Chapter 8
Black Yeasts in Cold Habitats

Laura Selbmann, G. Sybren de Hoog, Laura Zucconi, Daniela Isola and Silvano Onofri

Abstract Black yeasts have already been known since the end of the nineteenth century, but for a number of reasons, only few workers were familiar with them. That was since recently, until the wealth of biodiversity, stunning ecologies and potential applications have become apparent. Some remote and extreme locations, such as mountain tops, glaciers or polar areas, are now being investigated by mycologists. Many rock-colonizing fungi have been interpreted for long time as blackish fly-ash particles or dust on marble monuments or buildings. Black yeasts are easily overlooked in routine studies due to their very slow growth and poor competitive abilities. With the improvement isolation procedures, it has become clear that black yeasts are actually much more common and widespread than previously believed. Identification was hampered by their morphological plasticity, until molecular techniques became a routine approach in fungal systematic. In this chapter, the authors aim to give an overview of all the aspects concerning this unconventional group of fungi, from their peculiar ecology to their wide spectrum of biodiversity. Understanding about their ecological amplitude arose from impressive efforts in sampling remote habitats and concomitant sequencing activity during the last two decades.

Keywords Black yeasts · Dothideomycetes · Evolution · Cold adaptation

L. Selbmann (✉) · L. Zucconi · D. Isola · S. Onofri
Department of Ecological and Biological Sciences, University of Tuscia, Largo
dell'Università snc, 01100 Viterbo, VT, Italy
e-mail: selbmann@unitus.it

G. S. de Hoog
CBS Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD, Utrecht, The Netherlands

Contents

8.1 Introduction

The term "black yeasts" comprises melanized fungi able to reproduce by uni-cellular growth, at least for a part of their life cycle. The group is phylogenetically quite heterogeneous and includes mainly fungi belonging to divergent orders of *Ascomycota* (de Hoog and McGinnis 1987), sharing a number of basic features as melanized cell wall and formation of daughter cells by yeast-like or multilateral polar budding. Yet, since most black yeasts exhibit mycelial growth or may shift to meristematic development, and are phylogenetically diverse, there is no common descriptive term for these fungi. They have variously been indicated as black yeasts and relatives, meristematic fungi or microcolonial fungi. The term meri-stematic, originally introduced for black fungi by de Hoog and Hermanides-Nijhof (1977), describes non-disintegrating aggregations of thick-walled, melanized cells. Meristematic growth is infrequent in the fungal kingdom and is a specific response to stress: conversion towards isodiametric expansion can be induced in hyphal or yeast-like thalli of *Herpotrichiellaceae* by environmental factors such as acidifi-cation of the culture medium (Mendoza et al. 1993; Xi et al. 2009); similar observations have been made in *Dothideaceae* (de Hoog et al. 1999). In some cases, these may be stable mutants (Matsumoto et al. 1986) as for those black yeasts permanently living under extreme conditions (Selbmann et al. 2005, 2008).

This morphological plasticity, coupled in many cases with high polymorphism of synanamorphs and very poor differentiation, hampered morphological identi-fication. Molecular phylogeny supplied an impressive body of data during the last decade enabling to establish the position of black yeast in the fungal kingdom with much more accuracy. These studies revealed that, despite poor morphological differentiation, the biodiversity of black fungi is quite impressive and still far from being elucidated.

One of the characters shared in the whole group is the ability to tolerate or even adapt to extreme conditions. Black yeasts are commonly isolated from saltpans (Plemenitaš and Gunde-Cimerman 2005), acidic and hydrocarbon-contaminated sites (Baker et al. 2004; Seyedmousavi et al. 2011; Selbmann et al. 2012; Isola et al. 2013), exposed natural rocks (Ruibal et al. 2005) and monuments (Sert et al.

2007a, b, c; Marvasi et al. 2012), hot deserts (Staley et al. 1982) and very cold icy habitats (Selbmann et al. 2005, 2008; Turchetti et al. 2008; Zalar et al. 2008; Branda et al. 2010; Brunner et al. 2011). The human body is also an extreme environment, and meristematic growth and melanins represent virulence factors for some human opportunists (Matsumoto et al. 1984; van de Sande et al. 2006; Liu and Nizet 2009; Xi et al. 2009; de Hoog et al. 2011). Apparently, a basic set of traits, among which are melanization and meristematic development, are primarily suited to cope with and adapt to highly diverse environmental conditions.

8.2 Biodiversity, Locations and Substrate

Black fungi are worldwide distributed in cold habitats, thriving from subglacial ice to cold sea water, soils and rocks, one of the most frequently investigated substrata. Some species have a broad ecological amplitude and are found in alternative environments, while others occur specifically in cold habitats.

Aureobasidium pullulans is an osmotolerant black yeast that may thrive in a number of osmotically fluctuating environments including the phyllosphere (Andrews et al. 2002), polluted water (Vadkertiova and Slavikova 1995) and solar salterns (Gunde-Cimerman et al. 2000). Given the known adaptive ability of *A. pullulans* to low water activity (A_w) and oligotrophic conditions, it appeared likely that ice from cryocarstic formations and subglacial ice in polythermal glaciers may constitute a potential natural habitat.

Aureobasidium-like fungi were found among the dominant ascomycetous mycota in ice originating from glacial and subglacial environments as polythermal Arctic glaciers in Svalbard (Spitsbergen, Norway; Butinar et al. 2007, 2011; Sonjak et al. 2006). *A. pullulans* var. *pullulans* and *A. pullulans* var. *melanogenum*, both species with a global distribution, were found in Arctic glaciers using a multilocus approach (Zalar et al. 2008); moreover, an additional genotype, *A. pullulans* var. *subglaciale*, was found exclusively in Kongsfjorden glacial and subglacial ice and sea water (Zalar et al. 2008). The psychrotolerant nature of the last one allows active metabolism under conditions of permanently cold in Arctic glaciers. *A. pullulans* was isolated from other cold locations, such as ice-free Alaskan soils, sediments and melt water from glaciers of the Apennines (Turchetti et al. 2008; Branda et al. 2010; Brunner et al. 2011) and from soils and mosses from different locations of Antarctica, including different locations of the McMurdo Dry Valleys (Onofri et al. 2007).

