Anhydrobiotic rock-inhabiting cyanobacteria: potential for astrobiology and biotechnology*

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1 Introduction

Deciphering how microorganisms can adapt to what we consider, in an anthropocentric way, extreme, is not only challenging intellectually, but also an issue of intense social and commercial interest. The metabolism and physiology of extremophiles have such peculiar features as to be fascinating per se; however, their commercial potential, albeit long recognized, is far from being fully realized. Discovering the extremes at which life can occur has made more plausible the search for life on other planets, with many more discoveries likely to come due to improvements in exploration and analytical technology (Rothschild and Mancinelli 2001). The International Space Station provides a unique opportunity in establishing the limits of endurance of life as we know it; results of ongoing research will provide insights into the potential of life to survive beyond Earth (Rabbow et al. 2009).

In cold and hot deserts, such as the McMurdo Dry Valleys in Antarctica and the Atacama Desert in Chile, both considered the Earth's nearest equivalent to the Martian environment, life is pushed to its physical limits due to extreme water deficit and/or freezing temperatures. In these places organisms escaped from prohibitive external conditions by colonizing rocks, the last refuge for life: Such are the photosynthesis-based lithic communities. The discovery of these communities sheds light on the possible history of Martian microbial life, if it ever existed, and has led to identifying rock-inhabiting cyanobacteria as pioneers in the colonization of the ultimate desert – Mars (Grilli Caiola and Billi 2007).

*In memoriam of Imre Friedmann and Roseli Ocampo-Friedmann.

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2 Cyanobacteria in hot and cold desert rocks

Lithobionts (from the Greek, lithos: rock; bios: life) are mainly microbionts colonizing rock surfaces and rock interiors in a wide variety of environments. In dry environments, such as hot and cold deserts, lithic ecosystems harbor much of the extant life. Lithobionts are distinguished according to the location in respect to substrate and functional criteria: epilithics dwelling on the rock surface; hypolithics forming biofilms at the stone–soil interface; endolithics colonizing microscopic fissures (chasmoendoliths) and structural cavities (cryptoendoliths) of rocks; and finally euendoliths actively boring into rocks (Golubic et al. 1981). In hot and cold deserts, where life is pushed to its limits, members of the genus *Chroococcidiopsis* colonize porous rock or the stone–soil interface (Fig. 1a, b). The endurance of *Chroococcidiopsis* in the Dry Valleys in Antarctica as well as in the Atacama Desert in Chile, which are both considered terrestrial analogs of the two environmental extremes on Mars – cold and dryness

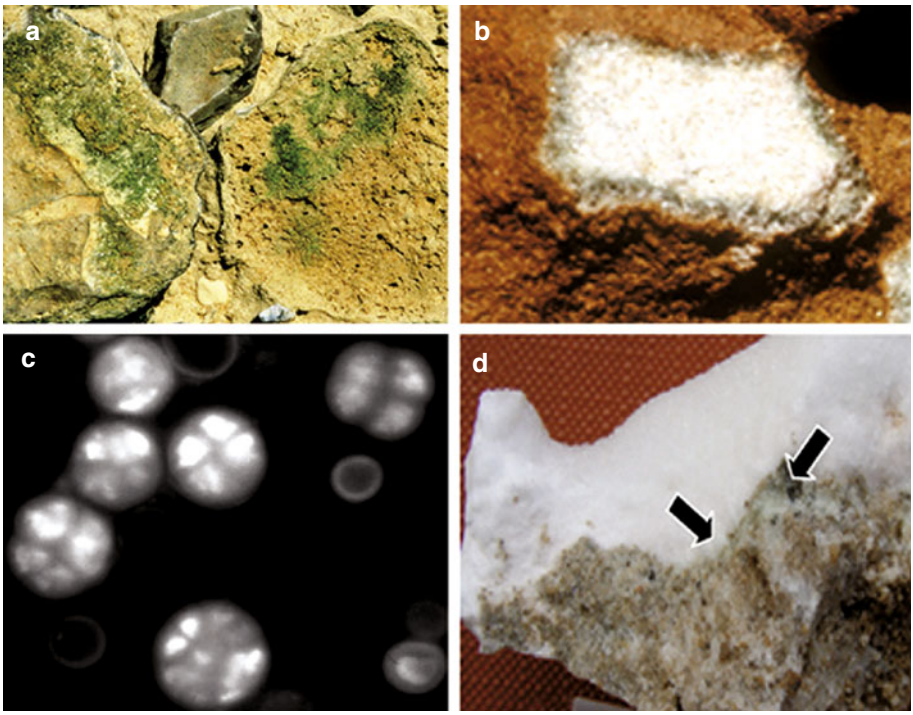

Fig. 1. Examples of photosynthesis-based lithic communities. (**a**) Hypolithics; (**b**) endolithics (photos: E. Imre Friedmann); (**c**) *Chroococcidiopsis* sp. isolated from endolithic growth in Nubian sandstone; (**d**) endoevaporitic growth (photo: Nunzia Stivaletta and Roberto Barbieri)

(Warren-Rhodes et al. 2006) – is remarkable: The photosynthesis-based lithic communities found offer unique model systems for microbial ecology, geobiology, and astrobiology (Walker and Norman 2007).

