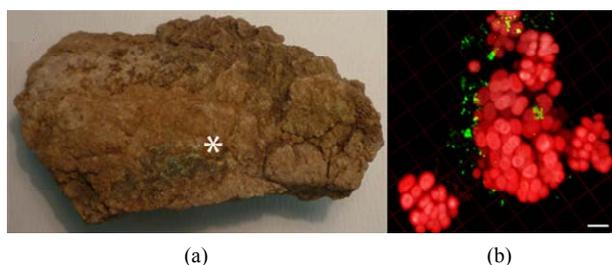


Figure 1. Hypolithic communities in a red-coated carbonate rock. (a): rock sample collected within the Silver Lake region of the eastern part of the Mojave Desert with phototrophic growth (asterix); (b): confocal laser scanning microscopy image of the hypolithic community dominated by *Chroococcidiopsis* sp. Bar = 10 μ m.

tremes [11]. Anhydrobiotes from extremely dry environments have developed protective/repair strategies so that they have the chance to survive in space. Their endurance under space conditions is still partially understood, although ascribable to mechanisms underlying desiccation tolerance that is usually paralleled by an extraordinary radio-resistance [27,28].

In nature rock-inhabiting phototrophic communities are wetted for only a few hours per year; thus they persist in an ametabolic dry and/or frozen state for the greater part of their life [19,29]. Under laboratory-desiccation desert strains of *Chroococcidiopsis* were reported to survive several years of dry storage [30]; the maintenance of sub-cellular integrities, namely genomic DNA and plasma membranes, in desiccation survivors indicated that protection mechanisms play a key role in desiccation tolerance, although repair mechanisms must take place upon rehydration to recover damages accumulated during prolonged desiccation, when oxidative damage continue even in the absence of metabolic activity [31]. Protection and repair mechanisms guarantee the survival of dried *Chroococcidiopsis* when experiencing additional environmental stressors [32,33]. The major effect caused by space vacuum is desiccation, while concerning solar and galactic cosmic radiation, desiccation-tolerant organisms exhibit a resistance towards UV and ionizing radiation; evidences suggested that such a resistance might be enhanced when cells are irradiated in the dried status, as reported for *Deinococcus radiodurans* [34].

Hydrated cells of *Chroococcidiopsis* were reported to survive up to 15 kGy of ionizing radiation, being capable of restoring the induced genome fragmentation [28]. Such doses are in the range of those occurring in LEO, even during solar flares [35]. The effect of ionizing radiation on dried cells of *Chroococcidiopsis* is currently under investigation. Hydrated cells of *Chroococcidiopsis* withstood UVC doses as high as 13 kJ/m²; this endurance was ascribed either to the occurrence of multicellular aggregates which attenuate the UVC radiation reaching inner cells, either to an oxidative stress defense and re-

pair of induced damage to the genome and photosynthetic apparatus [36].

Dried monolayers of *Chroococcidiopsis* survived 10 min (30 kJ/m²) under a simulated Martian UV flux, thus displaying a greater tolerance than *B. subtilis* spores; furthermore no effect on *Chroococcidiopsis* survival was detected, when overlaid by 1 mm of simulant Martian soil or gneiss, suggesting that on Mars organisms could survive and perhaps grow within lithic habitats in the presence of liquid water and nutrients [37]. Indeed the use of *Chroococcidiopsis* as photosynthetic pioneers for Mars' terraforming, to be inoculated into desert pavements and periodically wetted, has been suggested long before ground-based simulations of planetary conditions were performed [38].

Ground-based simulations carried out in preparation of the EXPOSE-E space mission [5] have shown that dried *Chroococcidiopsis* tolerate some space constraints; when shielded under 3 mm of sandstone they survived to simulated Mars-like UV radiation, UV radiation (200 - 400 nm) and space vacuum as expected for 1.5 years permanence in LEO [33]. Recent ground-based simulations performed for the EXPOSE-R2 space mission, set to be launched in April 2014, documented that dried biofilms of *Chroococcidiopsis* exhibited an enhanced survival compared to planktonic dried cells under space and Martian simulations [39,40]. While cellular components (genome and photosynthetic apparatus) of *Chroococcidiopsis* were protected against UVC radiation when mixed to a phyllosilicatic Mars regolith soil [39].

4. Desert Cyanobacteria under Space and Martian-Simulated Conditions in LEO

The first space experiment with rock-dwelling microbial communities was carried out in 2007, during the ESA BIOPAN 6 mission; after 10 days of permanence in space a single *Gloeocapsa*-like cyanobacterium was selected within a sample from coastal limestone cliff in Beer, Devon (UK) initially composed of *Pleurocapsales*, *Oscillatoriales* and *Chroococcales* [41]. During the same space mission the endurance of resting-state cells of cyanobacteria, the akinetes, dried onto rocks was tested; after return to Earth only samples exposed to vacuum with filtered UV radiation retained their germination capability [42]. By taking advantage of the possibility to allocate samples at the exterior of the Foton spacecraft, *Chroococcidiopsis* cells were inoculated within rocks, at a depth at which light is available for photosynthesis, and tested for survival upon re-entry into the Earth atmosphere [43]. These experiments are carried out in the context of the (litho)-Panspermia theory, *i.e.* the possibility of interplanetary transport of life by means of meteorites [44]. Since *Chroococcidiopsis* did not survive the re-entry process, due to the heating well above the upper

temperature limit for life, it was suggested that eventually non-photosynthetic organisms living deeper within rocks have a better chance of surviving the exit and entry processes [43].