The dimorphic genus *Exophiala* comprises numerous thermotolerant potential human opportunists or pathogens such as *E. dermatitidis* (Sudhadham et al. 2008), *E. spinifera* (Li et al. 2008) and *E. asiatica* (Li et al. 2009). In contrast, many *Exophiala* spp. lacking thermotolerance were found as pathogens in cold-blooded animals such as *E. salmonis*, *E. pisciphila* and *E. psychrophila* (Richards et al. 1978; Pedersen and Langvad 1989; de Hoog et al. 2009). Their remarkable association with monoaromatic hydrocarbons (Prenafeta-Boldú et al. 2006), which are structural analogues of neurotransmitters, allows these fungi to easily shift to

animals, including humans. Species belonging to this genus are normally difficult to recover from the environment as they grow very slowly and are frequently overlooked and require dedicated isolation methods (Prenafeta-Boldú et al. 2006; Sudhadham et al. 2008; Vicente et al. 2008; Zhao et al. 2010). Nonetheless, the genus *Exophiala* was also found in cold habitats. The species *E. nigra* was isolated from Antarctic soils (Lyakh and Ruban 1970). The thermophilic species *E. dermatitidis* was reported from the Antarctic continent associated with mummified seals and from air samples in McMurdo Station (Sun et al. 1978) and in Lake Fryxell (Greenfield 1981), as well as in sediments and meltwater of Calderone glaciers in the Apennines, Italy (Branda et al. 2010), but not all these strains have been confirmed by molecular data.

A number of black yeasts have been found associated with rocks in different cold environments, the majority of which still lack a formal description (Ruibal et al. 2009). Apparently, environmental pressure and geographical isolation promote speciation processes and unknown species are frequently encountered even during routine sampling. Black fungi were observed in the cryptoendolithic communities of the Antarctic (Friedmann 1982), living in spatial association with other extremotolerant microbes such as cyanobacteria and lichens. Several of these black yeasts have been described as endemic genera and species for the Antarctic: *F. endolithicus* (Onofri et al. 1999) and *F. simplex* (Selbmann et al. 2005) have hitherto been recorded exclusively in the Antarctic Victoria Land. The genus *Cryomyces*, reported to be restricted to the McMurdo Dry Valleys (Selbmann et al. 2005), was recently found in the Alps with the novel species *Cryomyces montanus* and *C. funiculosus* (Selbmann et al. 2013a). The genus *Saxomyces*, with still unresolved ancestry and endemic to alpine mountain tops, contains strictly psychrophilic species only (Selbmann et al. 2013a). *Recurvomyces mirabilis* and *Elasticomyces elasticus* were found associated with Antarctic endolithic communities (Selbmann et al. 2008) and reported on exposed rock in the Mediterranean or different cold sites as mountain tops in the Andes and Alps (Isola 2010). *E. elasticus* was also found associated with different species of epilithic psychrophilic Antarctic lichens including the endemic species *Lecanora fuscobrunnea* (Selbmann et al. 2013b). In the same study, further strains closely related to or conspecific with black fungi previously thought to be restricted to Antarctic endolithic microbial communities, such as *F. endolithicus*, were found associated with cosmopolitan lichen species. This ability to make association with different microbes according to location may give further advantage in adaptation and survival of the whole community. As heterotrophs, black fungi take advantage from the association with autotrophic organisms under oligotrophic conditions, but it is still unclear whether or not black fungi may supply benefits to epi- or endolithic lichens as well. It was suggested that black fungi may play a role in hydration or in protection of photobionts by dissipating excessive sunlight (Harutyunyan et al. 2008). This would particularly involve the black barrier just above the photobiont stratification in cryptoendolithic lichens (Selbmann et al. 2005). The presence of black fungi may therefore play a crucial role to allow survival under these highly stressful conditions.

8.3 Phylogenetic Assignment and Evolution of Black Yeasts

The abilities to shift to meristematic development and consistently produce melanin, typical of black yeasts, are not widespread in the fungal kingdom. Molecular phylogenetics revealed that black yeasts are mainly distributed in two classes: Dothideomycetes, where the order Capnodiales is particularly overrepresented, and to a lesser extent in the Eurotiomycetes, order Chaetothyriales. In the latter, with few exceptions, black fungi nearly exclusively thrive in hot, semi-arid climates. Black fungi in the order Chaetothyriales are known for their ability to metabolize aromatic compounds (Prenafeta-Boldú et al. 2006); this may explain their higher presence at sites much more influenced by human activities and rich in pollutants, such as coasts and urban environments rather than mountain tops or the Antarctic (Onofri et al. 2011). Moreover, the class Dothideomycetes shows a significant class-wide tendency to extreme survival with a number of lineages purely constituted of black-specialized extremophiles. To that class, for instance, belongs the halophilic species *Hortaea werneckii*, or the highly acidophilic fungus *Acidomyces acidophilus* (Selbmann et al. 2008) as well as most of the psychrophilic black yeasts mentioned above as the genera *Cryomyces*, *Friedmanniomyces* and *Saxomyces*. Chaetothyriales have instead evolved a larger spectrum of assimilative abilities including toxic organic compounds; this tendency, rather than extremotolerance, has led mainly to diversification and promoted shifting to opportunism (Gueidan et al. 2008) which is the main tendency within the order.

The reason why cold-adapted black yeasts are almost exclusively distributed in the class Dothideomycetes may also be found in the early evolutionary history of both fungal groups. It has been recently demonstrated that the class Dothideomycetes has evolved much earlier than chaetothyrialean lineages (Gueidan et al. 2011). The period of diversification of Dothideomycetes was estimated in the Silurian–Devonian, 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 an expansion of arid land masses, about 250 million years ago, when global temperatures were relatively high. Adaptations of extant Dothideomycetes and Eurotiomycetes clearly reflect this evolutionary process.

Both Dothideomycetes and chaetothyrialean fungi evolved in different times but have presumably a common ancestry on oligotrophic organisms living on rock surface or subsurface (Gueidan et al. 2011). At that time, exposed rocks were the most abundant natural substrate and tolerance to radiations could have helped black yeasts to survive and proliferate during historic periods of increased cosmic radiation, e.g. due to weakened or absent magnetic field of the Earth (Dadachova and Casadevall 2008).