Research into photosynthesis-based lithic communities was pioneered by E. Imre Friedmann and Roseli Ocampo-Friedmann, who first described the *Chroococcidiopsis*-dominated lithic communities in the Negev Desert, Israel (Friedmann et al. 1967). Later research was extended to hot and cold deserts worldwide, leading to the establishment of the Culture Collection of Microorganisms from Extreme Environments (CCMEE); currently about 250 desert strains of *Chroococcidiopsis* and a few related genera are maintained by this author at the University of Rome, Tor Vergata.

Members of the genus *Chroococcidiopsis* have a developmental cycle in which a spherical cell (called baeocyte) undergoes repeated binary fissions, yielding aggregates with only a few cells; thereafter, multiple fission occurs in almost all of the cells within an aggregate, followed by the release of numerous baeocytes (Fig. 1c). The phylogenetic relationships within the *Chroococcidiopsis* lineage remain to be resolved. Due to the high sequence divergences of 16S rRNA genes it has been suggested that some forms could be even regarded as different species or genera (Fewer et al. 2002). Based on the sequencing of 16S rRNA genes it was reported that four desert isolates of *Chroococcidiopsis* were divergent from other pleurocapsalean representatives (Billi et al. 2001). The extension of the phylogenetic analysis to 12 desert strains of *Chroococcidiopsis* revealed their clustering into different groups (Billi and Wilmotte, unpublished).

The Dry Valleys are the largest ice-free region on the Antarctic continent and were considered to be virtually sterile (Horowitz et al. 1972). Then came the discovery of the "lichen-dominated communities," formed by fungi, algae, bacteria, and cyanobacteria of the genera *Chroococcidiopsis* and *Gloeocapsa*, in sedimentary rocks (Friedmann and Ocampo-Friedmann 1976). As a consequence of culture-independent analysis based on molecular methods it is now well recognized that the bacterial diversity and abundance in the Dry Valleys is not as low as first assumed (Cary et al. 2010). Recently, a culture-independent survey of the microbial biodiversity in the McKelvey Valley showed that the greatest diversity occurred in endolithic and chasmolithic communities in sandstone, where *Chroococcidiopsis* was dominant. The soil, on the other hand, was relatively impoverished and lacked a significant photoautotrophic component, except for isolated islands of hypolithic cyanobacterial colonization on quartz rocks (Pointing et al. 2009). However, the finding of lichen-dominated communities in the Dry Valleys raised new hopes of finding life on Mars at a time when the Viking missions had shown the Martian soil to be lifeless and depleted in organic material. Since then considerable interest has been aroused concerning rock-inhabiting communities,

in the contest of identifying signature for past or present life forms Mars and to investigate the survival potential of terrestrial microbial life on Mars of present days (Cockell et al. 2005). It has been speculated that before their extinction Martian microbes, if they ever existed, could have escaped the adverse environmental conditions during the cooling of the planet by withdrawing into the rocks (Friedmannn 1986).

In hot deserts life must adapt to severe stress due to sudden changes between warm/humid and hot/dry, and this is thought to be the reason for the exclusion of eukaryotic organisms from endolithic communities in extremely dry deserts. It was reported that along an environmental gradient of hot and cold deserts in China the diversity of hypolithic communities was affected by the availability of liquid water rather than by temperature, *Chroococcidiopsis* being ubiquitous in different thermal and moisture conditions, and dominant in the most arid sites (Pointing et al. 2007). In the effort to identify the absolute dry limit of life on Earth, the Atacama Desert in Chile has become one of the most relevant hot places. This desert is so dry that a hyperarid core has been identified: decades without rain were recorded, along with the virtual absence of heterotrophic bacteria (Navarro-González et al. 2003). In the Atacama Desert, hypolithic cyanobacterial communities occur along the aridity gradient, which reaches its limit at the hyperarid core. Here rare *Chroococcidiopsis*-based communities exist in small spatially isolated islands amidst a microbially impoverished soil (Warren-Rhodes et al. 2006). When applied to Mars, the Atacama model predicts that if microhabitats exist (or have existed) on Mars, they will be difficult to detect, being dispersed in virtually lifeless surroundings (Warren-Rhodes et al. 2006). Remarkably, in the hyperarid zone of the Atacama, cyanobacteria of the genus *Chroococcidiopsis* colonize a peculiar habitat provided by halite deposits (Wierzchos et al. 2006). Such a finding suggests this cyanobacterium can achieve metabolic activity during periods of moisture availability, by adsorbing moisture from halite deliquescence, e.g., the absorption of moisture from the atmosphere (Davila et al. 2008). Recently, environmental 16S rRNA gene sequences obtained from endolithic growth in halite rock collected from the Atacama Desert (Fig. 1d) identified a cyanobacterial sequence closely related to hot desert strains of *Chroococcidiopsis* isolated from geographically distinct desert areas (Billi, Stivaletta and Barbieri, unpublished). Photosynthetic microorganisms within dry evaporate rocks have been considered as relevant models in the search for life within our Solar System (Wierzchos et al. 2006). Mars has been suggested as an intriguing location to search for halophiles (or their remnants) outside Earth, as it may have been a wetter and warmer place in the past and recent data suggest the presence of halite on Mars (Leuko et al. 2010).

