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Evolution and adaptation of fungi at boundaries of life

S. Onofri [a,*], L. Selbmann [a], G.S. de Hoog [b], M. Grube [c], D. Barreca [a], S. Ruisi [a], L. Zucconi [a]

[a] Dipartimento di Ecologia e Sviluppo Economico Sostenibile, Università della Tuscia, Largo dell'Università snc, 01100 Viterbo, Italy
[b] Centraalbureau voor Schimmelcultures, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands
[c] Institute für Pflanzenwissenschaften, Karl Franzens-Universität Graz, Holteigasse 6, Graz-8010, Graz, Austria

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Abstract

The Antarctic cryptoendolithic fungi of the ice-free desert could have evolved genetically and geographically isolated since the separation of the Continent from the Gondwanaland. The resulting harsh environmental conditions due to the migration of Antarctica to the South Pole led to a strong selective pressure possibly promoting adaptive radiation and speciation. Microorganisms evolved during this unique process are adapted to colonize what is known as the closest Martian environment on Earth. For this reason they have been already suggested as the best eukaryotic model for exobiological speculations. The results on freeze and thawing, UV exposure and osmotic stress tolerance here reported highlight an uncommon ability of surviving under these external pressures. Studies on their ability to withstand space conditions are in progress in view of the opportunity of direct space exposure on the International Space Station. The results could give new tools to solve the conflict concerning the Panspermia hypothesis.

Keywords: Antarctica; Rock black fungi; Cold-tolerance; Cryo-resistance; Osmotic stress; UV-B; Exobiology

1. Introduction

Survival of organisms in space conditions and the possible past occurrence of life forms on Mars are matters of great interest. The Antarctic ice-free desert, and in particular the McMurdo Dry Valleys, located in Southern Victoria Land (continental Antarctica), are considered to be, among the terrestrial 'extreme' environments, the closest analogue of Mars due to a combination of very cold and very dry conditions, very poor nutrient availability, and large fluxes of UV-light (Horneck, 2000; Finster et al., 2007; Onofri et al., 2007a,b).

The McMurdo Dry Valleys, approximately 4800 km^2 wide, is the largest ice-free area in the Antarctica. Only a few endolithic microorganisms, considered among the most extremotolerant, live in the harshest part of this area. Sampling and isolation of such microorganisms, and the study of their adaptive success, will provide interesting information in the perspective of the investigation for possible extraterrestrial life (Onofri et al., 2004). A great deal of interest has recently been focused on the extreme limits of life, and the above-mentioned microorganisms, being able to live in such extreme environments, have been considered the best experimental models.

The spreading of biological systems in the Antarctic ice-free desert is deeply restricted due to a combination of several environmental stresses, including very low temperatures, ranging in the McMurdo Dry Valleys from −20 to −50 °C in winter, with monthly mean temperatures always well below 0 °C, wide thermal fluctuations, frequent freeze/thaw cycles, extreme dryness, due to the lack of snow or ice cover and precipitation less than 100 mm WE (water equivalent) per year but practically absent in some zones, high salt concentrations due to high evaporation, low nutrient availability and, finally, high radiation, including high UV doses.

* Corresponding author.
E-mail address: onofri@unitus.it (S. Onofri).

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The result is an apparently absence of a biotic component in the most extreme areas; few lichens are however occasionally present, hidden in sheltered niches as crevices and fissures of rocks; among these are *Acarospora gwynnii* Dodge and Rudolph, *Aspicilia glacialis* Dodge, *Buellia grisea* Dodge and Baker, *Buellia pallida* Dodge and Baker, *Lecanora fuscobrunnea* Dodge and Baker, *Lecidea cancriformis* Dodge and Baker (Hale, 1987; Seppelt et al., 1995). Despite the practically sterile conditions of the outside, the endolithic life colonizing the (relatively) milder inside of rocks is even more widespread than one could expect. Its presence is often revealed by typical patched rock surfaces, due to a bio-weathering process, fundamental for spreading of the microbial components. Microorganisms living underneath the rock surfaces form well organised communities, the most widespread of which is the lichen-dominated cryptoendolithic community, discovered and described by Friedmann (Friedmann, 1982).

Among the fungal components of these communities, including lichenized and non-lichenized fungi, there is a group of black meristematic fungi, also known as microcolonial fungi (MCF), because of their slow growth rate (from 1 to 5 mm a month), colonizing the upper layer of the stratified rock colonization. They are also known as colonizers of other localities and substrates, such as rocks in hot climates or hypersaline environments, altogether characterized by the presence of environmental stress conditions (Sterflinger et al., 1999; Sterflinger, 2005; Gunde-Cimerman et al., 2005).

Up to date a quite large number of black meristematic fungi has been isolated from Antarctica, not exclusively from colonized rocks but also from lichen thalli collected in the ice-free areas of both Northern and Southern Victoria Land, including the McMurdo Dry Valleys. They share similar morphological characteristics, each representing a tool to survive in that environment. The colonies grow slowly and are cauliflower-like shaped which ensures the best surface/volume ratio (Wollenzien et al., 1995); cell walls are thick and heavily pigmented, to better resist desiccation and irradiation, a well visible extra-cellular polymeric layer envelopes the fungal structures, probably to protect them from desiccation and freeze and thaw cycles (Selbmann et al., 2002). Among them two new genera and four new species, possibly endemic, have been described up to date by means of morphological and molecular analyses: *Friedmanniomyces endolithicus* Onofri (Onofri et al., 1999), *Friedmanniomyces simplex* Selbmann et al., *Cryomyces minteri* Selbmann et al., *Cryomyces antarcticus* Selbmann et al. (Selbmann et al., 2005).

