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RESEARCH ARTICLE

Rock black fungi: excellence in the extremes, from the Antarctic to space

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Abstract This work focuses on rock-inhabiting fungi (RIF) of Antarctic rocky deserts, considered the closest to a possible Martian habitat, as the best example of adaptation to the extremes. The study of RIF ecophysiology, resistance and adaptation provides tools that shed light on the evolution of extremophily. These studies also help define the actual limits for life and provide insight for investigating its existence beyond our planet. The scientific results obtained from over 20 years of research on the biodiversity, phylogeny and evolution toward extremotolerance reviewed here demonstrate how these fascinating organisms can withstand conditions well beyond those in their natural environment. A final focus is given on results and perspectives arising from a recent proteomic approach, and from astrobiological experiments and their significance for future space exploration. These studies demonstrate that Antarctic RIF offer an excellent opportunity to investigate many basic, but also applicative areas of research on extremophily.

Keywords Adaptation · Astrobiology · Antarctica · Fungal collection · Extremophily · Extremotolerance · Rock black fungi · Stress

Introduction

Rock represents the earliest terrestrial niche for life since the Earth was first inhabited by microbes. At present, naked rock surfaces are easily colonized by microbial biofilms, which comprise algal, fungal, bacterial and cyanobacterial associations and are normally succeeded by macroscopic vegetation (Gorbushina 2007). Yet, under permanent stressing conditions, microbes remain the only settlers of this peculiar niche.

The Antarctic Continent provides excellent material for studying this ancient microbial lifestyle, because the history of the continent has been opposite to that of all the other land masses. At ~50 MYA, the continent was positioned at higher latitudes and its temperatures were milder. It was characterized by fertile soils and colonized by forested vegetation (Askin 1992; Francis and Poole 2002). Continental drift shifted the Antarctic Continent toward the South Pole, but this was not the only reason that it was eventually placed into the deep freeze. The widening of the sea between Antarctica and Australia and the split between South America and the Antarctic Peninsula were crucial for the establishment of the cold Circumpolar Current that circulates clockwise around the continent, preventing warm water from reaching the coast. Once ice on Antarctica began to spread, the process reinforced itself through positive feedback mechanisms. The ice increased sunlight reflection to space (higher albedo) and led to further cooling of the continent; as the ice sheets grew further, the white surface became wider and, consequently, cooler. These events caused the extinction of higher plants and animals. Soils were eroded by glacier movements and winds, leading to a regression to pristine conditions. For these reasons, at present, life in the Antarctic, more than any other continent, is dominated by microbes and rocks that are the

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main substratum for colonization (Nienow and Friedmann 1993).

Among other factors, rock colonization is strictly dependent on climatic conditions. Epi- and endolithic colonizations are both present in coastal sites, where epilithic mosses and lichens dominate. Moving toward the interior of the continent and at higher altitudes, conditions become more severe, and the biodiversity progressively decreases; epilithic growth becomes rare and represented by lichens only until almost complete disappearance, while endolithic colonization becomes predominant (Zucconi et al. 2014). The endolithic niche provides lichens and associated microbes such as bacteria and fungi (Selbmann et al. 2010, 2013a, b) more buffered conditions compared to the rock surface, and colonizers find a further chance of survival; endolithic lifestyle (Golubic et al. 1981) should be regarded as a border adaptation of life before extinction (Friedmann and Ocampo-Friedmann 1976; Nienow and Friedmann 1993). Both eukaryotic and prokaryotic endolithic communities have been reported (Friedmann et al. 1988), but the most widely distributed in the harshest ice-free areas of continental Antarctica is the cryptoendolithic lichen-dominated community (Fig. 1), described for the first time in the McMurdo Dry Valleys (Friedmann 1982). Here, conditions are extreme in a number of parameters; annual mean temperatures remain below the freezing point, ranging from −14.8 °C to −30.0 °C, depending on the site and period of measurement (Doran et al. 2002); aridity is also extreme: the potential evaporation greatly exceeds the annual snowfall which is less than 100 mm water per year in many sites (Nienow and Friedmann 1993), contributing to the ice-free nature of the area (Fountain et al. 1998, 2010).

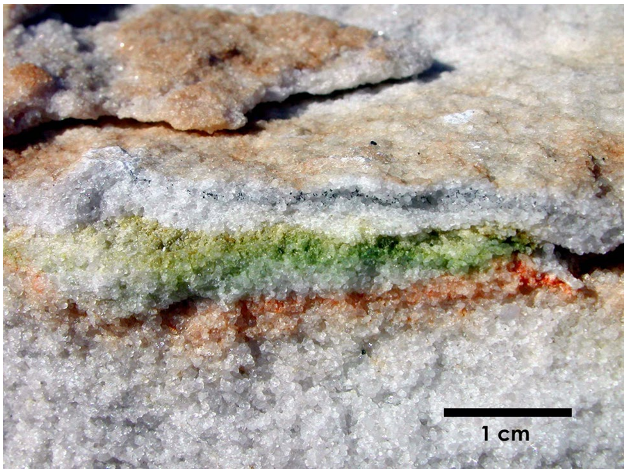


Fig. 1 Cryptoendolithic lichen-dominated community colonizing sandstone in the McMurdo Dry Valleys, Antarctica (bar 10 mm) (Onofri et al. 2006)

Because of the extreme cold and arid conditions, these valleys are considered as the best terrestrial analog of the conditions existing on Mars, for both climate and the physical processes shaping the surfaces (Andersen et al. 1992). The conditions of temperature and aridity of the McMurdo Dry Valleys, coupled with high solar irradiation and strong thermal fluctuations, prevent microbial settlement on rock surfaces that are virtually abiotic.

