Luiz Henrique Rosa *Editor*

Fungi of Antarctica

Diversity, Ecology and Biotechnological Applications

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Preface

Banner clouds associated with Antarctic mountain photographs in the Austral summer of 2013 onboard of the Brazilian Oceanographic Polar Ship Almirante Maximiano (H-41) on the Gerlache strait. (Photo Credits: LH Rosa)

Antarctica is one of the most pristine regions on Earth, spanning 13.8 million km^2 and including different types of extreme environments. Despite its apparently homogenous landscape of ice, snow, and occasional areas of exposed soil, Antarctica has a variety of marine and terrestrial environments that encompass cold, dry, and oligotrophic extreme conditions where cryptic microbes live. In Antarctica, the most well-known organisms include only a few species of animals, several mosses, lichens, and two endemic angiosperms. Yet, cryptic microbial communities of viruses, archaea, bacteria, microalgae, and fungi represent the largest reservoir of biodiversity capable of survival in the extremities of the Antarctic environment. The adaptations, ecology, physiology, genetics, and evolution of these microorganisms under extreme conditions have been studied for several decades.

Kingdom Fungi includes eukaryotic organisms that are present in nearly all environments on Earth. Indeed, they can survive, colonize, and disperse in many substrata worldwide. Traditionally, fungi have been represented in Antarctica by taxa of the known traditional phyla *Chytridiomycota*, traditional *Zygomycota*, *Glomeromycota*, *Ascomycota*, *Basidiomycota*, and its anamorph forms (Kirk et al. 2008).

According to Vincent (2000), some Antarctic environments have remained isolated for hundreds of thousands of years or greater and represent an opportunity for exploring microbial evolution. Möller and Dreyfuss (1996) reported that the initial microbiological studies in Antarctica began with the first expedition sent to explore the South Pole, as documented by Ekelöf (1908) and Tsiklinsky (1908). Subsequently, the first published studies of Antarctic fungi focused on those organisms that were present in soil and freshwater (Tubaki and Asano 1965; Boyd et al. 1966; Heal et al. 1967; Sugiyama et al. 1967; Sun et al. 1978; Bailey and Wynn-Williams 1982; Ellis-Evans 1985; Montemartini-Corte 1991). More recently, many fungal species have been described in various substrates, such as different types of soil, rocks, ice, snow, sediments, freshwater and seawater, and in association with plants, macroalgae, and animals. Fungal communities in Antarctica appear to play roles in nutrient and carbon cycling in terrestrial and marine ecosystems and act as symbionts, mutualists, pathogens, and saprobes to form a complex biological web along with other organisms, representing an interesting model for studying the co-evolution of symbiosis under extreme conditions.

Despite the massive size of Antarctica, only a few mycological studies have been performed to date. Furthermore, these studies have mainly focused on selected habitats, such as those of the ultra-extreme conditions in continental regions. Throughout Antarctica, fungal diversity, geographical distribution, and evolution related to climate changes, as well as biotechnological applications of Antarctic fungi, have yet to be thoroughly examined. The aim of this book is to present the vanguard aspects of diversity, ecology, and biotechnology of Antarctic fungi. Experts in Antarctic mycology from several countries (i.e., Brazil, Argentine, Chile, Uruguay, the United States, Italy, Belgium, England, and the Netherlands) describe and discuss fungal occurrence in nonliving and living substrates/habitats. Applied topics such as antibiotics and enzymes produces by fungi and genomics are also addressed.

In recent years, the capability of Antarctic fungi to produce compounds for use in biotechnological processes has been explored. Antibiotics, pesticides, antioxidant fatty acids, photoprotective pigments, enzymes, anti-freeze proteins, polysaccharides, and other useful biotechnological compounds produced by Antarctic fungi have great potential to be used in industrial, medicine, and agriculture sectors. The search for extremophile fungi, considered living factories of bioproducts, in Antarctica may represent a useful strategy for finding new eukaryotic metabolic pathways and, consequently, new compounds for use in biotechnology.