Information on the Antarctic microorganisms belonging to this enigmatic group of extremotolerant fungi is still scant, despite the potential interest of isolates from the Antarctic desert also as experimental models in spatial studies (Gorbushina, 2003; Onofri et al., 2007a,b). In this respect, molecular, phylogenetic, and physiological studies have been undertaken, in order to explain the origin, evolution and adaptation of these microorganisms in the most extreme terrestrial ice-free environment on Earth. In the perspective to expose these extremotolerant fungi to space conditions, ground-based experiments are in progress at the German Aerospace Centre (DLR, Cologne) to test their survival to different simulated space and Mars conditions.

2. Materials and methods

2.1. Freeze and thaw cycles

Two strains of *C. antarcticus* (CCFEE 515 and CCFEE 534) and one strain of *C. minteri* (CCFEE 5187), grown in Petri dishes (diameter 55 mm) on MEA medium (Malt Extract Agar: malt extract, powdered 30 g/l; peptone 5 g/l; agar 15 g/l) for 1 month at 15 °C (optimal temperature), were maintained at 15 ± 1 °C, 0 ± 2 °C, -5 ± 2 °C, -10 ± 2 °C, -20 ± 1 °C, and -25 ± 2 °C. Cultures were transferred monthly at ambient temperature and thawed for 12 h, the mycelium was utilized to seed a new plate with a punctiform inoculum using a sterile glass needle; cultures were frozen again and newly inoculated plates were then incubated at 15 °C. The experiment was performed in triplicate and cultures incubated at 15 °C were utilized as control. Vitality was expressed as diameter (mm) of the colony after 60 days of incubation.

2.2. UV resistance

Actively growing colonies of *C. antarcticus* (CCFEE 515 and CCFEE 534) and *C. minteri* (CCFEE 5187), respectively, grown on MEA at 15 °C, were collected and used to prepare fungal cell suspensions in physiological solution (NaCl 0.9%). 0.1 ml of suspension containing about 100 cells was uniformly spread on Petri dishes (diameter 90 mm) supplemented with MEA. Five plates of each strain were used as control, and others were UV-B irradiated for times ranging from 15 to 120 min (doses from 2.7 to 21.6 kJ/m^2), rate 15 min, and finally for 4, 6, and 10 h (doses of 43.2, 64.8, and 108 kJ/m^2). Plates were irradiated by using three fluorescent lamps (Sankyo Denki, Co., JP <http://www.sankyo-denki.co.jp/e2_09.html>), emitting ultraviolet rays between 280 and 360 nm (at peak 306 nm), placed on a metallic support so to assure a homogeneous exposition. Total irradiance, measured by a broadband UV-B radiometer SKU 430 (SKYE Instruments Ltd., UK), was 3.0 W/m^2, over five to eight times the Antarctic terrestrial UV-B irradiance (Skerratt et al., 1998; Hughes et al., 2003). Immediately after irradiation, dishes were incubated at 15 °C up to the appearance of micro-colonies and counting was performed under the stereomicroscope. The experiment was performed in five replicas and the percentage of viable propagules expressed as means of Colony Forming Units (CFU).

2.3. Osmotic stress

Cryomyces antarcticus (strains CCFEE 515 and CCFEE 534) and *C. minteri* (strain CCFEE 5187) were tested also

for resistance to osmotic stress, by culturing them on MEA 2% medium (MEA with 20 g/l of malt extract, powdered) supplemented with progressively increasing NaCl additions (rate 2–3.5%) up to 27% (w/v). Inoculated plates were incubated at 15 °C and the resistance was evaluated by measuring colony diameter (mm) after 10 weeks. The test was performed with five replicas.

2.4. Scanning and transmission electron microscopy

Controls, UV-irradiated and NaCl-treated colonies were prepared for ultrastructural analysis by scanning (SEM) and transmission (TEM) electron microscopy.

Samples for SEM were fixed with 5% glutaraldehyde/cacodylate sucrose buffer 0.1 M (pH 7.2) for 12 h at 4 °C, three times washed in the same buffer for 1 h each at 4 °C, post fixed in 1% OsO_4 + 0.15% ruthenium red in 0.1 M cacodylate buffer (pH 7.2) for 3 h at 4 °C, washed in distilled water (2 changes for 30 min each at 4 °C), block stained with 1% uranyl acetate in distilled water for 1 h at 4 °C, washed in distilled water (2 changes for 30 min each at 4 °C), dehydrated in ethanol series: 30%, 50%, 70% (15 min for each changes at room temperature) and 100% (1 h at room temperature) EtOH, critical point dried, coated with gold and examined with a Jeol 840 Scanning Electron Microscope.

Samples for TEM analysis were fixed and dehydrated as above, then infiltrated in ethanol 100%: LR White series with accelerator, in rotator, at 4 °C (2:1 for 3 h; 1:1 for 3 h, 1:2 overnight), and embedded in pure resin for 1 day and overnight; finally, they were included in pure resin in gelatinous capsule for 2 days at 48–52 °C.