There are evidences that symbiotic lifestyle may have played a pivotal role in the evolution of free-living rock ancestor for black yeasts. Molecular phylogeny revealed a certain affinity of some lichenized fungal lineages and different lineages

of black fungi since some black yeasts from rocks resulted basal to the large lichenized lineages of Arthoniomycetes and Verrucariales (Gueidan et al. 2008; Ruibal et al. 2009). Moreover, some black yeasts have been observed to develop into lichenoid structures within months when co-cultured with lichen algae (Gorbushina et al. 2005; Brunauer et al. 2007). These peculiar interactions with autotrophic organisms, which help black fungi to improve their carbon supply, may be interpreted as vestiges of the ancestral situation. Black yeasts maintained somehow their connection with this primitive life form and may be easily found associated with rocks or are commonly visible as lichen colonizers (Harutyunyan et al. 2008; Selbmann et al. 2013b).

8.4 Stress Tolerance

Black yeasts may actually be seen as the excellence in the extremes. Along with cold, they may cope with a number of different stresses and also a combination of them (Ruisi et al. 2007), ranging from wide temperature fluctuations to irradiation and osmotic stress (Palmer et al. 1990; Sterflinger 1998; Gorbushina et al. 2003, 2008; Onofri et al. 2007; Marvasi et al. 2012). Black yeasts from cold habitats, such as Antarctic and alpine species of the genera *Friedmanniomyces* and *Cryomyces* spp., have typical psychrophilic profiles (Van Uden 1984). Black yeasts from the Antarctic desert live in almost permanently frozen conditions, but during austral summer, temperature of rock surfaces may fluctuate across the freezing point over 14 times within 40 min, causing a repeated freeze–thawing stress to lithobionts. Antarctic black fungi may actually easily tolerate this stress: repeated freeze–thaw cycles (–20 °C/+20 °C) did not affect growth ability of these fungi (Onofri et al. 2007, 2008). Remarkably, psychrophilic black yeasts were proved to tolerate even very high temperatures. Germination ability of *Cryomyces* spp. is not affected after exposition at 90 °C for 1 h (Onofri et al. 2008). Yet, some black yeasts have an eurithermic behaviour with optimal growth temperature well above the normal ambient temperature of their natural environment. *C. funiculosus*, for instance, a species living in the Alps above 3,000 m a.s.l., grows in the range 0–35 °C with optimal growth at 25 °C. This apparent incongruence may be interpreted as an adaptation to very fluctuating environmental conditions; even in very cold environments, exposed rock surfaces may reach temperatures 20 °C above the air temperatures (Nienow and Friedmann 1993; Selbmann et al. 2013a).

Cold often implies a number of additional stressors such as osmotic stress since the formation of ice crystals leads to lack of water for active life. Black fungi are actually osmotolerant rather than halophiles but, since in some cold location such as the Antarctic desert evaporation may be incredibly high and salt may accumulate conspicuously on rock surface, some of them evolved specific adaptation and may tolerate even considerably high salt concentration. Even if salt tolerance is not comparable with that of a real halophilic fungus, *Cryomyces* spp. still maintain visible growth at NaCl concentration of 25 % (w/v) (Onofri et al. 2007).

Life on exposed rocks in cold environments, such as mountain tops or Antarctic deserts, implies resistance to strong solar radiations since exposition may be even more intense than under hot conditions due to thin atmosphere at mountain tops, or the ozone hole persisting particularly at the South Pole. Resistance to radiation has been largely documented in black yeasts. Antarctic species maintain their ability to germinate after high UV exposition (Onofri et al. 2007) and even space radiations (Onofri et al. 2012) by resisting, rather than repairing potential DNA damages (Selbmann et al. 2011).

8.5 Adaptations to Stress: The Importance of Being Simple

Black yeasts have evolved a number of morphophysiological characteristics and even adopted a lifestyle, allowing them to deal with a wide selection of external pressures. Their adaptations are so effective that their tolerance may push them well beyond the natural conditions they normally experience in their natural environments (Onofri et al. 2008).

Fungi in general have remarkable plasticity and may promptly shift from one growth form to another according to variation in physical–chemical conditions. They adopt unicellular growth when immersed in rapidly fermentable sugar or when they are in their infective phase, as most human pathogens or in vascular plant diseases; alternatively, they may grow as filamentous form, the "search for food" growth form, allowing them to inspect the surroundings. Yet, shifting to meristematic development is less frequent and is the answer of a group of fungi, particularly black yeasts, to stressing conditions. Many black yeasts may shift alternatively from filamentous to yeast-like organization or to meristematic growth according to the external conditions, but, for species living under permanent stress, the last may became a stable character. Examples of morphological organization of "cold" black yeasts are reported in Fig. 8.1. The advantage of meristematic development lies in optimizing the volume/surface ratio since it minimizes exposition to external stressors (Wollenzien et al. 1995).

The dimorphic fungus *A. pullulans* may switch promptly from small colourless yeast cells to thick-walled, heavily melanized, meristematic forms (Bermejo et al. 1981) to mitigate environmental stress. This process has been described at the molecular level (Kogej et al. 2005, 2006; Turk et al. 2007; Gostinčar et al. 2008).