Additionally, studies in Antarctica mycology indicate that, despite the extreme conditions, fungi display high diversity, which is still poorly understood. Further studies on its distribution, ecological role, adaptation to extreme conditions, behavior under the climate changes effects, intrinsic pathogenic potential for plants and humans, and biotechnological use are growing within the Polar sciences.

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Belo Horizonte, Minas Gerais, Brazil — Luiz Henrique Rosa

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Chapter 1
Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds

Luiz Henrique Rosa, Carlos Leomar Zani, Charles Lowell Cantrell, Stephen Oscar Duke, Patrick Van Dijck, Alessandro Desideri, and Carlos Augusto Rosa

1.1 Taxonomy, Diversity, and Ecology of Antarctic Fungi

In Antarctica, microorganisms dominate food chains in several different pristine ecosystems. In these ecosystems, fungi occur as two known basic forms (i) filamentous fungi and (ii) yeasts, which display colonies with different morphologies and colours (Fig. 1.1). Such colonies demonstrate a high degree of genetic plasticity that allows them to survive under extreme conditions of low temperatures, high UV irradiation, freeze-thaw cycles, different pH levels, strong winds, dehydration, osmotic stress, and low nutrient concentrations (Fell et al. 2006).

The fungal assemblages of Antarctica include taxa that belong to the major fungal groups, which were reported by Kirk et al. (2008) to be *Ascomycota*, *Basidiomycota*, traditional *Zygomycota*, *Chytridiomycota*, and *Glomeromycota*;

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Fig. 1.1 Antarctic fungi and their diverse colony macro-morphologies

some assemblages also include the stramenopiles (*Oomycota*) and slime moulds (*Mycetozoa*) traditionally studied by mycologists (Bridge and Spooner 2012). However, as modern taxonomic studies consider phylogenetic analysis and the characterisation of uncultivable taxa, this taxonomic hierarchy is changing. Tedersoo et al. (2018) proposed, using phylogenies and divergence time estimates, the following 18 phyla for fungi: *Ascomycota*, *Aphelidiomycota*, *Basidiobolomycota*, *Basidiomycota*, *Blastocladiomycota*, *Calcarisporiellomycota*, *Chytridiomycota*, *Glomeromycota*, *Entomophthoromycota*, *Entorrhizomycota*, *Kickxellomycota*, *Monoblepharomycota*, *Mortierellomycota*, *Mucoromycota*, *Neocallimastigomycota*, *Olpidiomycota*, *Rozellomycota*, and *Zoopagomycota*. In contrast to fungi from tropical and temperate environments, the fungi present in Antarctica still represent an unknown proportion of diversity and potentially new phyla that may yet be discovered. Bridge and Spooner (2012) estimated that over 1000 fungal species (without

lichens) have been previously recorded in Antarctica and suggested that the true diversity of Antarctic fungi may be far greater than currently estimated.

Most taxonomic studies of Antarctic fungi have included the use of molecular biology techniques. DNA is extracted from mycelia using protocols similar to those proposed by de Hoog et al. (2005) and Rosa et al. (2009), followed by sequencing of the internal transcribed spacer (ITS) region according to protocols established by White et al. (1990). The ITS represents the most common and accepted DNA barcoding marker for the identification of fungi, and ITS sequencing has been frequently used to identify fungi (Gazis et al. 2011). However, some fungal species of the genera *Penicillium*, *Aspergillus*, and *Cladosporium*, which represent abundant cosmopolitan cold-adapted taxa living in Antarctica, are genetically very similar, and the ITS region is not sufficiently variable to separate them at the species level. For this reason, other more variable DNA regions such as partial β-tubulin II (TUB2), γ-actin (ACT), translation elongation factor 1-α (TEF1α), RNA polymerase II (partial RPB2), elongation factor 3 (TEF3), topoisomerase I (TOPI), and phosphoglycerate kinase (PGK) can be sequenced to identify these fungi at the species level (Stielow et al. 2015). Although yeasts are also fungi, they are identified using different protocols. Yeasts are morphologically and physiologically characterised using standard methods (Kurtzman et al. 2011), followed by an analysis of the DNA region spanning the ITS-5.8S and rRNA gene D1/D2 domains according to protocols established by White et al. (1990) and Lachance et al. (1999).