3. Results

3.1. Freeze and thaw cycles

Results of 7 cycles are reported in Table 1. After seven repeated cycles of freezing and thawing all strains seemed to maintain the same growth ability as that of the controls; in fact, colonies of comparable diameters were obtained after all repeated cycles and at all different temperatures tested, after the same incubation time.

3.2. UV resistance

All the strains tested showed to be highly resistant to UV-B irradiation (Fig. 1), with a lethal effect on almost 50% of propagules after 90 min (16.2 kJ/m^2) of irradiation for *C. antarcticus* CCFEE 534, between 105 and 120 min (18.9 and 21.6 kJ/m^2) for *C. minteri* CCFEE 5187, and between 120 and 240 min (21.6 and 43.2 kJ/m^2) for *C. antarcticus* CCFEE 515. Irradiation times up to 10 h (108 kJ/m^2) were progressively more damaging, up to a total germination inhibition, first occurring in *C. minteri* CCFEE 5187.

No morphological modifications at both colony and cellular levels of all the strains were detected after UV-irradiation when observed at light and scanning electron microscopes, while some ultrastructural damages seemed

Table 1
Growth of *Cryomyces antarcticus* CCFEE 515 and CCFEE 534 and *Cryomyces minteri* CCFEE 5187 (colony diameter, in mm, recorded after 60 days of incubation) after seven repeated freeze and thaw cycles at six different temperatures (15 °C as control)

Cycle	15 ± 1 °C	0 ± 2 °C	−5 ± 2 °C	−10 ± 2 °C	−20 ± 1 °C	−25 ± 2 °C
Cryomyces antarcticus CCFEE 515						
1°	9.67 ± 0.76	9.80 ± 2.66	9.50 ± 1.26	9.67 ± 1.25	11.17 ± 1.83	10.50 ± 0.84
2°	9.33 ± 0.58	8.67 ± 0.58	10.00 ± 1.00	9.33 ± 0.58	10.67 ± 1.15	11.33 ± 2.52
3°	10.00 ± 0.00	8.67 ± 0.58	10.00 ± 1.00	8.33 ± 0.58	10.00 ± 1.53	10.33 ± 0.58
4°	12.00 ± 0.00	12.33 ± 1.53	14.00 ± 1.73	12.00 ± 1.00	10.67 ± 0.58	11.67 ± 1.53
5°	13.33 ± 1.04	13.00 ± 2.78	13.83 ± 1.04	12.83 ± 0.58	12.33 ± 1.00	13.17 ± 0.76
6°	9.33 ± 0.58	7.50 ± 0.50	9.33 ± 0.58	10.67 ± 1.53	9.67 ± 0.84	10.33 ± 0.58
7°	10.00 ± 1.00	10.33 ± 0.58	8.67 ± 1.15	7.67 ± 0.58	10.00 ± 0.84	9.67 ± 0.76
Cryomyces antarcticus CCFEE 534						
1°	9.00 ± 0.00	12.83 ± 1.17	9.17 ± 2.25	13.00 ± 2.47	12.17 ± 2.17	13.00 ± 1.10
2°	12.13 ± 0.58	11.67 ± 0.58	10.33 ± 1.15	12.33 ± 1.15	13.00 ± 0.00	12.00 ± 0.00
3°	9.67 ± 0.58	9.33 ± 0.58	9.33 ± 0.58	11.00 ± 2.65	10.33 ± 0.58	10.33 ± 0.58
4°	13.33 ± 1.15	13.33 ± 1.15	12.83 ± 0.76	13.33 ± 1.15	13.00 ± 1.00	13.33 ± 0.58
5°	14.17 ± 0.29	13.17 ± 2.75	14.67 ± 0.76	14.83 ± 1.15	14.00 ± 0.00	14.83 ± 1.61
6°	10.17 ± 1.61	10.50 ± 1.50	9.50 ± 0.50	10.50 ± 0.87	10.83 ± 0.76	10.83 ± 0.58
7°	9.00 ± 1.00	11.00 ± 1.00	10.00 ± 1.80	9.83 ± 2.08	7.67 ± 0.58	10.17 ± 1.15
Cryomyces minteri CCFEE 5187						
1°	11.33 ± 0.58	13.17 ± 0.98	13.67 ± 1.26	13.17 ± 1.96	12.17 ± 2.40	11.67 ± 0.52
2°	10.67 ± 1.53	13.67 ± 1.53	13.67 ± 0.58	14.67 ± 0.58	12.33 ± 1.53	13.00 ± 1.00
3°	12.00 ± 0.00	12.00 ± 1.00	11.00 ± 2.00	12.33 ± 0.58	11.33 ± 1.76	9.00 ± 2.00
4°	15.00 ± 0.00	13.00 ± 1.00	12.67 ± 1.15	15.00 ± 0.00	15.33 ± 0.58	13.33 ± 2.52
5°	16.67 ± 1.26	16.83 ± 0.58	15.67 ± 0.58	16.00 ± 0.50	11.83 ± 0.29	12.50 ± 0.50
6°	11.17 ± 0.58	10.67 ± 0.76	10.00 ± 0.00	14.83 ± 0.76	10.83 ± 0.29	12.13 ± 0.58
7°	10.83 ± 0.29	9.33 ± 0.58	7.17 ± 2.02	10.33 ± 1.04	10.00 ± 0.00	10.50 ± 0.87