Exophiala dermatitidis can shift into a meristematic form at low pH (de Hoog et al. 1994). However, several stress-tolerant strains of this species are known to irreversibly maintain their slowly expanding, meristematic morphology. The same has been noted even more frequently for the related species *E. phaeomuriformis* (Matsumoto et al. 1986). Meristematic and yeast-like strains do not differ in variable loci of their rDNA (Matos et al. 2003) in these cases, while in other fungal orders, such as *Capnodiales*, some sequence divergence has been seen indicating possible subsequent sympatric speciation (Selbmann, unpublished data).
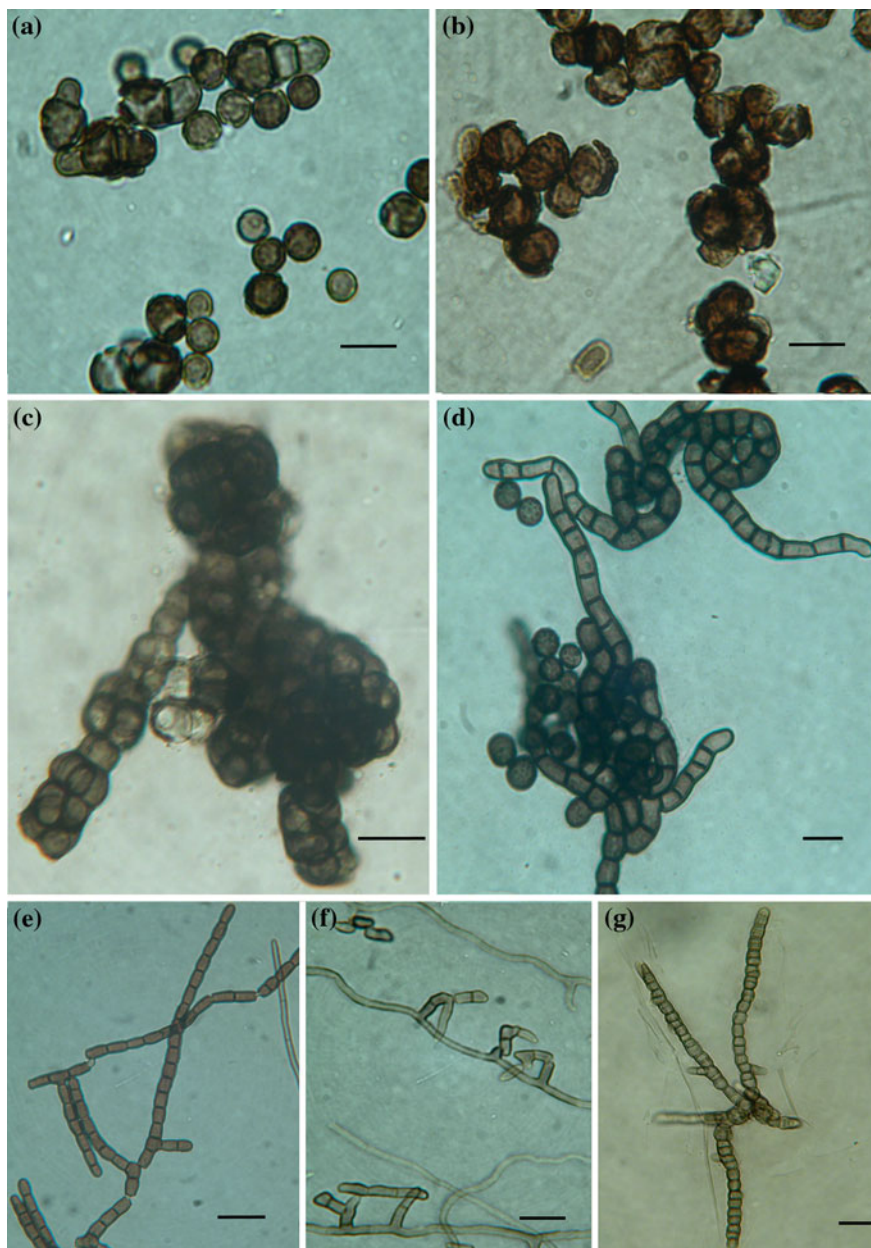
Fig. 8.1 *Cryomyces antarcticus* CCFEE 534 (**a**); *Cryomyces minteri* CCFEE 5187 (**b**); *Cryomyces funiculosus* CCFEE 5554 (**c**); *Saxomyces alpinus* CCFEE 5470 (**d**); *Elasticomyces elasticus* CCFEE 5319 (**e**); *Recurvomyces mirabilis* CCFEE 5264 (**f**); *Friedmanniomyces endolithicus* CCFEE 5208 (**g**). Scale bars = 10 μm

Most of cold-adapted rock black yeasts 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. To gain success, the extremes black yeasts have learned to save energy: simplification is essential for these fungi. Differentiation is minimal and they show very short life cycles, comprising just some black clumps of cells, to be completed during short periods of time when favourable conditions prevail (Selbmann et al. 2005). The life cycle in these fungi usually concerns just a few cells that subdivide and fall apart for passive dispersal. At most, some species exceptionally show some morphological differentiation, with tiny conidiophores as in *Recurvomyces mirabilis* (Selbmann et al. 2008). The absence in most black yeasts of active and abundant conidia formation hampered the efficiency of dispersal, and some of them have adopted surprising means to spread. Rock black yeasts, for instance, actively penetrate and break up the substratum and are actively dispersed with dust transported by winds. Current biogeographical data suggest that long-distance dispersal occurs in rock-inhabiting fungi (Gorbushina 2007; Selbmann et al. 2008, 2013a). However, in few cases, some rock-inhabiting taxa seem to be only present in unique localities or geographical areas (Selbmann et al. 2005).

Black yeasts, particularly in the class Dothideomycetes, are invariably asexual; the chaethothyrialean genus *Exophiala*, for which *Capronia* as teleomorph is known, represent an exception. Sex is costly, implying not only the trouble of finding a mate but also the cost to keep the genetic machinery for recombination running. Fungal teleomorphs tend to be elaborate, with fruit bodies and specialized cells in which recombination takes place. In the extreme, as in almost permanent frozen environments, there is no place and time for all this. Sexuality is a known driving force in evolution: recombination aids the spread of advantageous traits over populations and purges the genome of deleterious mutations. If this mechanism is absent, as is the case in clonally reproducing organisms, Muller's ratchet applies, harmful mutations accumulate in the course of time and gradually but inevitably lead to extinction (Muller 1964). Black yeasts seem to be a fundamental exception to this rule and do not show to miss their sexuality. Apparently, beyond one certain threshold of stress, asexuality, with perpetuation of few super-adapted genotypes, seems to be advantageous for survival.

Oligotrophy is another important adaptation of black yeasts, enabling these fungi to rely only on sparse, airborne nutrients available such as dust; even if some of them, particularly in the chaetothyrialean lineages, show remarkable degradative abilities and may digest pollutants such as aromatic compounds (Isola et al. 2013), dothidealean black yeasts are metabolically scarcely competent. They produce only few metabolites, which are restricted to substances essential for survival, such as extracellular polymeric substances (EPS), polyols and melanins (Selbmann et al. 2005; Sterflinger 2006). Considering oligotrophy of typical environments for black yeasts, 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 play a fundamental role increasing resistance to cold conditions by protecting cells from freeze–thawing damages (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 Rios et al. 2003). Cold often implies an osmotic stress due to the lack of liquid water and salt accumulation due to strong evaporation. A number of intracellular osmoregulators are accumulated at high concentrations without interfering with enzyme activity; among these compatible solutes, the disaccharide trehalose is obligate in several black yeasts (Sterflinger 1998). It 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).