3 Cyanobacterial adaptation to Earth's deserts

In extremely dry environments, such as the ice-free Ross desert or hyperarid hot deserts, rock-inhabiting communities of *Chroococcidiopsis* come into contact with water 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 (Friedmann et al. 1993; Warren-Rhodes et al. 2006). How they manage to survive is still a mystery.

Water is essential for life: Only a small but taxonomically diverse group of organisms can withstand desiccation by entering into a state of suspended animation, a phenomenon known as anhydrobiosis (from the Greek "life without water") (Van Leeuwenhoek 1702). Anhydrobiotic cyanobacteria can withstand desiccation without differentiating into any specialized cell types, as other anhydrobiotes do, e.g., certain bacteria, lichens, mosses, ferns, certain angiosperm genera (known as "resurrection plants"), representatives of rotifers, tardigrades, and nematode taxa. Other anhydrobiotes produce stage-specific anhydrobiotic forms, e.g., the spores of some bacteria and fungi, the embryonic cysts of brine shrimps, larvae of certain insects (chironomids), and some plant seeds and pollen (Alpert 2006). Anhydrobiotic cyanobacteria such as *Chroococcidiopsis* spp. and *Nostoc commune* are of particular significance since they need to prevent oxidative damage exacerbated by oxygenic photosynthesis (Billi and Potts 2000, 2002). Despite the interest in comprehending such a peculiar phenomenon, the mechanisms underlying anhydrobiosis have not been fully understood in any organism (Alpert 2006). Adaptation to desiccation has the singular distinction that dried cells enter full metabolic arrest. This raises intriguing questions as to the role of this function opposed to adaptation to extremes of pH, temperature, or pressure, once it is assumed that evolution is driven toward optimum function rather than maximum stability (Potts et al. 2005). In desiccation-sensitive cells the complete removal of water is lethal due to the damage induced at every level of cellular organization. The removal of the hydration shell from phospholipids of membrane bilayers increases the van der Waal's interactions between adjacent lipids, causing an increase in the phase transition temperature of membranes, and their transition to the gel phase at environmentally relevant temperatures (Crowe 2007). Oxidative damage to proteins, lipids, and nucleic acids is induced when the production of reactive oxygen species (ROS) due to dysfunction in enzymes and/or electron transport chains exceeds the antioxidant system (França et al. 2007). Other damage to proteins and nucleic acids results from metal-catalyzed Haber–Weiss and Fenton reactions as well as via the Maillard (browning) reaction (Potts et al. 2005).

The occurrence of live and dead cells in dried multicellular aggregates of *Chroococcidiopsis* is a feature of its response to desiccation that warrants emphasis. Cytological and ultrastructural studies carried out on samples dried for 5 years

identified cells retaining typical ultrastructural features and others with varying degrees of degeneration (Grilli Caiola et al. 1993, 1996a). Subsequent investigations based on molecular probes used with fluorescence microscopy highlighted the fact that desiccation surviving *Chroococcidiopsis* avoids and/or limits genomic fragmentation and covalent modifications, preserves intact plasma membranes and autofluorescence of photosynthetic pigments, and undergoes a spatially reduced ROS accumulation (Billi 2009a). The co-occurrence of live and dead cells within a given dried aggregate of *Chroococcidiopsis* poses intriguing questions: Is cell death the outcome of a passive externally driven process? Or does it result from programmed cell death, thus further corroborating the idea that desiccation resistance is not a simple process.

The capability of dried cells of *Chroococcidiopsis* to avoid and/or reduce subcellular damage indicates that protection mechanisms are relevant in the process of desiccation resistance. However, other evidence indicates that an efficient repair DNA systems is relevant to the ability of *Chroococcidiopsis* to survive prolonged desiccation, a condition in which oxidative damage continues even in the absence of metabolic activity, or when dried cells experience additional environmental stressors. Actively growing *Chroococcidiopsis* cells can repair the genomic fragmentation induced by 5 kGy of ionizing radiation (Billi et al. 2000a) while DNA damage caused in dried cells by a simulated unattenuated Martian UV flux is repaired upon rehydration (Cockell et al. 2005).