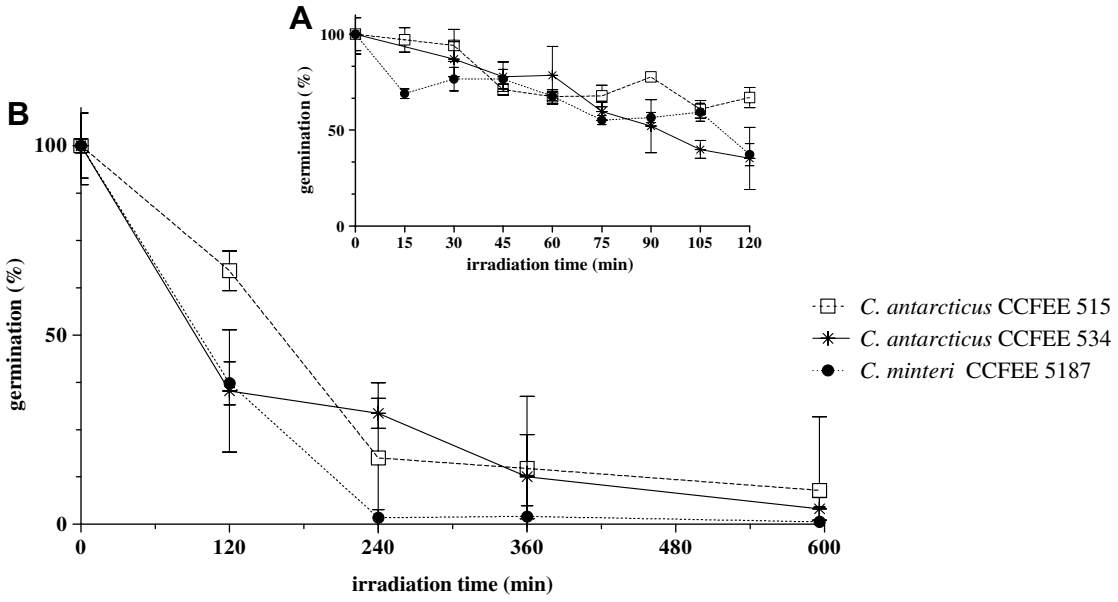

Fig. 1. Germination of *C. antarcticus* CCFEE 515 and CCFEE 534 and *C. minteri* CCFEE 5187 propagules, at increasing UV-B irradiation times; (A) from 15 to 120 min, rate 15 min; (B) for 2, 4, 6, and 10 h.

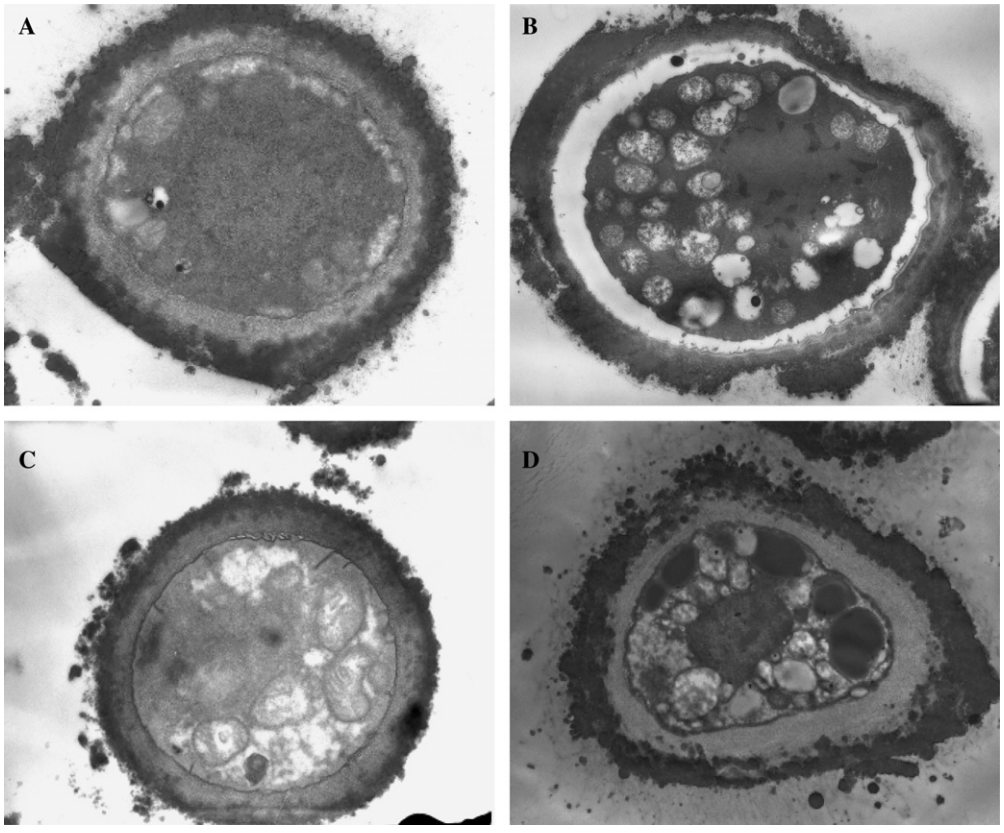

Fig. 2. TEM micrographs of fungal cells. (A) and (B): *Cryomyces minteri* CCFEE 5187 before (A, 15,000×) and after (B, 8000×) UV-B irradiation for 120 min; (C) and (D): *Cryomyces antarcticus* CCFEE 534 grown on MEA 2% without (C, 15,000×) and with (D, 10,000×) NaCl (24%).

to be induced. In fact, compared to the control (Fig. 2A), TEM images of fungal cells after UV-B irradiation (Fig. 2B) showed detached protoplasts from the thick multi-layered cell wall and the presence of large intracellular electron-dense globules in the cytoplasm.