Melanin, together with a thick cell wall with conspicuous incrustations, is for certain the most important factor to stress resistance in black yeasts. Melanins of black fungi are different types of high molecular weight pigments produced by enzymatic coupling of phenolic units; in black yeasts, these are reported as 1,8-dihydroxynaphthalene (Kogej et al. 2004). They are responsible for the typical dark green to brown or totally black colour of these fungi and confer them the ability to survive a number of different external pressures, such as excessive heat or cold, extreme pH or osmotic conditions, polychromatic UV radiation, and 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).

Melanin confers also tolerance to ionizing radiation: some melanized fungal species have been found in nuclear reactors and their cooling water systems (Zhdanova et al. 2000) which even feed on radiation by using melanins to convert ionizing gamma radiation into chemical energy by still unknown mechanisms (Dadachova et al. 2007).

Melanin has also a role in osmoadaptation of black yeasts; in *Hortaea werneckii*, for instance, it improves glycerol retention within the cells, which act as osmoregulator, by reducing size of pores in the cell wall (Plemenitaš et al. 2008). This may help to reduce the cost of supplying energy to transmembrane transporters to maintain intracellular concentration of compatible solutes (Gostinčar et al. 2011).

8.6 Nutrition

Cold-adapted black yeasts colonize environments, such as polar or alpine areas, which are strictly oligotrophic and largely unpolluted; therefore, they are adapted to this nutritional constraints relying on airborne nutrients or living in spatial association with autotrophic, similarly stress-resistant organisms. Yet, the environmental constraints exert a high selective pressure; therefore, black fungi do not have the trouble to sharpen their competitive abilities but rather to focus on stress tolerance and survival. The very slow growth rate and ability to shift to dormant

state even for long periods make them scarcely exigent from a nutritional point of view; they just need simple sugars for growth or easily degradable polymers such as starch. Some strains living above 3,000 m a.s.l. in the Alps were found to grow with scarce efficiency on potato dextrose agar (PDA) medium, rich in starch, if compared with media containing glucose oligomers. This is probably due to a reduced ability to produce amylases; in those environments, plants are sparse or absent at all and starch is expected to be rare. Therefore, these fungi may benefit from simpler carbon sources such as glucose and other oligomers resulting from photosynthesis of neighbouring lichens and algae (Selbmann et al. 2013b). Most black yeasts have a very slow growth rate with no significant increases even when cultured on rich media; some also maintain visible mycelial growth, even for short periods, when cultivated on media without carbon sources. This behaviour indicates an extreme adaptation to the oligotrophic conditions of their natural environments.

The ubiquity of black fungi in polar and alpine locations indicates that, in addition to their cohabitation with algae, the existence of unconventional pathways of carbon acquisition or energy gain may be supposed (Gostinčar et al. 2012). Atmospheric carbon dioxide fixation could exceptionally take place in fungal metabolism, although it lacks a Calvin cycle comparable with that found in plants. It was observed, using ^{14}C-labelling, that black fungi isolated from Antarctic cryptoendolithic communities of the McMurdo Dry Valleys, the closest terrestrial analogue for Mars, 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) as it was reported earlier for a number of fungi, but a definitive confirmation with a modern approach is still missing. 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.

As already mentioned, black yeasts can also gain energy from unconventional sources such as ionizing radiations which are then transformed into biochemical energy by means of melanins. All these unusual abilities to gain carbon and energy in black yeasts can, in their overall, explain not only the ability to survive, but even to thrive in environments normally neglected or precluded for most life forms, such as oligotrophic, exposed rock surfaces.

8.7 Conclusions

Black yeasts are a group of fungi that have remained rather unexplored since recently due to the difficulties in isolation for the slow growth rate and scarce competitive abilities, and the troubles in identification due to their poor differentiation and morphological plasticity. The "omics" revolution gave new insights into the biology of these unconventional organisms. Rapid progresses in sequencing technologies, for instance, allowed to look deeper into the genomes of

black yeasts, and we now have a clearer picture of the amplitude of their biodiversity (Ruibal et al. 2009), even if it still remains largely unknown, and considerable progresses have been done in shedding light on the origin and evolution of these organisms (Gueidan et al. 2008, 2011). Particularly in the class Dothideomycetes novel species of black yeasts, often with unclear phylogenetic relations at order level, are continuously discovered and described (Selbmann et al. 2013a). This suggests that species richness, at least within this class, remains woefully underestimated.

The understanding of the expression, function and regulation of the entire set of genes/proteins encoded by fungal genomes is an intriguing challenge, and proteomics 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 (Isola et al. 2011). Recent studies gave significant evidence that black yeasts have a peculiar response to temperature that differs considerably from the one of common mesophilic hyphomycetes. In particular, it was observed that black yeasts show a significant decrease in protein expression when exposed to temperatures above their growth optimum, indicating a downregulation of their metabolism, while the mesophilic fungus *Penicillium chrysogenum*, taken as control strain, expressed the highest number of proteins at 40 °C, indicating a temperature-induced reaction (Tesei et al. 2012). It is worth investigating in the future if this is a general stress response in this special group of fungi.

Black yeasts are an excellent material of study in many different fields. Their extraordinary stress tolerance and ability to grow inside the rocks suggested their possible use as model for astrobiological studies, such as searching life on cold planets such as Mars or the under ice oceans of Europa, or to study the possibility of interplanetary transfer of life from one planet to another within meteorites according to the Lithopanspermia theory (Onofri et al. 2008, 2009, 2012).

Among black yeasts, rock inhabitants equally colonize natural rock or monument, and due to their extraordinary capacity to penetrate minerals, they are an attractive subject for applied research in biodeterioration of monuments. Another fascinating field of research concerns the ability of some of them, particularly the chaetothyrialean strains, to metabolize aromatic compounds and their possible exploitation in bioremediation programs (Prenafeta-Boldú et al. 2006); this has encouraged the search of competent strains in new, unconventional habitats such as gasoline tanks or soap dispensers of washing machines (Isola et al. 2013). In the meantime, since assimilation of phenolic compounds and hydrocarbons may represent a virulence factor enabling to infect the central nervous system which has a high content of monoaromatic neurotransmitters, it is of utmost importance to shed light on phylogenetic relations and differences in physio-ecological traits of black yeasts within Chaetothyriales to control and prevent the spreading of biohazardous species.