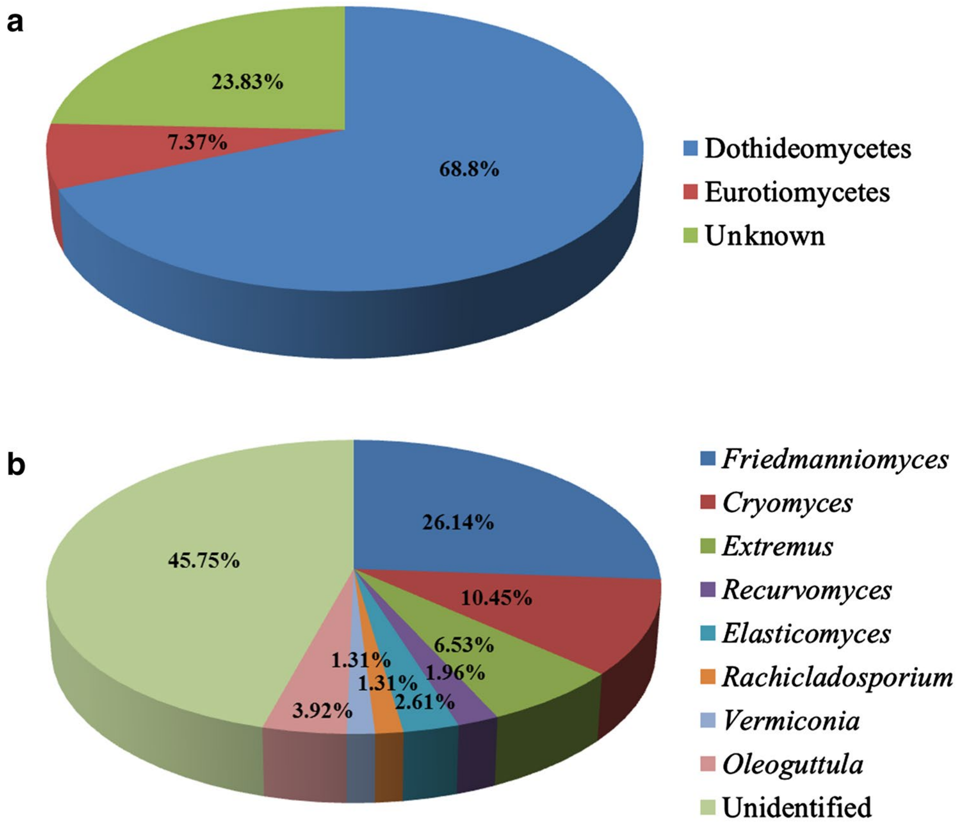
Black meristematic fungi are a recurrent presence in the Antarctic cryptoendolithic communities (Selbmann et al. 2005, 2008; Egidi et al. 2014). These organisms are polyphyletic, but constitute an ecological group of fungi specialized in extremotolerance and living in different stressing environments such as hot and cold deserts, saltpans, acidic sites and exposed rocks (Gunde-Cimerman et al. 2000; Baker et al. 2004; Ruibal et al. 2005; Sert et al. 2007a, b, c; Selbmann et al. 2005, 2008, 2013a; Isola et al. 2013). They are also known as black yeasts or, when living on rocks, microcolonial fungi (MCF) or rock inhabitant fungi (RIF) (Sterflinger 1998; Ruibal et al. 2009). In the cryptoendolithic communities of the Antarctic, they live in association with similarly stress-resistant organisms such as lichens (Onofri et al. 2007a; Harutyunyan et al. 2008; Selbmann et al. 2013b) and cyanobacteria (de los Ríos et al. 2014). More than 150 isolates are preserved in the Culture Collection of Fungi from Extreme Environments (CCFEE), Mycological section of the Italian National Museum for the Antarctic.

The strict geographical and genetic isolation over a timescale of evolutionary significance, in one of the harshest terrestrial environments on the planet, promoted adaptive radiation and speciation. A number of endemic taxa of black meristematic fungi, highly specialized to the extreme and with unique genotypes, have been described and continue to be found; these organisms are of utmost scientific value for understanding the possible limits for life as well as evolution and adaptation to extreme conditions. Here, we review the scientific work conducted over the past 20 years to elucidate the biodiversity, phylogeny and exceptional tendency to extremotolerance of these fascinating organisms, with a final focus on experiments for space exploration for which they represent optimal models. Due to their exceptional stress resistance and ability to grow inside rock, Antarctic rock-inhabiting fungi (RIF) were selected for astrobiological studies that gave new insights to the lithopanspermia theory and planetary protection (Onofri et al. 2009, 2012).