3.3. Osmotic stress

Growth, determined as diameter of colonies after 10 weeks of incubation, was inhibited by NaCl (Fig. 3); even if growth markedly decreased since from 7% NaCl supply,

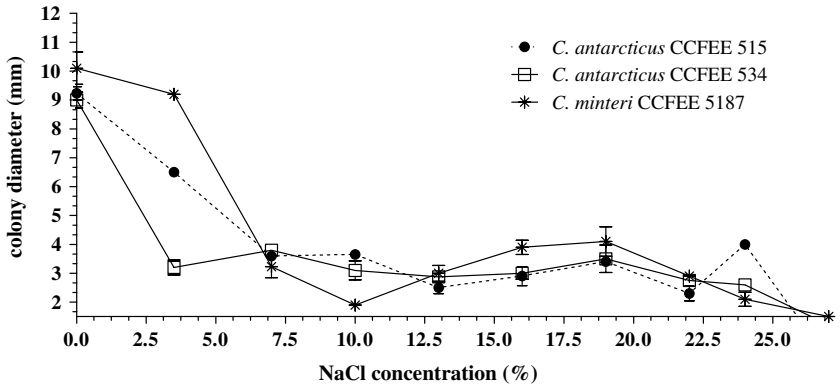

Fig. 3. Growth of *C. antarcticus* CCFEE 534 and CCFEE 515 and *C. minteri* CCFEE 5187 on MEA 2%, after 10 weeks of incubation at 15 °C, at increasing NaCl concentrations.

all the strains tested were able to grow up to 24% NaCl amended medium with a growth reduction up to about five times compared to the control. No growth was observed at the higher concentration tested (27%). Therefore, we can conclude that all the strains tested, even at different extent, showed a good NaCl tolerance.

Marked morphological differences between control and NaCl treated samples were detected by means of TEM observation (Fig. 2C and D). Compared to the control (Fig. 2C), treated samples showed high production of melanin covering the thick multi-layered cell wall (Fig. 2D). Moreover, osmotic stress seemed to induce the occurrence of intracellular globules, possibly related to the production of osmoregulators as trehalose and glycerol.

4. Discussion

Exobiology has focused attention for long time on prokaryote models (Horneck et al., 1994; Nicholson et al., 2000) because of their less complex organization, their earlier emergence, and putative higher resistance to stresses with respect to eukaryotes. However, it is now clear that many eukaryotes are able to live or even thrive in different extreme environments (Amaral Zettler et al., 2002) and, among them, black meristematic fungi are one of the most interesting examples (Gunde-Cimerman et al., 2005; Sterflinger, 2005). The Antarctic cryptoendolithic black fungi colonizing environments best resembling those on Mars have been already suggested as the best eukaryotic model for exobiological speculations, although their actual limits of surviving and their tolerance to different stresses were still unknown (Onofri et al., 2004).

Molecular and physiological studies have been recently carried out on these fungi in order to highlight, on the basis of their phylogenetic relationships, the evolutionary processes which, under extreme conditions, lead to the arising of these highly extremotolerant organisms (Selbmann et al., 2005) and to highlight how much they can outstretch their ability of surviving.

Up to date 26 strains of Antarctic cryptoendolithic black fungi from both Northern and Southern Victoria Land have been studied phylogenetically (Selbmann et al., 2005). The analyses suggested the presence of diverse lineages of uncertain or isolated position, which belong to possibly different undescribed orders in the subclass *Dothideomycetidae*. Only one strain was included in the order *Hysteriales* showing phylogenetic affinities with other rock fungi in the genus *Coniosporium* Link isolated from monuments in the Mediterranean basin. Among the new entities described, the genus *Cryomyces* Selbmann et al., with the species *C. antarcticus* Selbmann et al. and *C. minteri* Selbmann et al., remained well isolated in the phylogenetic tree, without any obvious close relatives in other fungal groups. The recently described genus *Friedmanniomyces* Onofri (Onofri et al., 1999) was confirmed as a monophyletic taxon by molecular results, including two different cryptoendolithic species, *F. endolithicus* Onofri and *F. simplex* Selbmann et al.

Experiments here reported point out a high resistance of these fungi to all the conditions tested. All strains have been previously referred as psychrophiles, most of them having an optimum growth temperature in the range of 10–20 °C, and in many cases growth was still well detectable at 0 °C (Selbmann et al., 2005), even if difficulties do exist in determining the lower limits of temperatures for growth. Here we demonstrate resistance to seven repeated freezing and thawing cycles, after which their ability of germinating and forming colonies remains practically unaffected (Table 1). The ability to tolerate extremely low as well as rapid and frequent changes of temperatures is most important for a microorganism colonizing an environment characterized by very strong thermal fluctuations such as the Antarctic desert where, during the Austral Summer, at the rock surface the temperature can cross the freezing point even 14 times within only 42 min (Nienow and Friedmann, 1993). The interest resulting from proving a high resistance to repeated freeze/thaw cycles also lies in the frequent and high thermal fluctuations, within the range −20 °C/20 °C, to which these samples will be exposed during the space flight.