However, such molecular biology taxonomy procedures are dependent on the parameters used by each mycologist. For these reasons, Godinho et al. (2013) proposed uniform criteria to interpret sequences from the GenBank database: for query coverage and sequence identities ≥99%, the genus and species were accepted; for coverage and sequence identities = 98%, the genus and species were accepted, but the term 'cf.' (Latin for confer = compares with) is used to indicate that the specimen resembles, but has certain minor features not found in the reference species; for query coverage and sequence identities between 95% and 97%, only the genus was accepted; for query coverage and sequence identities ≤95%, the isolates were labelled with the order or family name or as 'unknown' fungi. Additionally, phylogenetic analyses can be conducted to estimate the evolutionary distance between the sequences of Antarctic fungi and those sequences of type species deposited in the GenBank database. Molecular biology methods are also recommended for those fungi that do not produce conidia or spores when cultured on common mycological media.

Although molecular biology methods represent the main taxonomic tool used to identify Antarctic fungi, classical macro- and micro-morphological and physiological methods can be necessary in some cases. Some cosmopolitan cold-adapted fungi show the same query coverage and identities percentage, so they have been assigned to two or more species when compared with sequences deposited in GenBank. For these cases, it is necessary to characterise fungal macro- and micro-morphological structures and perform a physiological characterisation on different mycological media, such as potato dextrose, cornmeal, malt extract, potato dextrose, Sabouraud dextrose, and yeast extract sucrose agars, to define the species level.

Those studies conducted to date show that fungal diversity differed between the Antarctica Peninsula and continental Antarctica environments. The extreme conditions of the Antarctic Peninsula are milder than those of the continental regions. Additionally, the Antarctic Peninsula has more life forms, such as plants, macroalgae, invertebrates, and vertebrates, supplying this region with organic matter and nutrients and, consequently, creating several ecological niches and microenvironments for different fungal webs to survive. In contrast, in continental Antarctica, organic matter and nutrients are extremely scarce because of the near absence of life forms; moreover, the soils, rocks, snow, and ice are ultra-oligotrophic. According to Rao et al. (2012), life in continental Antarctica is restricted to the rare occurrence of some species of lichens, mosses, invertebrates, and soil microbial communities. Fungi in continental Antarctica have been more often described in lichen symbioses, while the occurrence of free-living fungi in soils remains poorly understood (Godinho et al. 2015).

Cold habitats are dominated by cold-adapted (psychrophilic) and cold-tolerant (psychrotolerant) microorganisms (Harding et al. 2011). Mycological studies in Antarctica have reported the occurrence of few endemic, psychrophilic species, with the majority being cosmopolitan psychrotolerant taxa. According to Ruisi et al. (2007), the endemic Antarctic fungal species are characterised as true psychrophilic fungi that are only able to actively grow and reproduce in specific Antarctic environments. The cosmopolitan psychrotolerant fungi are ecotypes with mesophilic-psychrotolerant behaviour resulting from an adaptation to the cold Antarctic climate (Zucconi et al. 1996). According to these criteria, species like *Metschnikowia australis*, *Antarctomyces psychrotrophicus*, *Antarctomyces pellizariae*, *Cryomyces antarcticus*, *Friedmanniomyces simplex*, *Friedmanniomyces endolithicus*, *Mortierella antarctica*, *Penicillium antarcticum*, *Penicillium tardochrysogenum*, *Thelebolus globosus*, *Thelebolus ellipsoideus*, *Thelebolus balaustiformis*, and *Thelebolus spongiae* are considered to be endemic psychrophilic species. By contrast, different species of *Penicillium* (e.g. *P. chrysogenum*), *Aspergillus* (*A. fumigatus*), *Cladosporium* (*C. sphaerospermum)*, *Colletotrichum* (*Co. gloeosporioides*), and *Rhodotorula* (*R. mucilaginosa*) are considered to be cosmopolitan cold-tolerant taxa that have adapted to the cold Antarctic climate. Additionally, some psychrophilic taxa of polar or temperate occurrence occur in Antarctica such as *Pseudogymnoascus destructans* and *Mortierella alpina*. The ecological, biochemical, physiological, and genetic peculiarities of certain endemic, cosmopolitan cold adapted and polar fungi have been studied. Among them are *M. australis*, *A. psychrotrophicus* and *A. pellizariae* (endemic), *P. destructans* (cold regions), and *P. chrysogenum* (cosmopolitan cold adapted).