Since the time when black yeasts were just a subject for few specialists, they have now become an ever-expanding field of study, offering research opportunities in many basic fields, such as microbial ecophysiology, evolution and adaptation to

extremes, as well as in applied research, such as human pathogenicity, bioremediation, biodeterioration of monuments and exobiology.

References

Andrews JH, Spear RN, Nordheim EV (2002) Population biology of *Aureobasidium pullulans* on apple leaf surfaces. Can J Microbiol 48:500–513

Baker BJ, Lutz MA, Dawson SC, Bond PL, Banfield JF (2004) Metabolically active eukaryotic communities in extremely acidic mine drainage. Appl Environ Microbiol 70:6264–6271

Bermejo JM, Dominguez JB, Goni FM, Uruburu F (1981) Influence of pH on the transition from yeast-like cells to chlamydospores in *Aureobasidium pullulans*. A van Leeuwenhoek 47:385–392

Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P (2010) Yeast and yeast-like diversity in the southernmost Glacier of Europe (Calderone glacier, Apennines, Italy). FEMS Microbiol Ecol 72:354–369

Brunauer G, Blaha J, Hager A, Türk R, Stocker-Wörgötter E, Grube M (2007) Lichenoid structures in vitro of a cultured lichenicolous fungus. Symbiosis 44:127–136

Brunner I, Plotze M, Rieder S, Zumsteg A, Furrer G, Frey B (2011) Pioneering fungi from the Damma glacier fore field in the Swiss Alps can promote granite weathering. Geobiology 9:266–279

Butinar L, Spencer-Martins I, Gunde-Cimerman N (2007) Yeasts in high Arctic glaciers: the discovery of a new habitat for eukaryotic microorganisms. A van Leeuwenhoek 91:277–289

Butinar L, Strmole T, Spencer-Martins I, Gunde-Cimerman N (2011) Relative incidence of ascomycetous yeasts in Arctic coastal environments. Microb Ecol 61:832–843

Dadachova E, Casadevall A (2008) Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Curr Opin Microbiol 11:525–531

Dadachova E, Bryan RA, Huang X, Moadel T, Schweitzer AD, Aisen P, Nosanchuk JD, Casadevall A (2007) Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. PLoS ONE 2:e457

de Hoog GS, Hermanides-Nijhof EJ (1977) The black yeasts and allied hyphomycetes. Stud Mycol 15:1–222

de Hoog GS, McGinnis MR (1987) Ascomycetous black yeasts. Stud Mycol 30:187–199

de Hoog GS, Takeo K, Yoshida S, Gottlich E, Nishimura K, Miyaji M (1994) Pleoanamorphic life cycle of *Exophiala* (*Wangiella*) *dermatitidis*. A van Leeuwenhoek 65:143–153

de Hoog GS, Zalar P, Urzì C, de Leo F, Yurlova NA, Sterflinger K (1999) Relationships of dothideaceous black yeasts and meristematic fungi based on 5.8S and ITS2 rDNA sequence comparison. Stud Mycol 43:31–37

de Hoog GS, Guarro J, Figueras MJ, Gené J (2009) Atlas of clinical fungi. 3rd CD-ROM ed. CBS-KNAW Fungal Biodiversity Centre, Utrecht/Universitat Rovira I Virgili, Reus

de Hoog GS, Vicente VA, Harrak MJ, Najafzadeh MJ, Badali H, Seyedmousav S (2011) Waterborne *Exophiala* species causing disease in cold-blooded animals. Persoonia 27:46–72

de los Ríos A, Wierzchos J, Sancho LG, Ascaso C (2003) Acid microenvironments in microbial biofilms of Antarctic endolithic microecosystems. Eviron Microbiol 5:231–237

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

Gadd GM, de Rome L (1988) Biosorption of copper by fungal melanin. Appl Microbiol Biotechnol 29:610–617

Gorbushina AA (2007) Life on the rocks. Environ Microbiol 9:1613–1631

Gorbushina AA, Whitehead K, Dornieden T, Niesse A, Schulte A, Hedges JI (2003) Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. Can J Bot 81:131–138

Gorbushina AA, Beck A, Schulte A (2005) Microcolonial rock inhabiting fungi and lichen photobionts: evidence for mutualistic interactions. Mycol Res 109:1288–1296

Gorbushina AA, Kotlova ER, Sherstneva OA (2008) Cellular responses of microcolonial rock fungi to long-term desiccation and subsequent rehydration. Stud Mycol 61:91–97

Gostinčar C, Turk M, Trbuha T, Vaupotič T, Plemenitaš A, Gunde-Cimerman N (2008) Expression of fatty-acid modifying enzymes in halotolerant black yeast *Aureobasidium pullulans* (de Bary) G. Arnaud under salt stress. Stud Mycol 61:1–59

Gostinčar C, Grube M, Gunde-Cimerman N (2011) Evolution of fungal pathogens in domestic environments? Fungal Biol 115:1008–1018

Gostinčar C, Muggia L, Grube M (2012) Polyextremotolerant black fungi: oligotrophism, adaptive potential, and a link to lichen symbioses. Front Microbiol 3:390

Greenfield L (1981) Soil microbiological studies. In: Greenfield L, Wilson G (eds) University of Canterbury, Antarctic Expedition no. 19. Christchurch, pp 4–22

Gueidan C, Ruibal C, de Hoog GS, Gorbushina AA, Untereiner WA, Lutzoni F (2008) A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. Stud Mycol 61:111–119

Gueidan C, Ruibal C, de Hoog GS, Schneider H (2011) Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. Fungal Biol 115:987–996

Gunde-Cimerman N, Zalar P, de Hoog S, Plemenitaš A (2000) Hypersaline waters in salterns: natural ecological niches for halophilic black yeasts. FEMS Microbiol Ecol 32:235–240

Harutyunyan S, Muggia L, Grube M (2008) Black fungi in lichens from seasonally arid habitats. Stud Mycol 61:83–90