Systematics: data from the CCFEE

The abilities to shift to meristematic development, or adapt this growth form as a stable character as in RIF, and produce high amounts of melanin, are not widespread features

Fig. 2 Frequency (%) of dothideomycetous, eurotiomycetes and unknown Antarctic RIF (**a**). Frequency of RIF genera in the CCFEE (**b**)



in the fungal kingdom. Morphological identification of RIF is largely affected by their very poor differentiation and even polymorphism of synanamorph in some cases (Sterflinger et al. 1999). Yet, molecular phylogeny reveals that RIF are distributed into two classes, Dothideomycetes, mainly in the order Capnodiales, and Eurotiomycetes, particularly within the order Chaetothyriales (Gueidan et al. 2008; Ruibal et al. 2009). Almost 70 % of the isolates preserved in the CCFEE, the fungal collection hosting the largest number of Antarctic RIF, belong to the class Dothideomycetes. Only 7.37 % of the remaining strains belong to the Eurotiomycetes (order Chaetothyriales) and 23.83 % are still undetermined (Fig. 2a).

The large predominance of dothideomycetous cold-adapted Antarctic RIF is most probably related to the early evolutionary history of the group. The class Dothideomycetes, in fact, has evolved in the Silurian–Devonian period, about 430 million years ago when temperatures were much cooler than today. In contrast, the origin of chaetothyrialean lineages was during a period of recovery after the Permian–Triassic mass extinction and expansion of arid land masses, about 250 million years ago, when global temperatures were relatively high (Gueidan et al. 2011). Dothideomycetous and chaetothyrialean RIF evolved in different times, but presumably have a common ancestry on oligotrophic rock-colonizing organisms (Gueidan et al. 2011), as exposed rocks are the most abundant natural substrate; tolerance to radiation could have helped black yeasts to survive and proliferate on exposed rocks during historic periods of increased cosmic radiation, e.g., due to a weakened or absent magnetic field of the Earth (Dadachova and Casadevall 2008).

The class Dothideomycetes shows a significant class-wide tendency to extreme survival with a number of lineages purely comprising black-specialized extremophiles. The halophilic species *Hortaea werneckii* (Horta) Nishim. and Miyaji, for instance, or the highly acidophilic fungus *Acidomyces acidophilus* (Sigler and J.W. Carmich.) Selbmann, de Hoog and De Leo (Selbmann et al. 2008) belongs to that class as well as most of the psychrophilic RIF from highest mountain tops worldwide, not just from the Antarctic (Selbmann et al. 2014a).

Moreover, Chaetothyriales, showing a larger spectrum of assimilative abilities including toxic organic compounds, are frequently found on monuments in urban polluted environments; this tendency, rather than extremotolerance, drove the evolution of the group and promoted its shifting to opportunism (Gueidan et al. 2008) which is the main tendency of some families within the order.

A number of RIF genera and species have been reported in the Antarctic (Fig. 3), some of which are endemic with no reported occurrences elsewhere. The genus