The capability of desert strains of *Chroococcidiopsis* to repair genome fragmentation resembles that reported for *Deinococcus radiodurans* (Cox and Battista 2005). Since naturally occurring environments result in exposures exceeding 400 mGy per year, it has been proposed that radioresistance is a consequence of the adaptation to DNA-damaging conditions, such as desiccation (Cox and Battista 2005). However, unlike *D. radiodurans*, *Chroococcidiopsis* does not undergo genome fragmentation upon desiccation (Billi 2009a) as reported for *N. commune* (Shirkey et al. 2003). In dried cells of *Chroococcidiopsis* at least a subset of proteins, namely phycobilisomes and esterase, were protected against oxidative damage (Billi 2009a). This might have a bearing on the desiccation tolerance of *Chroococcidiopsis*, given that oxidative protein damage, but not DNA damage, has been proposed to determine bacterial survival of DNA-damaging conditions. Indeed, protein damage is thought to kill irradiation-sensitive bacteria after exposure to low doses of ionizing radiation which cause less than one DNA double-strand break per genome (Daly 2009).

How *Chroococcidiopsis* can manage to resist oxidative stress remains largely unknown. The accumulation of an iron superoxide dismutase has been observed in dried cells of *Chroococcidiopsis* (Grilli Caiola et al. 1996b) while an abundant, active iron superoxide dismutase has been described in dried *N. commune* (Shirkey et al.

2000). Recently, high Mn (II) contents have been correlated with the resistance of *D. radiodurans* to both ionizing radiation and desiccation (Daly 2009). A close correlation between high Mn (II) concentration, high levels of resistance to ionizing irradiation and low susceptibility to desiccation-induced protein oxidation have also been reported in bacteria isolated from desert environments (Fredrickson et al. 2008).

In the effort to decipher the structural, physiological, and molecular mechanisms of anhydrobiosis it is becoming clear that they are both numerous and highly diverse. A crucial structural mechanism in the adaptation of *Chroococcidiopsis* to anhydrobiosis is the production of abundant polysaccharide-rich envelopes (Grilli Caiola et al. 1996a). It has been proposed that extracellular polysaccharide (EPS) is a key component in cyanobacterial desiccation tolerance by providing a repository for water as well as a matrix which stabilizes desiccation-related enzymes and molecules (Wright et al. 2005). In anhydrobiotic cyanobacteria the EPS production might act in synergy with trehalose accumulation in the cytoplasm. This non-reducing disaccharide is produced in large amounts by most anhydrobiotes and, by substituting water molecules, prevents the phase transition of cellular membranes and stabilizes dried proteins (Crowe 2007). It is not yet known whether *Chroococcidiopsis* accumulates trehalose upon drying, although the involvement of this disaccharide in the desiccation resistance of *N. commune* has been reported (Shirkey et al. 2003).

Although hints on desiccation tolerance of *Chroococcidiopsis* suggest an interplay between protection and repair mechanisms, it is necessary to employ DNA microarray and proteomic tools to decipher the genetic basis of its anhydrobiotic adaptation. Remarkably *Chroococcidiopsis* is the only anhydrobiotic cyanobacterium suitable for genetic manipulation (Billi et al. 2001), for which gene activation was attempted (Billi 2009b). When the genome sequences of hot and cold desert strains of *Chroococcidiopsis*, namely CCMEE 029 from the Negev desert and CCMEE 134 from Beacon Valley (Antarctica), along with a Tibetan and Taklimakan isolate, will be completed (Billi and Pointing, unpublished), insights into the molecular aspects of desiccation tolerance are likely.

4 Survival of desert cyanobacteria beyond Earth

It is widely recognized that the capability of anhydrobiotes to withstand desiccation is often associated with an extraordinary resistance to other environmental stressors. Such a feature makes them ideal for investigating the survival potential of terrestrial organisms for outer space conditions or Mars, both of which require extreme tolerance to vacuum (imposing extreme dehydration), cold, and radiation (Baglioni et al. 2007).

Notably, spores of *Bacillus subtilis* survived space conditions in low Earth orbit for 6 years on the Long Duration Exposure Facility (Horneck 1993), while dried lichens and tardigrades survived in space for 2 weeks during the Biopan experiments (Sancho et al. 2007; Jönsson et al. 2008). Ground-based simulations of Martian and space conditions demonstrated the potential endurance of Antarctic rock-inhabiting fungi and desert strains of *Chroococcidiopsis* in extraterrestrial environments (Onofri et al. 2008; Billi et al. 2008). The mechanisms behind the tolerance of anhydrobiotes to outer space have not yet been revealed; however, they are considered to be a consequence of the resistance to desiccation and radiation (Jönsson et al. 2008).