The yeast *M. australis* is always reported in marine ecosystems (Fell and Hunter 1968; Gonçalves et al. 2017), similar to *Euphausia superba*, as species that colonise the stomach of Antarctic krill (Donachie and Zdanowski 1998) and macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014) in lakes next to the sea that have influence on marine spray (Vaz et al. 2011; Gonçalves et al. 2012). The isolation of *M. australis* in abundant association with several Antarctic macroalgae from different areas of the Antarctic Peninsula supports the possibility that this

yeast may have a specific ecological association with Antarctic macroalgae in the marine environment.

The genus *Antarctomyces* has only two species reported to be endemic for Antarctica: *Antarctomyces psychrotrophicus* (isolated for the first time from the soil) and *A. pellizariae* (from snow) in the South Shetland Islands, King George Island (Stchigel et al. 2001; de Menezes et al. 2017). *Antarctomyces psychrotrophicus* produces an antifreeze protein, similar to those produced by polar fish, with potential uses in biotechnological processes (Xiao et al. 2010). *Antarctomyces pellizariae* (Fig. 1.2) produces a rare, blue pigment with possible uses in the food industry (de Menezes et al. 2017).

Pseudogymnoascus species are abundant in Antarctica and occur in different substrates and environments, including the soils (Mercantini et al. 1989), mosses (Tosi et al. 2002), leaves of *Colobanthus quitensis* (Rosa et al. 2010), thalli of macroalgae (Loque et al. 2010), freshwater lakes (Gonçalves et al. 2012), and lichens

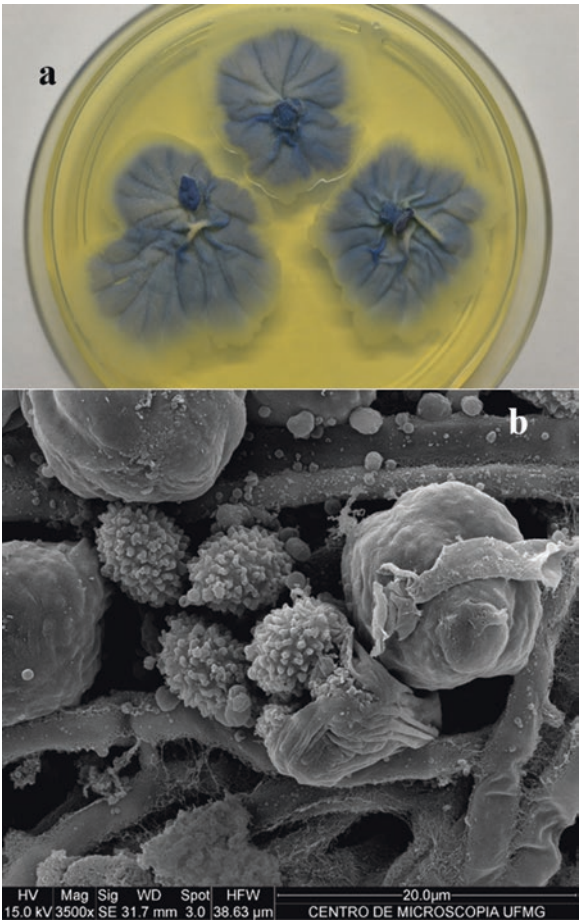

Fig. 1.2 (**a**) Blue colony of A*ntarctomyces pellizariae* and (**b**) a scanning electron microscopic image of its ascospores and ascus. (Photos Credits: LH Rosa)