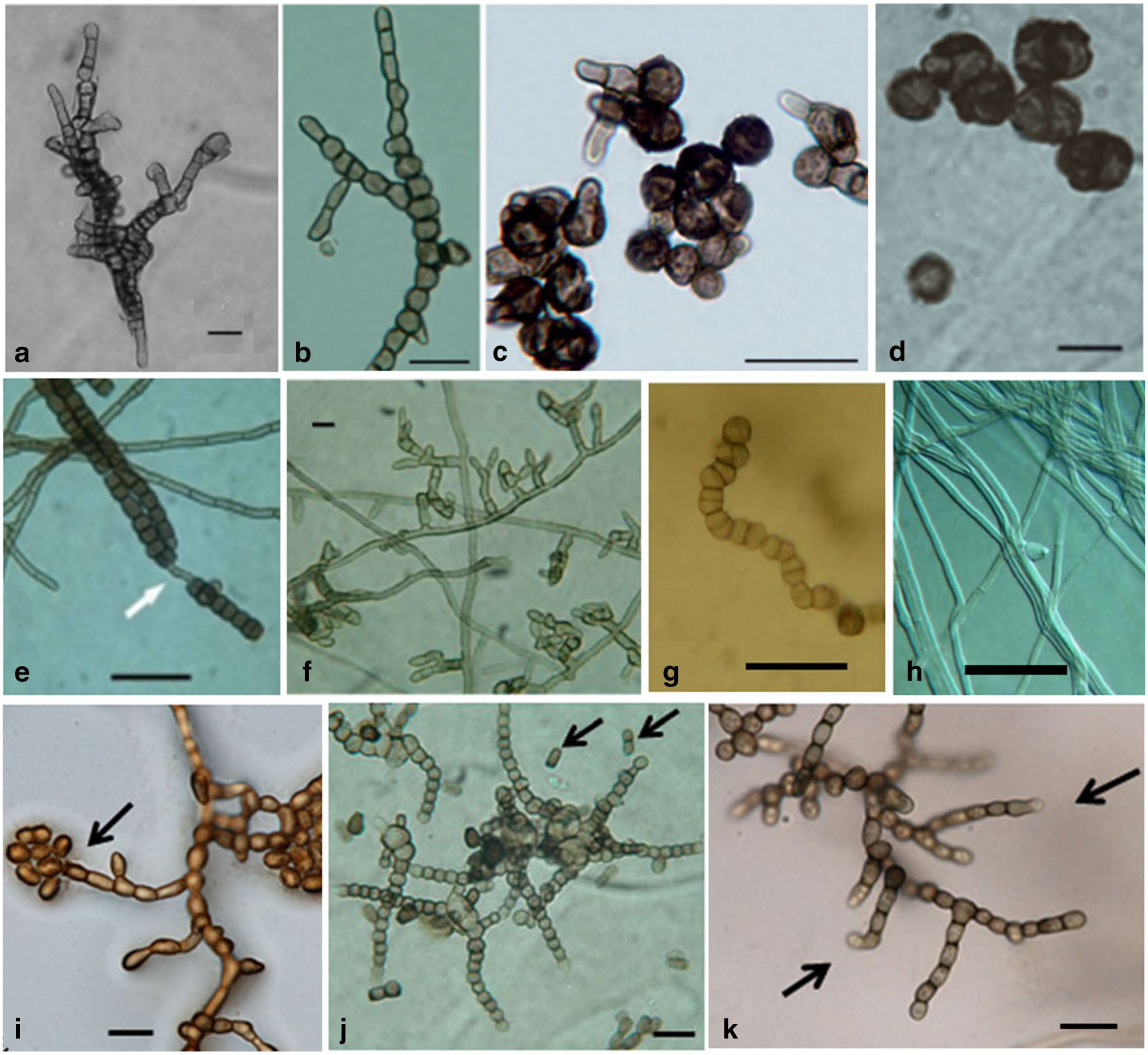


Fig. 3 **a** *Friedmanniomyces simplex*, CCFEE 5184: hyphal growth and terminal swelling cells. **b** Melanized hypha of *Friedmanniomyces endolithicus* CCFEE 5208. **c** Meristematic growth of *Cryomyces antarcticus* CCFEE 534. **d** *Cryomyces minteri,* CCFEE 5187: *thick-walled*, strongly melanized propagules. **e** *Elasticomyces elasticus,* CCFEE 5313: incomplete disarticulation of arthric conidia and hyphal fragments remaining joined by connectives (*white arrow*). **f** *Recurvomyces mirabilis*, CCFEE 5264 (=CBS 119434): hyphae with branched and unbranched conidiophores producing 0–1 septate conidia. **g** *Vermiconia antarctica*, CCFEE 5488 (=CBS 136107): septate, thick-walled cells showing meristematic growth. **h** *Extremus antarcticus* (=*Devriesia antarctica*), CCFEE 451: hyphae. **i** *Oleoguttula mirabilis*, CCFEE 5523: phialides with wide openings and very short collarettes and conidia aggregating in dense clusters at phialide tips. J. *Rachicladosporium mcmurdoi,* CCFEE 5211 (= CBS 119432): chained conidia with 1−2-celled conidia (*arrows*). K. *Rachicladosporium antarcticum*, CCFEE 5527: torulose, branched hyphae and enteroblastic proliferation (*arrows*). *Scale bars* **a–c**, **f–k** = 10 μm; *E* = 20 μm (Selbmann et al. 2005, 2008; Egidi et al. 2014)

Friedmanniomyces Onofri, for example, with the two species *F. endolithicus* Onofri and *F. simplex* Selbmann et al., occurs exclusively in Victoria Land and is the most represented genus among Antarctic RIF in CCFEE, with a frequency of 26 % (Fig. 2b). *Cryomyces* Selbmann et al., is found in the McMurdo Dry Valleys with the species *C. antarcticus* Selbmann et al. and *C. minteri* Selbmann et al.; yet, recently two new species, *C. funiculosus* Selbmann and de Hoog and *C. montanus* Isola and Zucconi, 8–10 % distant in ITS ribosomal gene sequences from the Antarctic species, have been found in the Alps at about 3200 m a.s.l. (Selbmann et al. 2014a). Despite the restricted distribution, this is the second most frequent RIF genus in the Antarctic with a frequency in the CCFEE of 10 %. The genus *Oleoguttula* Selbmann and de Hoog, with the only species *O. mirabilis* Selbmann and de Hoog, also occurs only in the Antarctic (Egidi et al. 2014) and represents 3.9 % of the RIF in the CCFEE collection. Other genera, such as *Extremus* Quaedvlieg and Crous, *Vermiconia* Egidi and Onofri, *Rachicladosporium* Crous, U. Braun and C.F. Hill, *Recurvomyces* Selbmann and de Hoog and *Elasticomyces* Zucconi and Selbmann, are less represented in the continent with the exception of *Extremus*, which showed a wider distribution spanning different continents in both hemispheres (Selbmann et al. 2008; Egidi et al. 2014; Quaedvlieg et al. 2014). Other species were hitherto found only in the Antarctic, such as *V. antarctica* Egidi and Selbmann, *Rachicladosporium antarcticum* Onofri and Egidi, *R. mcmurdoi* Selbmann and Onofri and *E. antarcticus* (Selbmann and de Hoog) Quaedvlieg and Crous, revealing local evolution and adaptation. Two exceptions were found for the taxa *Recurvomyces mirabilis* Selbmann and de Hoog and *E. elasticus* Zucconi and Selbmann with a worldwide distribution at the species level, even if always connected with cold locations. About 45 % of the isolates preserved in the CCFEE belong to still unknown species; these include all the strains in the order Chaetothyriales (Eurotiomycetes).