In recognition of its environmental flexibility *Chroococcidiopsis* has long been identified as a photosynthetic model organism for space research (Friedmann and Ocampo-Friedmann 1995), at a time when NASA established the Astrobiology Program (Cockell 2002). It was proposed that *Chroococcidiopsis* could be used as a photosynthetic pioneer for Mars terraforming, if inoculated in proper desert pavements and periodically wetted (Friedmann and Ocampo-Friedmann 1995). The expected capability of *Chroococcidiopsis* to withstand environmental stressors not currently met in nature was corroborated by the finding that strains from hot and cold deserts survive doses of ionizing radiation as high as 15 kGy (Billi et al. 2000a). Remarkably, a monolayer of dried cells of *Chroococcidiopsis* survived 15-min exposure to an attenuated Martian UV flux (Cockell et al. 2005), thus proving more resistant than *B. subtilis* spores (Schuerger et al. 2003) and akinetes of *Anabaena cylindrica* (Olsson-Francis et al. 2009). It was also reported that the survival of dried cells of *Chroococcidiopsis* shielded under 1 mm of Martian soil simulant or gneiss was unaffected by 4 h of exposure to Martian UV radiation. The endurance of dried *Chroococcidiopsis* under simulated Martian UV radiation further supported its use in future approaches to mimic endolithic Martian exposure and allowed the speculation that it could survive and perhaps grow within lithic habitats in the presence of a source of liquid water and essential nutrients (Cockell et al. 2005). Dried cells of *Chroococcidiopsis* under a few millimeters of Antarctic sandstone were reported to withstand UV radiation corresponding to 1.5 years permanence in space (Billi et al. 2008; Billi et al. 2011). This corroborates the importance of lithic communities in the context of lithopanspermia, the transfer of living material inside rocks between planets (Nicholson 2009). Lithopanspermia is currently divided into three stages: (i) launch of microbe-bearing rocks from a donor planet into space; (ii) transit through space to a recipient planet; and (iii) entry into a recipient planet. Survival of launch pressures simulating the estimated values of Martian meteorites during escape was reported for spores of *B. subtilis* as well as the photobiont and mycobiont partners of the lichen *Xanthoria elegans* which, embedded in a Martian analog rock, encompassed shock pressures up to 40 GPa

(Horneck et al. 2008). By contrast, *Chroococcidiopsis* survived shock pressures ranging from 5 to 10 GPa. Therefore, given the low frequency of weakly shocked meteorites, the chances for interplanetary transport of cyanobacteria-type organisms seem reduced (Horneck et al. 2008). *Chroococcidiopsis* cells inoculated into rock to the depth at which they occur in nature, and mounted on the heat shield of a FOTON-M2 recoverable orbital capsule, did not survive the re-entry into the atmosphere (Cockell et al. 2007). In fact, during the atmospheric re-entry, the photosynthetic lithic community located at a depth at which light is available for photosynthesis, was heated to well above the upper temperature limit for life; this suggests that nonphotosynthetic organisms living deep within rocks have a better chance of surviving the exit and entry process (Cockell 2007).

5 Biotechnological exploitation of anhydrobiosis

Since the air-dried state is characterized by enhanced biostability there is considerable interest in the industrial applications of conferring desiccation tolerance to otherwise desiccation-sensitive cells. Logistical problems and costs associated with preservation and storage at ultra-low temperatures necessitate the development of novel technologies for air-dried pharmaceuticals, dried cell-based biosensors, and cell and tissue banks (Potts et al. 2005). With the understanding of the role played by the accumulation of trehalose and sucrose in the majority of anhydrobiotes, a whole field of research has been opened up. Embedding in trehalose or sucrose has been used successfully to stabilize dry membranes and enzymes, as well as intact bacterial cells (Crowe 2007). In view of the simplicity of the biosynthetic pathway of trehalose and sucrose the metabolic engineering of desiccation-sensitive cells has been attempted. The expression of a cyanobacterial *sps*A gene encoding for a sucrose-6-phosphate synthase in *Escherichia coli* led to a marked increase in survival after air drying, freeze-drying and chemical desiccation over phosphorus pentoxide (Billi et al. 2000b). However, when as an extension of this principle the *sps*A gene was expressed in human kidney cells, only a low percentage of the cells underwent growth; moreover, this occurred with significantly less vigor than in cells that had never been desiccated (Bloom et al. 2001). Thus, even if it is in principle possible to stabilize air-dried human cells, additional studies are required to fully optimize such a process. In striking contrast to the possibility of enabling prokaryotes and isolated cells to tolerate desiccation, there is a lack of success in achieving desiccation tolerance of whole, desiccation-sensitive, multicellular animals and plants (Alpert 2005). While trehalose loading could be used to usefully stabilize human blood platelets, the survival of human embryonic kidney cells to air drying was enhanced only by the synergetic action of trehalose loading and expression of a gene codifying for the stress protein p26 obtained from an anhydrobiotic organism (Crowe et al. 2005).