(Santiago et al. 2015). Several published studies have reported several *Pseudogymnoascus* taxa identified only at the genus level. Moreover, many of these unidentified taxa may represent new species, different from those reported from the northern hemisphere, resulting from a lack of a critical taxonomic evaluation of the diversity of *Pseudogymnoascus*. Within the genus *Pseudogymnoascus*, *P. destructans*, which is characterised as a psychrophilic pathogenic fungus that has led to a reduction in bat populations as the causative agent of white-nose syndrome (WNS) in temperate regions (Lorch et al. 2011), is abundantly found in different substrates and regions of Antarctica. Zukal et al. (2016) reported that symptoms caused by *P. destructans* in the bats of North America and Europe/Palearctic Asia are different. However, no data about the virulence of *P. destructans* strains from Antarctica is yet available, which represents a major gap in knowledge owing to concern about their pathogenic potential (Gomes et al. 2018). Since Lorch et al. (2013) and Minnis and Lindner (2013) suggested that the diversity of *Pseudogymnoascus* seems to be greater than previously reported, those species found in Antarctica may represent new endemic species that play a previously unknown ecological role in Antarctic environments.

1.2 Effects of Climate Changes on Fungi that Are Resident to Antarctica

In recent years, concerns about global climate change have increased worldwide. Several studies demonstrated that Arctic regions and the Antarctic Peninsula represent two of the regions on Earth with the fastest changing climates, and the warming of these regions is likely to have a profound influence on terrestrial and marine environments (Clarke et al. 2006). Consequently, all biota living in these ecosystems are subject to varying effects of climate change. Another concern about Antarctica is the introduction of alien species owing to both climate changes and tourism, which can affect the resident Antarctic biota in terms of distribution and ecological roles.

Mycological studies have demonstrated that the fungal assemblages of different environments of Antarctica are composed of both cold-adapted and endemic taxa, which display interesting dynamics of richness, dominance, and similarity patterns. According to Fell et al. (2006), the different types of soils of Antarctica offer an interesting opportunity to investigate the regional to global environmental effects of microbial webs on community structures.

Godinho et al. (2013) and Furbino et al. (2014) observed that algicolous fungi assemblages, associated with endemic Antarctic macroalgae of the Antarctic Peninsula, are composed of few endemic and many cosmopolitan cold-adapted fungi. Based on these taxonomic and ecological observations, they proposed that the reduction of endemic or cold-adapted fungal species, associated with an increase of mesophilic cosmopolitan taxa within the fungal assemblages associated with endemic macroalgae, may reflect the influence of climate change in the maritime

Antarctic Peninsula. They also proposed that analyses of the balance and dynamics of richness, dominance, and distribution among endemic, cold-adapted, or cosmopolitan fungal taxa could be used as model to understand the influence of climate change on maritime Antarctica.

However, since Antarctica represents one of the most pristine extreme environments of the planet, there is a consensus that several new, undescribed microorganism taxa or strains may occur in its different regions, which could display novel physiological, biochemical, and genetic characteristics. Since the discovery of *Bacillus anthracis* in thawing permafrost of Siberia in Russia (Revich and Podolnaya 2011), different studies have focused on the microorganisms present in pristine environments under the effects of global climate change and their potential capabilities to cause diseases in animals or crop plants.

In Antarctica, few studies have been published to date on the pathogenic potential of resident microbiota, especially resident fungi. However, published studies on Antarctic fungal diversity have shown the presence of taxa phylogenetically near to fungi that are capable of causing diseases in plants and animals.