Although the mechanisms for this resistance have not yet been investigated, we can hypothesize a possible protec-

tive role of extra-cellular polymeric substances (EPSs) surrounding the hyphae of the black meristematic fungi. A thick crust of EPSs matrix, enveloping a colony of *C. minteri* CCFEE 5187, is well visible in Fig. 4. The protective role of EPSs to repeated freeze/thaw cycles has been already reported for an Antarctic strain of *Phoma herbarum* Westend, a filamentous fungus isolated from soil; in fact, when EPSs were present, the mycelium maintained a high growth capacity after repeated freeze/thaw cycles, while in the absence of EPSs it was markedly reduced (Selbmann et al., 2002). High tolerance of the cryptoendolithic fungi might include additional mechanisms, such as thick cell wall and, probably, production of compatible endocellular cryoprotectants such as glycerol (Brown, 1978), trehalose (Thevelein, 1984; Weinstein et al., 2000) or mannitol (Feofilova et al., 1994). A mucilaginous layer, possibly encrusted with melanin, covers the outer layer of the cell wall, as shown in both control and treated samples (Fig. 2). Furthermore, the involvement of cold shock proteins cannot be excluded (De Croos and Bidochka, 2001; Zhang et al., 2006).

The springtime reduction of stratospheric ozone levels over Antarctica has increased studies on abilities of both aquatic and terrestrial biota to tolerate the increased UV-B radiation level. Results of germination tests reported in Fig. 1 are explained as further adaptation of the strains tested to their natural habitat. The UV radiation effects on spore suspensions of a European strain of *Arthrobotrys oligospora* Fresen. and a strain of the Antarctic species *Arthrobotrys ferox* Onofri and Tosi were previously investigated (Zucconi et al., 2002); the spores of *A. ferox* appeared more tolerant compared with those of the European strain, as they showed higher germination rates and larger amounts of UV-protecting substances (Arcangeli et al., 1997; Arcangeli and Cannistraro, 2000). Here, the effects of enhanced UV-B irradiation on propagules' germination were examined on Antarctic strains of cryptoendolithic meristematic black fungi that, as a rule, colonize translucent sandstone and are exposed at very high solar radiation for several months during the Austral summer. Their tolerance to enhanced irradiation levels appears to be well higher than the one showed by *A. ferox*; in fact, the 50% of surviving was reached after an exposure time ranging from 90 to 120 min, while spores of *A. ferox*, at lower UV doses (2.5 W/m^2 for time ranging from 15 to 120 min), showed a higher death rate, with about 50% of damaged spores after 30 min of irradiation, and about 75% after 2 h of irradiation. This higher resistance of the strains tested could be the consequence of marked morphological and physiological differences characterizing them, such as highly pigmented thick walls and presence of EPSs (Butler and Day, 1998; Ruisi et al., 2007). These results again confirm *Cryomyces* strains as good candidates to face up to the space conditions. The great germinative capacity of UV-C-irradiated ascospores ($\lambda = 254$ nm) of the lichen species *Fulgensia bracteata* and *Xanthoria elegans* even after a dose of 2.88 kJ/m^2 makes the lichen mycobionts good candidates for space experiments as well (de Vera et al., 2003).

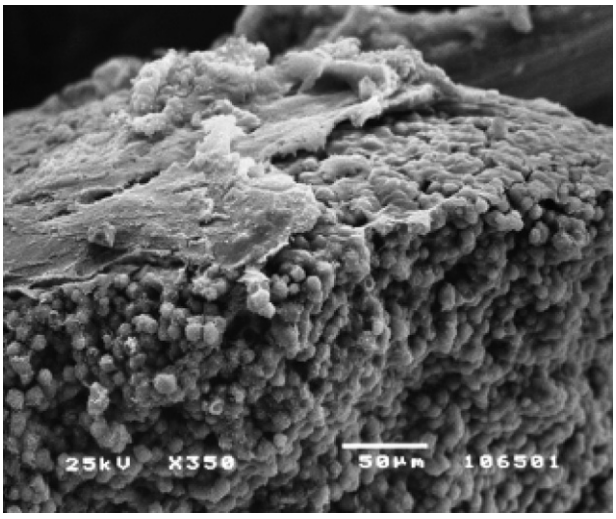

Fig. 4. SEM micrograph showing extra-cellular polymeric substances (EPSs) on the surface of a colony fragment of *Cryomyces minteri* CCFEE 5187.