Mechanisms identified as relevant in the adaptation to desiccation of the cyanobacterium *N. commune* were exploited to stabilize desiccation-sensitive cells as well. It was first proved that the extracellular glycan of *N. commune* can be used to inhibit fusion of membrane vesicles during desiccation and freeze-drying (Hill et al. 1997). Indeed cyanobacteria produce EPS that are so varied in their gel and sol properties that they may actually have properties similar to those described for glass-forming polymers and thus allow the stabilization of cell membranes during periods of desiccation (Pereira et al. 2009). In fact, in the amorphous state molecular diffusion is reduced and uncontrolled reactions that would be disastrous over the prolonged desiccation are avoided (Crowe et al. 1998). Unlike bacterial EPS which contain less than four different monomers, cyanobacterial EPS are complex heteropolysaccharides composed of more than six different monosaccharides, whose alternative composition is relevant to a variety of industrial applications (Pereira et al. 2009). It was also reported that the overexpression in the aquatic desiccation-sensitive cyanobacterium *Anabaena* sp. PCC 7120 of the group 3 sigma factor *sigJ* gene obtained from the desiccation-tolerant cyanobacterium *Nostoc* sp. HK-01 resulted in an increased ESP production and acquisition of desiccation tolerance (Yoshimura et al. 2007). When subsequently the exploitation of the extracellular glycan of *N. commune* was attempted in order to dry and revive nucleated mammalian cells, the cells proved to be nonviable despite structural preservation after several weeks of desiccation (Bloom et al. 2001). The use of exogenous addition of water stress proteins, abound in the extracellular matrix of dried cells of *N. commune*, for air-dry stabilization of *E. coli* proved evidence that a two-step rehydration protocol must be followed to avoid deleterious effects of rapid rewetting (Potts et al. 2005).

Until now, the composition and properties of EPS produced by desert strains of *Chroococcidiopsis* remain unknown as do any changes in their proteome following desiccation. However, unlike *N. commune*, which is refractory to gene transfer, desert strains of *Chroococcidiopsis* have been identified as suitable to genetic manipulation by means of electroporation and conjugation (Billi et al. 2001). The identification of a pDU1-based plasmid capable of autonomous replication in *Chroococcidiopsis* along with the possibility of driving gene expression by using an inducible promoter of *E. coli* (Billi et al. 2001), will contribute to the biotechnological exploitation of this anhydrobiotic cyanobacterium. Air-dried cells of *Chroococcidiopsis* could be used to develop novel biosensors for detecting DNA-damaging conditions, which only requires water for re-activation. The ability to withstand prolonged desiccation and high doses of ionizing radiation make *Chroococcidiopsis* an effective candidate for biosensor fabrication, important performance criteria in this regard being robustness, resistance to environmental extremes, and portability. The exploitation of genetically modified cells of *Chroococcidiopsis* has real potential in space research:

Dried cells of *Chroococcidiopsis* could be used to develop an air-dried bank of a wide varieties of metabolites with useful applications within the framework of human space exploration.

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References

Alpert P (2005) The limits and frontiers of desiccation-tolerant life. Integr Comp Biol 45:685–695

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584

Baglioni P, Sabbatini M, Horneck G (2007) Astrobiology experiments in low earth orbit – facilities, instrumentation and results. In: Rettberg P, Horneck G (eds) Complete course in astrobiology. Wiley-VCH, Berlin, pp 273–319

Billi D (2009a) Subcellular integrities in *Chroococcidiopsis* sp. CCMEE 029 survivors after prolonged desiccation revealed by molecular probes and genome stability assays. Extremophiles 13:49–57

Billi D (2009b) Loss of topological relationships in a Pleurocapsalean cyanobacterium (*Chroococcidiopsis* sp.) with partially inactivated *ftsZ*. Ann Microbiol 59:235–238

Billi D, Potts M (2000) Life without water: responses of prokaryotes to desiccation. In: Storey KB, Storey JM (eds) Cell and molecular response to stress: environmental stressors and gene responses. Elsevier Science, Amsterdam, pp 181–192

Billi D, Potts M (2002) Life and death of dried prokaryotes. Res Microbiol 153:7–12

Billi D, Friedmann EI, Hofer KG, Grilli Caiola M, Ocampo-Friedmann R (2000a) Ionizing-radiation resistance in the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. Appl Environ Microbiol 66:1489–1492

Billi D, Wright DJ, Helm RF, Prickett T, Potts M, Crowe JH (2000b) Engineering desiccation tolerance in *Escherichia coli*. Appl Environ Microbiol 66:1680–1684

Billi D, Friedmann EI, Helm RF, Potts M (2001) Gene transfer to the desiccation-tolerant cyanobacterium *Chroococcidiopsis*. J Bacteriol 183:2298–2305

Billi D, Ghelardini P, Onofri S, Cockell CS, Rabbow E, Horneck G (2008) Desert Cyanobacteria under simulated space and Martian conditions. In: European Planetary Science Congress, Abstr EPSC2008-A-00474

Billi D, Viaggiu E, Cockell CS, Rabbow E, Horneck G, Onofri S (2011) Damage escape and repair in dried *Chroococcidiopsis* spp. from hot and cold deserts exposed to simulated space and Martian conditions. Astrobiology 11:65–73.