The first studies of fungi associated with Antarctic plants (mosses and the angiosperms *Deschampsia antarctica* and *Colobanthus quitensis*) and lichens were published by Pugh and Allosopp (1982), Fletcher et al. (1985), Gamundi and Spinedi (1988), Onofri and Tosi (1989), Del Frante and Caretta (1990), Baublis et al. (1991), and Möller and Dreyfuss (1996). All of these studies detected taxa of the genera *Alternaria*, *Botrytis*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phaeosphaeria*, and *Phoma* but reported no correlation with potential plant pathogens. Additionally, the endophytes most frequently recovered from the angiosperms *D. antarctica* (Rosa et al. 2009) and *Colobanthus quitensis* (Rosa et al. 2010) are strains of *Alternaria*, *Fusarium*, *Microdochium*, *Mycocentrospora*, and *Phaeosphaeria*, which represent genera that can cause diseases in important crop plants worldwide (Prasada and Prabhu 1962; Thomma 2003). Species of *Microdochium* have been described as pathogens able to cause diseases in cereal and turf grasses of cold regions (Mahuku et al. 1998), *Fusarium* spp. are well-known plant pathogens (Zhong-shan et al. 2008), and *Mycocentrospora* spp. have widespread distribution that can be pathogenic in several plant species (Ananda and Sridhar 2002).

Recently, Gonçalves et al. (2017), Sousa et al. (2017), and Alves et al. (2019) studied fungal diversity in rocks and ornithogenic soils of Antarctica and the physiological opportunistic virulence potential in vitro of the fungi for animals. Gonçalves et al. (2017) recovered from rocks of continental Antarctica the fungal taxa *Acremonium* sp., *Debaryomyces hansenii*, *P. chrysogenum*, *P. citrinum*, *P. tardochrysogenum,* and *R. mucilaginosa*, which are able to grow at 37 °C. Additionally, different isolates of *P. chrysogenum*, *P. citrinum*, and *P. tardochrysogenum* could produce partial haemolysis on blood agar at 37 °C; *Acremonium* sp., *P. citrinum*, and *P. tardochrysogenum* had spore sizes ranging from 2.81 to 5.13 μm in diameter at 37 °C; and *P. chrysogenum* and *P. tardochrysogenum* displayed macro- and micro-morphological dimorphism. Additionally, from 50 rock samples of the Antarctic Peninsula, Alves et al. (2019) obtained 155 fungi able to grow at 37 °C, which were identified as *P. chrysogenum*, *Fusarium* sp., and *R. mucilaginosa*.

Additionally, 103 fungi exhibited haemolytic activity, 81 produced proteinase and 9 phospholipase, and 25 were dimorphic with spore diameters ≤4 μm.

Sousa et al. (2017) recovered 50 fungi that were able to grow at 37 °C, from the ornithogenic soil nests of bird species *Phalacrocorax atriceps*, *Macronectes giganteus*, *Pygoscelis antarcticus*, and *P. papua* in the Antarctic islands. Among the different species, *A. fumigatus*, *P. chrysogenum*, *Cryptococcus laurentii*, and *R. mucilaginosa* were the most abundant. Isolates of *A. fumigatus* and *Cr. laurentii* were able to grow at different pH values. *Aspergillus fumigatus* (Fig. 1.3) produced spores ≤1 μm, and the amphotericin B minimum inhibitory concentration (MIC) for this species varied from 0.5 and 1 μg mL^{-1}. *Cryptococcus laurentii* produced phospholipase and had haemolytic activities; they also produced a capsule, and the