All the species mentioned above are strictly psychrophilic (Selbmann et al. 2005, 2008; Egidi et al. 2014); *C. antarcticus* in particular has been shown to be resistant to a wide number of physical and chemical stresses in the laboratory (Onofri et al. 2007b, 2008; Selbmann et al. 2011) and was selected for space experiments in the frame of a number of European Space Agency (ESA) and Italian Space Agency (ASI) projects.

Stress tolerance

A number of studies proved the high resistance of black meristematic fungi in terms of extremes of temperatures, acidity, osmotic stress and salinity, dehydration, solar and UV irradiation and even radioactivity (Sterflinger 1998, 2006, 2012; Gorbushina et al. 2003, 2008; Dadachova et al. 2007; Onofri et al. 2007b, 2008; Selbmann et al. 2008). RIF from the Antarctic desert have evolved and adapted to one of the most hostile terrestrial habitats on earth and, among them, *Cryomyces antarcticus* is one of the most extremotolerant eukaryotic organisms known to date, able to survive even real space exposure and simulated Martian conditions (Onofri et al. 2008, 2012). Living in almost permanently frozen conditions, active life is possible for a very restricted window of the year; yet, during the austral summer, temperature of rock surfaces may fluctuate across the freezing point over 14 times within 40 min with a consequent repeated freeze–thawing stress for lithobionts, even more dangerous for cells than permanent frost (Nienow and Friedmann 1993). Antarctic RIF, showing typical psychrophilic profiles (Van Uden 1984), not only tolerate repeated freeze–thaw cycles (−20 °C/+ 20 °C) in laboratory conditions, but easily withstand very high temperatures (Onofri et al. 2007a, b, 2008); germination efficiency of *Cryomyces* spp., for instance, was not affected after exposure of actively growing colonies at 90 °C for 1 h (Onofri et al. 2008).

Along with temperature, in their natural environment they must cope with a number of different stresses and a combination of them (Ruisi et al. 2007). Cold often implies a number of additional injuries such as osmotic stress, since water sink in ice crystals causes a depletion of liquid water available for active life. Yet, in some cold locations such as the Antarctic desert, evaporation may be very high and salt may accumulate conspicuously on rock surfaces. Black fungi tend to be halotolerant rather than halophiles; the evolution in icy conditions acted as pre-adaptation to osmotic stress and some Antarctic RIF may tolerate even considerably high salt concentrations. Although salt tolerance in RIF is not comparable with that of a real halophilic fungus, *Cryomyces* spp. still maintains visible growth at NaCl concentrations of 25 % (w/v, 4.28 M) (Onofri et al. 2007b).

Even if about 70 % of fresh water accumulates in the Antarctic, it is trapped as ice and the whole continent is basically a desert; the ice-free locations, such as the McMurdo Dry Valleys, are among the driest areas on Earth. Precipitation, represented exclusively by snow, is typically less than 100 mm W/E per year that is mostly sublime or blown away (Nienow and Friedmann 1993). Antarctic RIF evolved under this permanent environmental pressure and are well adapted to desiccation, being able to overcome long periods of deep dehydration. Desiccated colonies of *C. antarcticus* may be easily revived after both simulated and real space vacuum, even with just a rehydration of a few hours in a physiological solution (Onofri et al. 2008, 2012).

Life on exposed rocks in Antarctic deserts implies resistance to strong solar radiation; during the austral summer, the exposition may be even more intense than under hot conditions due to the ozone hole persisting at the South Pole. Antarctic RIF species are very resistant to radiation; they have been shown to maintain the ability to germinate after UV-B exposition at lethal doses for yeasts and without a detectable effect on DNA integrity (Onofri et al. 2007b; Selbmann et al. 2011). This high resistance is thought to be a consequence of the strong screen effect of melanin and thick cell walls, rather than to an efficient DNA repairing system.

It was also suggested that black fungi may play a role in hydration or in protection of photobionts in epilithic lichens by dissipating excessive sunlight (Harutyunyan et al. 2008); in the Antarctic cryptoendolithic communities, black fungi form a barrier just above the photobiont stratification (Fig. 1) that may play a crucial role in photobionts protection by dangerous sunlight exposition (Selbmann et al. 2013b).

Naked rocks in the Antarctic are also extremely oligotrophic. Being heterotrophs, Antarctic RIF take advantage for nutrition from the spatial association with autotrophic, similarly stress-resistant organisms, and benefit from simple carbon sources such as glucose resulting from photosynthesis of neighboring algae and cyanobacteria (Selbmann et al. 2013b). Yet, black fungi are extremely well adapted to rely on the few nutrients available: their metabolism and growth rates are extremely slow and cannot be increased even when cultured on rich media and optimal physical conditions. Their slow growth rate, coupled with the ability to shift to a dormant state even for long periods, makes them scarcely exigent from a nutritional point of view.

It was observed, using ^{14}C-labeling, that Antarctic RIF may actually uptake CO_2 (Palmer and Friedman 1988). The authors supposed that Antarctic fungi may actually incorporate CO_2 by carboxylation of pyruvate (Moses et al. 1959). Such mechanisms might be irrelevant for fungi living in nutrient-rich habitats, but could represent a life-sustaining option for slow-growing oligotrophic black yeasts in competitor-free environments (Selbmann et al. 2014b).