Bloom F, Price RP, Lao GF, Xia JL, Crowe JH, Battista JR, Helm RF, Slaughter S, Potts M (2001) Engineering mammalian cells for solid-state sensor applications. Biosens Bioelectron 16:603–608

Cary SC, McDonald IR, Barrett JE, Cowan DA (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. Nat Rev Microbiol 8:129–138

Cockell CS (2002) Astrobiology – a new opportunity for interdisciplinary thinking. Space Policy 18:263–266

Cockell CS (2007) The interplanetary exchange of photosynthesis. Orig Life Evol Biosph 38:87–104
Cockell CS, Schuerger AC, Billi D, Friedmann EI, Panitz C (2005) Effects of a simulated Martian UV flux on the cyanobacterium, *Chroococcidiopsis* sp. 029. Astrobiology 5:127–140
Cockell CS, Brack A, Wynn-Williams DD, Baglioni P, Brandstatter F, Demets R, Edwards HG, Gronstal AL, Kurat G, Lee P, Osinski GR, Pearce DA, Pillinger JM, Roten CA, Sancisi-Frey S (2007) Interplanetary transfer of photosynthesis: an experimental demonstration of a selective dispersal filter in planetary island biogeography. Astrobiology 7:1–9
Cox MM, Battista JR (2005) *Deinococcus radiodurans* – the consummate survivor. Nat Rev Microbiol 3:882–892
Crowe JH (2007) Trehalose as a "chemical chaperone": fact and fantasy. Adv Exp Med Biol 94:143–158
Crowe JH, Carpenter JF, Crowe LM (1998) The role of vitrification in anhydrobiosis. Annu Rev Physiol 60:73–103
Crowe JH, Crowe LM, Wolkers WF, Oliver AE, Ma X, Auh J-H, Tang M, Zhu S, Norris J, Tablin F (2005) Stabilization of dry mammalian cells: lessons from nature. Integr Comp Biol 45:810–820
Daly MJ (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans*. Nat Rev Microbiol 7:237–245
Davila AF, Gómez-Silva B, de los Rios A, Ascaso C, Olivares H, McKay CP, Wierzchos J (2008) Facilitation of endolithic microbial survival in the hyperarid core of the Atacama Desert by mineral deliquescence. J Geophys Res 113. DOI: 10.1029=2007JG000561
Fewer D, Friedl T, Büdel B (2002) *Chroococcidiopsis* and heterocyst-differentiating cyanobacteria are each other's closest living relatives. Mol Phylogenet Evol 23:82–90
França MB, Panek AD, Eleutherio EC (2007) Oxidative stress and its effects during dehydration. Comp Biochem Physiol A Mol Integr Physiol 146:621–631
Fredrickson JK, Li SMW, Gaidamakova EK, Matrosova VY, Zhai M, Sulloway HM, Scholten JC, Brown MG, Balkwill DL, Daly MJ (2008) Protein oxidation: key to bacterial desiccation resistance? Int J Syst Evol Microbiol 2:393–403
Friedmann EI (1986) The Antarctic cold desert and the search for traces of life on Mars. Adv Space Res 6:265–268
Friedmann EI, Ocampo-Friedmann R (1976) Endolithic blue-green-algae in dry valleys – primary producers in Antarctic desert ecosystem. Science 193:1247–1249
Friedmann EI, Ocampo-Friedmann R (1995) A primitive cyanobacterium as pioneer microorganism for terraforming Mars. Adv Space Res 15:243–246
Friedmann EI, Lipkin Y, Ocampo-Paus R (1967) Desert algae of the Negev (Israel). Phycologia 6:185–199
Friedmann EI, Kappen L, Meyer MA, Nienow JA (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. Microb Ecol 25:51–69
Golubic S, Friedmann EI, Schneider J (1981) The lithobiontic ecological niche, with special reference to microorganisms. J Sediment Petrol 51:475–478
Grilli Caiola M, Billi D (2007) *Chroococcidiopsis* from desert to Mars. In: Seckbach J (ed) Algae and cyanobacteria in extreme environments, vol 11. Cellular origin, life in extreme habitats and astrobiology. Springer-Verlag, Berlin, pp 553–568
Grilli Caiola M, Ocampo-Friedmann R, Friedmann EI (1993) Cytology of long-term desiccation in the cyanobacterium *Chroococcidiopsis* (Chroococcales). Phycologia 32:315–322
Grilli Caiola M, Billi D, Friedmann EI (1996a) Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (Chroococcales). Eur J Phycol 31:97–105
Grilli Caiola M, Canini A, Ocampo-Friedmann R (1996b) Iron superoxide dismutase (Fe-SOD) localization in *Chroococcidiopsis* (Chroococcales, Cyanobacteria). Phycologia 35:90–94

Hill DR, Keenan TW, Helm RF, Potts M, Crowe LM, Crowe JH (1997) Extracellular polysaccharide of *Nostoc commune* (Cyanobacteria) inhibits fusion of membrane vesicles during desiccation. J Appl Phycol 9:237–248

Horneck G (1993) Responses of *Bacillus subtilis* spores to the space environment: results from experiments in space. Orig Life Evol Biosph 23:37–52

Horneck G, Stöffler D, Ott S, Hornemann U, Cockell CS, Moeller R, Meyer C, de Vera JP, Fritz J, Schade S, Artemieva NA (2008) Microbial rock inhabitants survive hypervelocity impacts on Mars-like host planets: first phase of lithopanspermia experimentally tested. Astrobiology 8:17–44

Horowitz NH, Cameron RE, Hubbard JS (1972) Microbiology of the Dry Valleys of Antarctica. Science 176:242–245