Finally all the strains tested showed high tolerance against NaCl, being still able to grow also at 24% of salt concentration (Fig. 3); nevertheless their growth ability is not comparable with a real halophilic fungus such as a strain of *Hortaea wernekii* (Horta) Nishim. and Miyaji, isolated in the sea spray areas of Delos, which has an NaCl optimum at 7% and grows in the range 0–27% (Sterflinger, 1998). The Antarctic desert is one of the driest environments on Earth; the very low amount of precipitations, represented only by snow, mostly sublime or is blown away by the strong catabatic winds. As a consequence of the high evaporation, salts accumulate on the rock surfaces and represent a further stress for microbial life (Ruisi et al., 2007); even if we could surmise a certain resistance to osmotic stresses of the Antarctic rock inhabitant fungi, data obtained are well beyond any expectations. The increase degree of cell wall melanization is in agreement with previous observations; it was observed in *Trimmatostroma salinum* Zalar, de Hoog and Gunde-Cim. grown on saline media and this response was proposed as one of the mechanisms enabling growth of halophilic and halotolerant black yeasts at hypersaline conditions (Sterflinger, 1998; Kogej et al., 2006). Melanin is known to play an important role in fungal protection against different types of stress preserving cells, for instance from both UV and desiccation damages (Butler and Day, 1998; Gorbushina, 2003; Gorbushina et al., 2003). The accumulation of large intracellular globules (Fig. 2D) under NaCl stress conditions is possibly related to intracellular production of compatible solutes as trehalose and glycerol, as already observed (Sterflinger, 1998).

On the basis of these results concerning the stress tolerance of Antarctic rock black fungi further experiments to test their ability to withstand space conditions are in progress.

5. LIFE – LIchens and Fungi Experiments

The opportunity to expose biological samples, including Antarctic black meristematic fungi, to space conditions outside of the International Space Station for at least six months, has imposed the necessity to assess their resistance to some space conditions; they include space vacuum, solar UV, and cosmic radiation. Even if not complete information are available neither on the number and type of components of the cryptoendolithic communities nor on the exact ecological role of each component of the community itself, as well as the relationships existing among the components each others and among epi- and endolithic colonizers, the already reported increased ecological valence of the entire community respect to its single components (Friedmann and Sun, 2005; Sun and Friedmann, 2005), has suggested to assess the long-term resistance to space conditions of either the entire Antarctic community or single fungal components.

LIchens and Fungi Experiments (LIFE programme), to be accommodated in the EXPOSE facility of ESA, which is part of the European Technology Facility (EuTEF), has the aim to assess the long-term resistance of dried cryptoendolithic Antarctic communities, Antarctic strains of *C. antarcticus* and *C. minteri*, and samples of the lichens *X. elegans* and *Rhizocarpon geographicum* to space and simulated Mars conditions, including space vacuum, solar UV, and cosmic radiation.

Ground-based experiments are in progress at the German Aerospace Centre (DLR, Cologne, Germany); two kinds of simulation tests (Experiment Verification Tests, EVTs) are programmed, during which samples will be exposed to the following conditions: vacuum (10^{-5} Pa for 1 h and 1 week), repeated freeze and thaw cycles (from −20 °C/20 °C for 2 weeks), UV-C (245 nm at 10, 100, and 1000 Jm^{-2}) and total UV (200–400 nm, at 1.5, 1.5×10^3, and 1.5×10^5 kJm^{-2}) in the EVT-E1 (first experiment), and to vacuum plus total UV, and Mars atmosphere plus total UV in the EVT-E2 (second experiment).

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References

Amaral Zettler, L.A., Gómez, F., Zettler, E., et al. Microbiology: eukaryotic diversity in Spain's River of Fire. Nature 417, 137, 2002.

Arcangeli, C., Cannistraro, S. In situ Raman microspectroscopic identification and localization of carotenoids: approach to monitoring of UV-B irradiation stress on Antarctic fungus. Biospectr. 57, 179–186, 2000.

Arcangeli, C., Zucconi, L., Onofri, S., et al. Fluorescence study on whole Antarctic fungal spores under enhanced UV irradiation. J. Photochem. Photobiol. B Biol. 39, 258–264, 1997.

Brown, A.D. Compatible solutes and extracellular water stress in eukaryotic microorganisms. Adv. Microb. Physiol. 17, 181–242, 1978.

Butler, M.J., Day, A.W. Fungal melanins. Can. J. Microbiol. 44, 1115–1136, 1998.

De Croos, J.N.A., Bidochka, M.J. Cold-induced proteins in cold-active isolates of the insect-pathogenic fungus *Metarhizium anisopliae*. Mycol. Res. 105, 868–873, 2001.

de Vera, J.-P., Hornack, G., Rettberg, P., et al. The potential of the lichen symbiosis to cope with extreme conditions of outer space – I. Influence of UV radiation and space vacuum on the vitality of lichen symbiosis and germination capacity. Intern. J. Astrobiol. 1 (4), 285–293, 2003.

Feofilova, E.P., Tereshina, V.M., Gorova, I.B. Changes in carbohydrate composition of fungi during adaptation to thermostress. Microbiology 63, 442–445, 1994.

Finster, K., Hansen, A.A., Liengaard, L., et al. Mars simulation experiments with complex microbial soil communities, in: Cockell, C.S. (Ed.), Microorganisms and Martian Environment, ESA Special Publication, Chapter 5, 2007.

Friedmann, E.I. Endolithic microorganisms in the Antarctic Cold Desert. Science 215, 1045–1053, 1982.

Friedmann, E.I., Sun, H.J. Communities adjusting their temperature optima by shifting producer to consumer ratio, shown in lichens as models: I. Hypothesis. Microb. Ecol. 49 (4), 523–527, 2005.

Gorbushina, A. Methodologies and techniques for detecting extraterrestrial (microbial) life. Microcolonial fungi: survival potential of terrestrial vegetative structures. Astrobiol. 3, 543–554, 2003.