Mechanisms of tolerance and response to stresses

A number of morphophysiological characteristics allow dealing with a wide selection of stresses and sustain Antarctic RIF even well beyond the natural conditions they normally have to face in their natural environment (Onofri et al. 2008). Fungi in general have remarkable plasticity and promptly shift from one growth form to another according to variation in physical–chemical conditions. The shift to meristematic development is less frequent and is typical particularly of black yeasts under stressing conditions; this peculiar polar growth optimizes the volume/surface ratio and minimizes exposition to external stressors (Wollenzien et al. 1995). In RIF living under permanent stress, meristematic growth becomes a stable character.

Most of the cold-adapted RIF reproduce by isodiametric enlargement with subdividing cells by production of both longitudinal and horizontal septa. Some, such as *Cryomyces* spp., have mostly a yeast-like organization. Saving energy is the main issue for the success of black yeasts in the extremes. Simplification is essential for these fungi: life cycles are simple enough to be completed during short periods of time when favorable conditions prevail. Differentiation is also minimal and mostly concerns a few black clumps of cells that subdivide and fall apart for passive dispersal (Selbmann et al. 2005). At most, some species are exceptional in showing some morphological differentiations, with tiny conidiophores as in *R. mirabilis* (Selbmann et al. 2008). Due to the general absence of conidiation, Antarctic RIF adapt peculiar solutions to maximize their efficient dispersal; they actively penetrate and break up the lithic substratum and the colonized rock powder is then transported by winds. Contrary to current biogeographical data suggesting long-distance dispersal for RIF (Gorbushina 2007; Selbmann et al. 2008, 2013b), few taxa seem to be present exclusively in specific localities or geographic areas (Selbmann et al. 2005).

RIF are invariably asexual. Sex is costly as it implies the production of complicated structures and the need to find a mate; all this cannot be maintained when energy must be employed for survival and stress resistance. RIF seem to not miss sexuality, even if is a known driving force in evolution aiding the spread of advantageous traits and purging the genome of deleterious mutations. As a rule, clonal reproduction leads to accumulation of deleterious mutations that, over time, lead invariably to extinction (Muller's ratchet, Muller 1964). In the far extreme, where RIF thrive, this rule does not apply, probably because harmful mutations are immediately selected out and do not accumulate. In those conditions, perpetuation of few, but super-adapted genotypes is apparently more advantageous for survival.

The need for safe energy induces RIF to produce only a few metabolites and they are generally very poorly competent as degraders (Ruibal 2004). In addition, some metabolically very expensive substances essential for survival, such as extracellular polymeric substances (EPS), polyols and melanins (Selbmann et al. 2005; Sterflinger 2006), are produced. Antarctic RIF's environment is highly oligotrophic and the high metabolic costs for synthesizing all these compounds significantly affect growth velocity of these fungi, which typically show a very slow growth rate. EPS are conspicuously produced by Antarctic RIF where colonies may appear totally embedded by a slimy coat as for *Cryomyces* spp. (Fig. 4, arrow). EPS play a fundamental role in increasing resistance to cold conditions by protecting cells from freeze–thaw damage (Selbmann et al. 2002); moreover, they contribute in creating buffered physicochemical conditions around the cells and their hygroscopic nature increases success under dry conditions (de los Ríos et al. 2003).

Osmoregulators that also accumulate at high concentrations do not interfere with enzyme activity; among these compatible solutes, disaccharide trehalose is obligate in several black yeasts (Sterflinger 1998). It is very efficient as cryoprotectant in the freezing process or as enzyme and phospholipid bilayers stabilizer during desiccation

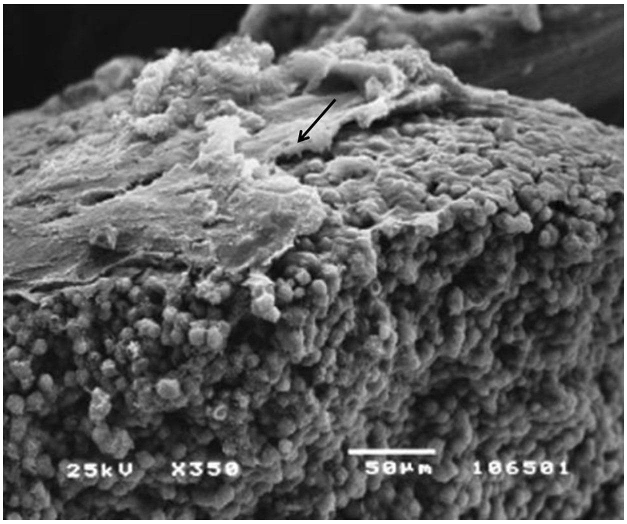


Fig. 4 SEM micrograph showing extracellular polymeric substances (EPSs, *arrow*) on the surface of a colony fragment of *Cryomyces minteri* CCFEE 5187 (Onofri et al. 2007a, b). A JEOL JSM 5200 Scanning Electron Microscope with an applied secondary electron detector

(Weinstein et al. 2000; Sterflinger 2006), allowing these surprising organisms to survive even complete dehydration (Onofri et al. 2012). It also is very efficient for its cryoprotective effects during freezing or desiccation (Weinstein et al. 2000), acting as stabilizer of enzyme conformation and phospholipid bilayers of membranes, allowing these surprising organisms to survive complete dehydration (Onofri et al. 2012).