Isola D (2010) Biodiversity, phylogeny and evolution of rock black fungi. PhD thesis, Università degli Studi della Tuscia, Viterbo

Isola D, Marzban G, Selbmann L, Onofri S, Laimer M, Sterflinger K (2011) Sample preparation and 2-DE procedure for protein expression profiling of black microcolonial fungi. Fungal Biol 115:971–977

Isola D, Selbmann L, de Hoog GS, Fenice M, Onofri S, Prenafeta-Boldú FX, Zucconi L (2013) Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. Mycopathologia. doi:10.1007/s11046-013-9635-2

Kogej T, Wheeler MH, Rižner TL, Gunde-Cimerman N (2004) Evidence for 1,8-dihydroxy-naphthalene melanin in three halophilic black yeasts grown under saline and non-saline conditions. FEMS Microbiol Lett 232:203–209

Kogej T, Ramos J, Plemenitaš A, Gunde-Cimerman N (2005) The halophilic fungus *Hortaea werneckii* and the halotolerant fungus *Aureobasidium pullulans* maintain low intracellular cation concentrations in hypersaline environments. Appl Environ Microb 71:6600–6605

Kogej T, Gostinčar C, Volkmann M, Gorbushina AA, Gunde-Cimerman N (2006) Mycosporines in extremophilic fungi—novel complementary osmolytes? Environ Chem 3:105–110

Li DM, de Hoog GS, Lindhardt Saunte DM, Gerrits van den Ende AHG, Chen XR (2008) *Coniosporium epidermidis* sp. nov., a new species from human skin. Stud Mycol 61:131–136

Li DM, Li RY, de Hoog GS, Wang YX, Wang DL (2009) *Exophiala asiatica*, a new species from a fatal case in China. Med Mycol 47:101–109

Liu GY, Nizet V (2009) Color me bad: microbial pigments as virulence factors. TIM 17:406–413

Lyakh SP, Ruban EL (1970) Antarctic "black yeasts" *Nadsoniella nigra* var. *hesuelica* (characteristics and identification of strain 365). Izv Akad Nauk Biol 4:581–592

Marvasi M, Donnarumma F, Frandi A, Mastromei G, Sterflinger K, Tiano P, Perito B (2012) Black microcolonial fungi as deteriogens of two famous marble statues in Florence, Italy. Int Biodet Biodeg 68:36–44

Matos T, Haase G, Gerrits van den Ende AH, de Hoog GS (2003) Molecular diversity of oligotrophic and neurotropic members of the black yeast genus *Exophiala*, with accent on *E. dermatitidis*. A van Leeuwenhoek 83:293–303

Matsumoto T, Matsuda T, McGinnis MR, Ajello L (1984) Clinical and mycological spectra of *Wangiella dermatitidis* infections. Mycoses 36:145–155

Matsumoto T, Padhye AA, Ajello L, McGinnis MR (1986) *Sarcinomyces phaeomuriformis*: a new dematiaceous hyphomycete. J Med Vet Mycol 24:395–400

Mendoza L, Karuppayil SM, Szaniszlo PJ (1993) Calcium regulates in vitro dimorphism in chromoblastomycotic fungi. Mycoses 36:157–164

Moses V, Holm-Hansen O, Calvin M (1959) Non photosynthetic fixation of carbon dioxide by three microorganisms. J Bacteriol 77:70–78

Muller HJ (1964) The relation of recombination to mutational advantage. Mutat Res 1:2–9

Nienow JA, Friedmann EI (1993) Terrestrial litophytic (rock) communities. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, pp 343–412

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

Onofri S, Selbmann L, de Hoog GS, Grube M, Barreca D, Ruisi S, Zucconi L (2007) Evolution and adaptation of fungi at the boundaries of life. Adv Space Res 40:1657–1664

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

Onofri S, Selbmann L, Barreca D, Isola D, Zucconi L (2009) Fungal survival in space conditions: new insights for lithopanspermia. Plant Biosyst 143:S85–S87

Onofri S, Anastasi A, Del Frate G, Di Piazza S, Garnero N, Guglielminetti M, Isola D, Panno L, Ripa C, Selbmann L, Varese GC, Voyron S, Zotti M, Zucconi L (2011) Biodiversity of rock, beach and water fungi in Italy. Plant Biosyst 145:978–987

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

Palmer RJ, Friedman EI (1988) Incorporation of inorganic carbon by Antarctic cryptoendolithic fungi. Polarforschung 58:189–191

Palmer FE, Staley JT, Ryan B (1990) Ecophysiology of microcolonial fungi and lichens on rocks in Northeastern Oregon. New Phytol 116:613–620

Pedersen OA, Langvad F (1989) *Exophiala psychrophila* sp. nov., a pathogenic species of the black yeasts isolated from Atlantic salmon. Mycol Res 92:153–156

Plemenitaš A, Gunde-Cimerman N (2005) Cellular responses in the halophilic black yeast *Hortaea werneckii* to high environmental salinity. In: Gunde-Cimerman N, Oren A, Plemenitaš A (eds) Adaptation to life at high salt concentrations in Archaea, Bacteria, and Eukarya. Springer, Dordrecht, pp 455–470

Plemenitaš A, Vaupotič T, Lenassi M, Kogej T, Gunde-Cimerman N (2008) Adaptation of extremely halotolerant black yeast *Hortaea werneckii* to increased osmolarity: a molecular perspective at a glance. Stud Mycol 61:67–75

Prenafeta-Boldú FX, Summerbell RC, de Hoog GS (2006) Fungi growing on aromatic hydrocarbons: biotechnology's unexpected encounter with biohazard. FEMS Microbiol Rev 30:109–130

Richards RH, Holliman A, Helgason S (1978) *Exophiala salmonis* infection in Atlantic salmon *Salmo salar* L. J Fish Dis 1:357–368

Ruibal C, Gonzalo P, Bills GF (2005) Isolation and characterization of melanized fungi from limestone formations in Mallorca. Mycol Prog 4:23–38

Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, Groenewald JZ, Muggia L, Grube M, Isola D, Schoch CL, Staley JT, Lutzoni F, de Hoog GS (2009) Phylogeny of rock inhabiting fungi related to Dothideomycetes. Stud Mycol 64:123–133