Gorbushina, A., Whitehead, K., Dornieden, T., et al. Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. Can. J. Bot. 81, 131–138, 2003.

Gunde-Cimerman, N., Oren, A., Plemenitaš, A. Adaptation to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya. Springer, The Netherlands, 2005.

Hale, M.E. Epilithic lichens in the Beacon Sandstone formation, Victoria Land, Antarctica. Lichenol. 19, 269–287, 1987.

Horneck, G. The microbial world and the case of Mars. Planet. Space Sci. 48, 1053–1063, 2000.

Horneck, G., Bucker, H., Reitz, G. Long-term survival of bacterial spores in space. Adv. Space Res. 14, 41–45, 1994.

Hughes, K.A., Lawley, B., Newsham, K.K. Solar UV-B radiation inhibits the growth of Antarctic terrestrial fungi. Appl. Environ. Microbiol. 69, 1488–1491, 2003.

Kogej, T., Gorbushina, A., Gunde-Cimerman, N. Hypersaline conditions induce changes in cell wall melanization and colony structure in a halophilic and a xerophilic black yeast species of the genus *Trimmatostroma*. Mycol. Res. 110, 713–724, 2006.

Nicholson, W.L., Munacata, N., Horneck, G., et al. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. 64, 548–672, 2000.

Nienow, J.A., Friedmann, E.I. Terrestrial lithophytic (rock) communities, in: Friedmann, E.I. (Ed.), Antarctic Microbiology. Wiley-Liss, New York, pp. 343–412, 1993.

Onofri, S., Pagano, S., Zucconi, L., et al. *Friedmanniomyces endolithicus* (Fungi, Hyphomycetes), anam.-gen. and sp.nov., from continental Antarctica. Nova Hedwigia 68, 175–181, 1999.

Onofri, S., Selbmann, L., Zucconi, L., et al. Antarctic microfungi as models for exobiology. Planet. Space Sci. 52, 229–237, 2004.

Onofri, S., Zucconi, L., Selbmann, L., et al. Fungi from Antarctic desert rocks as analogues for Martian life, in: Cockell C.S. (Ed.), Microorganisms and Martian Environment, ESA Special Publication, Chapter 6, 2007a.

Onofri, S., Zucconi, L., Selbmann, L., et al. Fungal association at the cold edge of life, in: Seckbach, J. (Ed.), Algae and Cyanobacteria in Extreme Environments: Cellular Origin, Life in Extreme Habitats and Astrobiology. Springer-Verlag, Berlin, 2007b.

Ruisi, S., Barreca, D., Selbmann, L., et al. Fungi in Antarctica. Rev. Environ. Sci. Biotechnol. 6, 127–141, 2007.

Selbmann, L., Onofri, S., Fenice, M., et al. Production and structural characterization of the exopolysaccharide of the Antarctic fungus *Phoma herbarum* CCFEE 5080. Res. Microbiol. 153, 585–592, 2002.

Selbmann, L., de Hoog, G.S., Mazzaglia, A., et al. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. Stud. Mycol. 51, 1–32, 2005.

Seppelt, R.D., Green, T.G.A., Schroeter, B. Lichens and mosses from the Kar Plateau, Southern Victoria Land, Antarctica. N. Z. J. Bot. 33, 203–220, 1995.

Skerratt, J.H., Davidson, A.D., Nichols, P.D., et al. Effect of UV-B on lipid content of three Antarctic marine phytoplankton. Phytochemistry 49, 999–1007, 1998.

Sterflinger, K. Temperature and NaCl-tolerance of rock inhabiting meristematic fungi. Antonie van Leeuwenhoek 74, 271–281, 1998.

Sterflinger, K. Black yeasts and meristematic fungi: Ecology, diversity and identification, in: Rosa, C., Gabor, P. (Eds.), Yeast Handbook: Biodiversity and Ecophysiology of Yeasts. Springer, New York, pp. 505–518, 2005.

Sterflinger, K., de Hoog, G.S., Haase, G. Phylogeny and ecology of meristematic ascomycetes. Stud. Mycol. 43, 5–22, 1999.

Sun, H.J., Friedmann, E.I. Communities adjusting their temperature optima by shifting producer to consumer ratio, shown in lichens as models: II. Experimental verification. Microb. Ecol. 49 (4), 528–535, 2005.

Thevelein, J.M. Regulation of trehalose mobilization in fungi. Microbiol. Rev. 48, 42–59, 1984.

Weinstein, R.N., Montiel, P.O., Johnstone, K. Influence of growth temperature on lipid and soluble carbohydrate synthesis by fungi isolated from fellfield soil in the maritime Antarctic. Mycologia 92, 222–229, 2000.

Wollenzien, U., de Hoog, G.S., Krumbein, W.E., et al. On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. Sci. Total Environ. 167, 287–294, 1995.

Zhang, S., Schisler, D., Jackson, M., et al. Cold shock during liquid production increases storage shelf-life of *Cryptococcus nodaensis* OH 182.9 after air-drying. Biocontrol Sci. Technol. 16, 281–293, 2006.

Zucconi, L., Ripa, C., Selbmann, L., et al. Effects of UV on the spores of the fungal species *Arthrobotrys oligospora* and *A. ferox*. Polar Biol. 25, 500–505, 2002.