Melanins are among the most important substances for enabling stress tolerance. Melanins in black fungi have different types of high molecular weight pigments produced by enzymatic coupling of phenolic units and are reported as 1,8-dihydroxynaphthalene DOPA-melanins (3,4-dihydroxyphenylalanine) or more frequently as DHN-melanins (Kogej et al. 2003, 2004). They are responsible for the typical dark green to brown or totally black color of these fungi and protect them from excessive heat or cold, extreme pH or osmotic conditions and polychromatic UV radiation; melanins also seem to mediate tolerance toward metals (Gadd and de Rome 1988; Gunde-Cimerman et al. 2000; Onofri et al. 2008; Selbmann et al. 2011; Sterflinger et al. 2012). They also confer tolerance to ionizing radiation: some melanized fungal species were found in nuclear reactors and their cooling water systems after the Chernobyl disaster (Zhdanova et al. 2000) and were feeding on radiation using melanins to convert ionizing gamma radiation into chemical energy through still unknown mechanisms (Dadachova et al. 2007). Melanins also have a role in osmo-adaptation of black yeasts. In the halophilic fungus *Hortaea werneckii*, for instance, they minimise glycerol loss from the cells and act as an osmoregulator, by reducing the size of pores in the cell wall (Plemenitaš et al. 2008). This implies a reduction of the cost for transmembrane transporters to maintain intracellular concentration of compatible solutes (Gostinčar et al. 2011). This process may also explain the osmotolerance observed in *Cryomyces* spp., as reported above.

Melanins are always coupled with the presence of a thick cell wall; particularly, in *Cryomyces* spp. conspicuous incrustations are present, conferring additional protection to the cell. During dehydration the volume of the cell is reduced, the plaques converge and weld to each other and the cell assumes the appearance of a small stone (Fig. 3d). Once water becomes available anew, the cells swell and the plaques move apart leaving cross-like scars allowing cell germination (Fig. 3c).

Even if there is a general understanding of the phenotypic and biological aspects sustaining RIF in the extremes, the study of the expression and function/regulation of the entire set of genes/proteins is still a virgin field of research. A key to understanding the ecology of these fungi is the question about the metabolic activity *versus* dormancy in the natural environments.

Proteome analyses performed with a few Antarctic RIF cultured in sub-optimal conditions revealed a general down-regulation of their metabolism. The Antarctic species *Friedmanniomyces endolithicus* decreases the number of protein spots when exposed to temperatures far above its growth optimum, indicating a lack of a heat-shock response at the protein level (Tesei et al. 2012). It can be concluded that the basic set of proteins necessary to survive high temperature is stable without the help of HSPs. The fungus is endemic in a permanently cold habitat where a heat-shock response was not developed during evolution (Hofmann et al. 2000). Desiccation produced a similar response in these fungi; in contrast to other rock fungi from the Mediterranean area that responded to dehydration by producing small proteins, the Antarctic RIF *Cryomyces antarcticus* down-regulates its metabolism (Zakharova et al. 2013). The function of these small proteins, called heat-shock proteins (sHSPs) or molecular chaperones, is to cluster with enzymes and stabilize their conformation (Kregel 2002; Åkerfelt et al. 2010).

The process of down-regulation is less energy consuming compared to the production of HSPs and much more targeted for the oligotrophic Antarctic environment. Besides, it requires a very specialized basic set of proteins that would not be affected by temperature or desiccation. Alternatively, protein protection may be mediated by the involvement of other protective factors such as sugars or fatty acids (Zakharova et al. 2013). Indeed, recent 2D-gel electrophoresis and Maldi-TOF/TOF mass spectrometry demonstrated that the proteome of *C. antarcticus* deviates significantly from other fungi (Zakharova et al. 2014a).

Fig. 5 Experiment hardware and biological samples of the LIFE experiment. **a** EXPOSE-E facility (*black arrow*) outside the Columbus module of the ISS during orbital flight, accommodating the LIFE samples. **b** Samples of the LIFE experiment (lichens and Antarctic RIF) accommodated in one of the compartments of the EXPOSE-E facility (Scalzi et al. 2012)

Astrobiological investigations

Astrobiology is the focus of many scientific investigations, aiming to answer questions on the possible existence of life on other planets, survival in outer space, interplanetary transfers, detecting signatures of extinct life and planetary protection. This last item refers to preserving the planetary integrity of natural processes by preventing the introduction of Earth-originated life that may mislead future life detection missions (COSPAR 2011).

The discovery of organisms inhabiting extreme environments adapted to cope with a number of extreme chemical–physical conditions, as high temperatures, salinity, acidity or dehydration, gave rise to the hope for locating life outside our planet (Miller 2005). Antarctic rock-inhabiting fungi, and *C. antarcticus* in particular, have been suggested as eukaryotic models for investigations on the lithopanspermia theory (transfer of life from one planet to another via colonized meteorites) and the biological exploration of Mars (Onofri et al. 2004, 2008, 2012; De Vera et al. 2012; Scalzi et al. 2012). These fungi, living in the closest terrestrial analog for the Red Planet and having the rock substratum as a natural niche, may faithfully mimic a putative life form that might be present under the icy surfaces of some of Jupiter's moons, in the underground caverns of Mars (Mustard et al. 2008), or in a Martian meteorite landed by chance on Earth.