Ruisi S, Barreca D, Selbmann L, Zucconi L, Onofri S (2007) Fungi in Antarctica. Rev Environ Sci Biotech 6:127–141

Selbmann L, Onofri S, Fenice M, Federici F, Petruccioli M (2002) Production and structural characterization of the exopolysaccharide of the Antarctic fungus *Phoma herbarum* CCFEE 5080. Res Microbiol 153:585–592

Selbmann L, de Hoog GS, Mazzaglia A, Friedmann EI, Onofri S (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic deserts. Stud Mycol 51:1–32

Selbmann L, de Hoog GS, Zucconi L, Isola D, Ruisi S, Gerrits van den Ende AHG, Ruibal C, De Leo F, Urzì C, Onofri S (2008) Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. Stud Mycol 61:1–20

Selbmann L, Isola D, Zucconi L, Onofri S (2011) Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. Fungal Biol 115:937–944

Selbmann L, Egidi E, Isola D, Onofri S, Zucconi Z, de Hoog GS, Chinaglia S, Testa L, Tosi S, Balestrazzi A, Lantieri A, Compagno R, Tigini V, Varese G (2012) Biodiversity, evolution and adaptation of fungi in extreme environments. Plant Biosyst 147:237–246

Selbmann L, Isola D, Egidi E, Zucconi L, Gueidan C, de Hoog GS, Onofri S (2013a) Mountain tips as reservoirs for new rock-fungal entities: *Saxomyces* gen. nov. and four new species from the Alps. Fungal Divers doi:10.1007/s13225-013-0234-9

Selbmann L, Grube M, Onofri S, Isola D, Zucconi L (2013b) Antarctic epilithic lichens as niches for black meristematic fungi. Biology 2:784–797

Sert HB, Sümbül H, Sterflinger K (2007a) Microcolonial fungi from antique marbles in Perge/Side/Termessos (Antalya/Turkey). A van Leeuwenhoek 91:217–227

Sert HB, Sümbül H, Sterflinger K (2007b) *Sarcinomyces sideticae*, a new black yeast from historical marble monuments in Side (Antalya, Turkey). Bot J Linn Soc 154:373–380

Sert HB, Sümbül H, Sterflinger K (2007c) A new species of *Capnobotryella* from monument surfaces. Mycol Res 111:1235–1241

Seyedmousavi S, Badali H, Chlebicki A, Zhao J, Prenafeta-Boldu′ FX, de Hoog GS (2011) *Exophiala sideris*, a novel black yeast isolated from environments polluted with toxic alkyl benzene and arsenic. Fungal Biol 115:1030–1037

Sonjak S, Frisvad JC, Gunde-Cimerman N (2006) *Penicillium* mycobiota in Arctic subglacial ice. Microb Ecol 52:207–216

Staley JT, Palmer F, Adams JB (1982) Microcolonial fungi: common inhabitants on desert rocks? Science 215:1093–1095

Sterflinger K (1998) Temperature and NaCl-tolerance of rock inhabiting meristematic fungi. A van Leeuwenhoek 74:271–281

Sterflinger K (2006) 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

Sterflinger K, Tesei D, Zakharova K (2012) Fungi in hot and cold deserts with particular reference to microcolonial fungi. Fungal Ecol 5:453–462

Sudhadham M, Prakitsin S, Sivichai S, Chaiwat R, Menken SBJ, Dorrestein GM, de Hoog GS (2008) The neurotropic black yeast *Exophiala dermatitidis* has a possible origin in the tropical rain forest. Stud Mycol 61:145–155

Sun SH, Huppert M, Cameron RE (1978) Identification of some fungi from soil and air of Antarctica. Antarct Res Ser 30:1–26

Tesei D, Marzban G, Zakharova K, Isola D, Selbmann L, Sterflinger K (2012) Alteration of protein patterns in black rock inhibiting fungi as a response to different temperatures. Fungal Biol 116:932–940

Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C, Smiraglia C, Vaughan-Martini A (2008) Psychrophilic yeasts in glacial environments of Alpine glaciers. FEMS Microbiol Ecol 63:73–83

Turk M, Abramovic Z, Plemenitas A, Gunde-Cimerman N (2007) Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. FEMS Yeast Res 7:550–557

Vadkertiova R, Slavikova E (1995) Killer activity of yeasts isolated from the water environment. Can J Microbiol 41:759–766

Van de Sande W, de Kat J, Ahmed A, Verbrugh H, van Belkum A (2006) Melanin protects *Madurella mycetomatis* against itraconazole and ketoconazole, firstline treatment agents against mycetoma. Ned Tijdschr Med Microbiol 14:520–521

Van Uden N (1984) Temperature profiles of yeasts. Adv Microbiol Physiol 25:195–251

Vicente VA, Attili-Angelis D, Pie MR, Queiroz-Telles F, Cruz LM, Najafzadeh MJ, de Hoog GS, Pizzirani-Kleine AR (2008) Environmental isolation of black yeast-like fungi involved in human infection. Stud Mycol 61:137–144

Weinstein RN, Montiel PO, Johnstone K (2000) Influence of growth temperature on lipid and soluble carbohydrate synthesis by fungi isolated from fellfield soil in the maritime Antarctic. Mycologia 92:222–229

Wollenzien U, de Hoog GS, Krumbein WE, Urzì C (1995) On the isolation of microcolonial fungi occurring on and in marble and other calcareous rocks. Sci Tot Environ 167:287–294

Xi L, Lu C, Sun J, Li X, Liu H, Zhang J, Xie Z, de Hoog GS (2009) Chromoblastomycosis caused by a meristematic mutant of *Fonsecaea monophora*. Med Mycol 47:77–80

Zalar P, Gostincar C, de Hoog GS, Ursic V, Sudhadham M, Gunde-Cimerman N (2008) Redefinition of *Aureobasidium pullulans* and its varieties. Stud Mycol 61:21–38

Zhao J, Zeng J, de Hoog GS, Attili-Angelis D, Prenafeta-Boldú FX (2010) Isolation of black yeasts by enrichment on atmospheres of monoaromatic hydrocarbons. Microb Ecol 60:149–156

Zhdanova NN, Zakharchenko VA, Vember VV, Nakonechnaya LT (2000) Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. Mycol Res 104:1421–1426