Jönsson KI, Rabbow E, Schill RO, Harms-Ringdahl M, Rettberg P (2008) Tardigrades survive exposure to space in low Earth orbit. Curr Biol 18:R729–R731

Leuko S, Rothschild LJ, Burns BP (2010) Halophilic archaea and the search for extinct and extant life on Mars J. Cosmology 5:940–950

Navarro-González R, Rainey FA, Molina P, Bagaley DR, Hollen BJ, de la Rosa J, Small AM, Quinn RC, Grunthaner FJ, Cáceres L, Gomez-Silva B, McKay CP (2003) Mars-like soils in the Atacama Desert, Chile, and the dry limit of microbial life. Science 302:1018–1021

Nicholson WL (2009) Ancient micronauts: interplanetary transport of microbes by cosmic impacts. Trends Microbiol 17:243–250

Olsson-Francis K, de la Torre R, Towner MC, Cockell CS (2009) Survival of akinetes (resting-state cells of Cyanobacteria) in Low Earth Orbit and simulated extraterrestrial conditions. Orig Life Evol Biosph 39:565–579

Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, Horneck G, de Vera JP, Hatton J, Zucconi L. 2008. Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. Stud Mycol 61:99–109

Pereira S, Zille A, Micheletti E, Moradas-Ferreira P, De Philippis R, Tamagnini P (2009) Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. FEMS Microbiol Rev 33:917–941

Pointing SB, Warren-Rhodes KA, Lacap DC, Rhodes KL, McKay CP (2007) Hypolithic community shifts occur as a result of liquid water availability along environmental gradients in China's hot and cold hyperarid deserts. Environ Microbiol 9:414–424

Pointing SB, Chan Y, Lacap DC, Lau MCY, Jurgens JA, Roberta L, Farrell RL (2009) Highly specialized microbial diversity in hyper-arid polar desert. Proc Natl Acad Sci USA 106:19964–19969

Potts M, Slaughter SM, Hunneke FU, Garst JF, Helm RF (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. Integr Comp Biol 45:800–809

Rabbow E, Horneck G, Rettberg P, Schott J-U, Panitz C, L'Afflitto A, von Heise-Rotenburg R, Willnecker R, Baglioni P, Hatton J, Dettmann J, Demets R, Reitz G (2009) EXPOSE, an astrobiological exposure facility on the International Space Station – from proposal to flight. Orig Life Evol Biosph 39:581–598

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Sancho LG, de la Torre R, Horneck G, Ascaso C, de los Rios A, Pintado A, Wierzchos J, Schuster M (2007) Lichens survive in space: results from the 2005 LICHENS experiment. Astrobiology 7:443–454

Schuerger AC, Mancinelli RL, Kern RG, Rothschild LJ, McKay CP (2003) Survival of *Bacillus subtilis* on spacecraft surfaces under simulated Martian environments: implications for the forward contamination of Mars. Icarus 165:253–27

Shirkey B, Kovarcik DP, Wtight DJ, Wilmoth G, Prickett TF, Helm RF, Gregory EM, Potts M (2000) Active Fe-containing superoxide dismutase and abundant *sodF* mRNA in *Nostoc commune* (Cyanobacteria) after years of desiccation. J Bacteriol 182:189–197

Shirkey B, McMaster NJ, Smith SC, Wright DJ, Rodriguez H, Jaruga P, Birincioglu M, Helm RF, Potts M (2003) Genomic DNA of *Nostoc commune* (Cyanobacteria) becomes covalently modified during long-term (decades) desiccation but is protected from oxidative damage and degradation. Nucl Acids Res 31:2995–3005

Van Leeuwenhoek A (1702) On certain animalcules found in the sediments in gutter of the roofs of houses. Letter 144 to Hendrik van Bleyswijk, dated 9 February 1702. In: The select works of Anton van Leeuwenhoek, vol 2. London, pp 207–213

Walker JJ, Norman RP (2007) Endolithic microbial ecosystems. Annu Rev Microbiol 61:331–47

Warren-Rhodes KA, Rhodes KL, Pointing SB, Ewing SA, Lacap DC, Gómez-Silva B, Amundson R, Friedmann EI, McKay CP (2006) Hypolithic cyanobacteria, dry limit of photosynthesis, and microbial ecology in the hyperarid Atacama Desert. Microb Ecol 52:389–398.

Wierzchos J, Ascaso C, McKay CP (2006) Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. Astrobiology 6:415–422

Wright DJ, Smith SC, Joardar V, Scherer S, Jervis J, Warren A, Helm RF, Potts M (2005) UV irradiation and desiccation modulate the three-dimensional extracellular matrix of *Nostoc commune* (Cyanobacteria). J Biol Chem 280:40271–40281

Yoshimura H, Okamoto S, Tsumuraya Y, Ohmori M (2007) Group 3 sigma factor gene, *sigJ*, a key regulator of desiccation tolerance, regulates the synthesis of extracellular polysaccharide in cyanobacterium *Anabaena* sp. strain PCC 7120. DNA Res 14:13–24