In the frame of European Space Agency (ESA) and Italian Space Agency (ASI) programs, Antarctic RIF (*Cryomyces antarcticus* and *C. minteri*) as well as the entire endolithic community representing their natural niche were selected for a number of experiments focused on exobiological exploration. The first space experiment in which Antarctic RIF were included was LIFE (Lichens and Fungi Experiment) with the objective of testing the ability of selected organisms to survive long-term travel in space. The experiment was launched into space on the Space Shuttle STS and accommodated outside the Columbus module of the International Space Station (ISS) (Fig. 5a) where test organisms were exposed to space conditions for 565 days using the EXPOSE-E facility (Fig. 5b) that was part of the European Technology Exposure Facility (EuTEF) and designed to expose a variety of biological systems (Rabbow et al. 2009, 2012). After retrieval, a number of laboratory analyses indicated that Antarctic RIF were still alive; in particular the viability of *C. antarticus*, the most resistant one, was around 12.5 % measured by cultural tests (Onofri et al. 2012).

One of the primary goals for astrobiologists is the definition of bio-signatures and biomarkers to be used as reference for future space exploration programs. In this frame, the BIOlogy and Mars EXperiment (BIOMEX) aim to detect signatures of extinct or extant life using sensitive and not-destructive approaches. A number of biological models, among other extremotolerant/extremophilic organisms, and the Antarctic RIF *C. antarcticus*, were selected for this experiment. The Expose-R2 Facility is being used to expose the biological models for about 2 years; the launch to the International Space Station occurred on 24 July 2014.

The prime objective of BIOMEX is to measure to what extent biomolecules maintain their stability under space and Mars-like conditions to develop a biosignature database for detecting life traces on Mars future space missions. The secondary scientific objective is to analyze survival of test organisms cultivated on terrestrial, moon and Mars rock analogs. Melanins were chosen as potential biomarkers for RIF, being typical of this group of fungi. The results will contribute to the development of life detection strategies, supporting future exploration missions.

It was recently demonstrated that *C. antarcticus* not only survives extraterrestrial conditions in a dehydrated state, but also remains in active growth form. It reacts to simulated Martian conditions by down-regulating protein expression and recovers quickly its ability to grow as soon as optimal conditions return (Zakharova et al. 2014b).

Extraterrestrial parameters do not seem to kill Antarctic RIF, rather they maintain metabolic activity even under Martian conditions. Since they do not react by producing protective stress-induced HSPs, as already demonstrated when exposed to sub-optimal temperature or desiccation (Tesei et al. 2012; Zakharova et al. 2013), it may be surmised that their proteins have special conformations for working even at minimum water content (Moeller et al. 2012; Zakharova et al. 2013).

Conclusions

Among rock-inhabiting fungi, Antarctic RIF evolved in complete geographic and genetic isolation for a timescale of evolutionary significance and in one of the harshest environments on Earth. Being scarcely differentiated, there was little progress on their biodiversity and systematics at first discovery, but with the "omics" revolution rapid progress in sequencing technologies allowed us to look deeper into the genomes of these fungi and we are now beginning to have a clearer picture of the amplitude of their biodiversity in the Antarctic Continent (Selbmann et al. 2005, 2008, 2013b; Egidi et al. 2014). Moreover, new sampling campaigns invariably lead to the discovery and description of new taxa suggesting that RIF species in Antarctica remain woefully underestimated. Yet, living at the edge of their biological potential, Antarctic RIF are prone to any possible climate change that may easily alter or compromise their actual biodiversity or lead to the extinction of extreme specialists (Selbmann et al. 2012). It is therefore of utmost importance to investigate and preserve their actual biodiversity before than any change will affect the natural equilibrium.

Evidence concerning the uncommon stress resistance of Antarctic RIF has considerably stretched our imagination on the limits for life and on the possibility for eukaryotic life beyond our planet (Onofri et al. 2008). This research provided considerable input for a number of experiments, in a framework of scientific international consortia, on exobiological exploration (Onofri et al. 2009; De Vera et al. 2012), and new insights on the possibility of transfer of life among planets and planetary protection (Onofri et al. 2012; Scalzi et al. 2012).

The proteomic approach is recently opening a window for having insights on expression, functioning and regulation of the genes/proteins encoded by the genomes of these fungi (Isola et al. 2011; Marzban et al. 2013). This is a recent, promising approach to investigate the presence of stress-associated genes and expressed products and their role in polyextremotolerance and oligotrophism in black yeasts. Recent studies have already provided significant evidence that black yeasts have a peculiar response to stress that differs considerably from those of common mesophilic hyphomycetes; this understanding is fundamental for understanding mechanisms that pushed these fascinating organisms to the deep freeze and the deep extremes in general (Tesei et al. 2012; Zakharova et al. 2013, 2014a, b). The study of Antarctic RIF is therefore well beyond a subject for just a few specialists; RIFs are becoming an ever-expanding group for study, offering research opportunities in many basic fields as microbial ecophysiology, evolution and adaptation to extremes as well as to the recent and fascinating field of astrobiology.

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