More recently, a longer permanence in space (548 days) of rock-inhabited communities was achieved during the EXPOSE-E mission; within an epilithic community augmented with akinetes of *Anabaena cylindrical* and vegetative cells of *Nostoc commune* and *Chroococcidiopsis*, only the former survived after exposure to space vacuum and extraterrestrial ultraviolet spectrum [45].

Taking advantage of the UV spectrum present in LEO, the future EXPOSE-R2 space mission will expose selected organisms not only to space conditions, but also to a simulated Mars-like environment (CO₂ atmosphere and UV > 200 nm). In this space mission two experiments, BOSS (Biofilm Organisms Surfing Space) and BIOMEX (BIOlogy and Mars EXperiment) will be performed which focus on desiccation-tolerant organisms, including halophytes, bacteria and cyanobacteria, fungi, and their cellular components. BOSS aims to investigate the microbial resistance when developing biofilm compared to planktonic counterparts. Whereas in BIOMEX extremophiles and their constituents (pigments, cell wall components, etc.) embedded with Martian and lunar mineral analogues will be investigated in order to investigate life endurance in the contest of the (litho)-Panspermia theory and to create a biosignature database for searching life beyond Earth [46].

5. Contribution of Desert Cyanobacteria to Human Space Exploration

Apart from the fundamental comprehension of the resistance mechanisms of desert cyanobacteria to a truly unexpected environment, the knowledge gathered during space missions and ground-based simulations could find application to support human space exploration, including the settlement on the Moon or Mars. Space biotechnology aims to develop life support systems for future human space exploration in order to achieve an efficient recycling of waste generated during long stays in space [47]. Such system is currently studied in the frame of the MELiSSA project (Micro-Ecological Life Support System Alternative) combining four different bioreactors able to produce food, fixing CO₂ and releasing O₂ [48]. One of the main interrogations in designing such systems is its resistance to space constraints, especially to radiation. A typical mission to Mars for example, would take 400 days travelling in interplanetary space where cosmic radiations and Solar flares are especially hazardous to biological systems [49,50]. Therefore, as strategies are being developed for the crew protection, the resistance of onboard life support systems should also be considered.

Several problems for future human space exploration could be solved by using the resources (regolith) available on the planetary surfaces for oxygen production, fuel production, biomass production, nutrient extraction, or feedstock [51]. The use of cyanobacteria for *in-situ* resource utilization was first approached by growing different strains of commercially available and extremophilic cyanobacteria on different Martian and Lunar mineral analogues [52].

As mentioned above, considering the *Chroococcidiopsis* resistance to certain space parameters it could represent a choice organism for life support systems, where highly resistant photosynthetic organisms are needed. However, the key point of using such photosynthetic organisms is to make sure that the photosynthetic activity is not degraded or reduced due to a long stay in space by exposure to radiations and other space constraints, like microgravity. Experiments are planned to test the hypothesis that as a by-product of its extreme-tolerance *Chroococcidiopsis* has the potential to thrive in the spaceflight environment by avoiding and/or repairing oxidative damage induced by microgravity, thus contributing to the study of photosynthesis in space; in addition by forming multicellular aggregates it is a suitable model organism to investigate the effects of microgravity on cell division process and cell-cell communication.

In the frame of prolonged permanence in Low Earth Orbit, long-term space expeditions and human settlement on other planets, e.g. Mars, it seems also crucial the development of a biological system to store the genetic information to produce, drugs or nutritional compounds: *Chroococcidiopsis* possesses unique features to achieve this task. It efficiently repair and/or protect its genome under DNA-damaging conditions, such as extreme desiccation, ionizing radiation and UV exposure, and last but not least, among desiccation-tolerant cyanobacteria it is the only one suitable to genetic manipulation [53,54]. Hence it is challenging the use of this cyanobacterium, in the air-dried status as a gene space cargo without using freeze-drying techniques. This technology might guarantee prolonged periods of DNA storage in dried cells as opposite to purified DNA, that even when immobilized in the presence of trehalose (a non reducing sugar accumulated by anhydrobiotes to stabilized dried cellular component), cannot be stored for period longer than 2 months [55].

6. Conclusion

As a proof of concept the stability of two plasmids able to replicate in *Chroococcidiopsis* was tested for after 18 months of permanence in dried cells of *Chroococcidiopsis*; evidences suggested that strategies employed by this cyanobacterium to stabilize dried chromosomal DNA were efficient in protecting plasmids [56]. This

finding has implications for space research, for example when developing life support systems based on phototrophs with genetically enhanced stress tolerance as well as engineered to produce drugs, which can be stored in the air-dried state for prolonged periods.

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