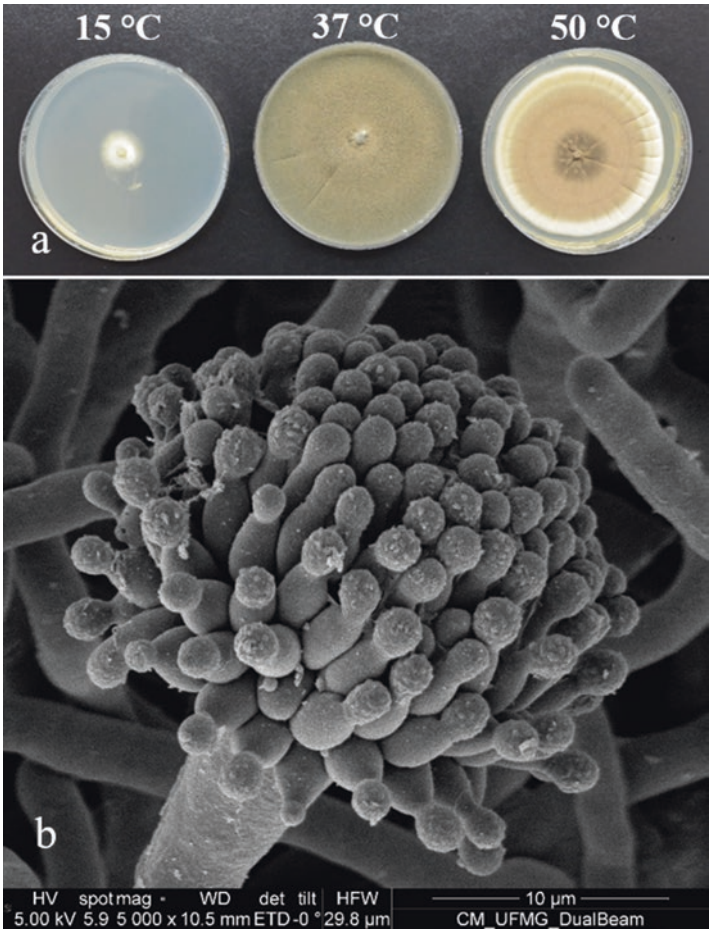

Fig. 1.3 *Aspergillus fumigatus* isolated from ornithogenic soil of Antarctica. (**a**) Colonies at different growth temperatures and (**b**) details of asexual reproductive structures are revealed by scanning electron microscopy. (Photos Credits: LH Rosa)

amphotericin B MIC for this species was 2 μg mL^{-1}. Additionally, isolates of *P. chrysogenum* could grow at 37 °C at different pH ranges, cause partial haemolysis and display polymorphism of its colonies and macro- and micro-morphologies. The spores were ≤3.07 μm, and the amphotericin B MIC for this species was 2 μg mL^{-1}.

These studies demonstrate that the Antarctic environments shelter different fungi that are phylogenetically close to species pathogenic to plants and animals. Since Antarctica is subject to the effects of the global climate changes, unreported and cryptic fungi, including those with innate pathogenic potential, may disperse from Antarctica by animals (birds), air, and tourists and then mainly spread to South America and Oceania.

1.3 Potential of Antarctic Fungi as a Pipeline to New Drugs and Agrochemicals

Microorganisms, including fungi, are promising sources of useful, new pharmaceuticals and agrochemicals. Some Antarctic bioprospecting studies have been conducted, and different organisms with potential uses in biotechnological processes have been identified. According to Santiago et al. (2012), the ability of Antarctic fungi to survive in extreme conditions suggests that they may display unusual biochemical pathways that allow them to generate new compounds. Within the Antarctic fungal communities, some taxa were detected as promising producers of bioactive secondary metabolites with potential uses as prototypical structures to develop new drugs and agrochemicals. Among them, we can report species of *Aspergillus*, *Cladosporium*, *Penicillium*, *Pseudogymnoascus*, *Phaeosphaeria*, *Microdochium*, *Mortierella*, and *Purpureocillium*.

Recently, different screening studies have been conducted with Antarctica fungi in recent years that have demonstrated their great potential in drug discovery programmes. Santiago et al. (2012) studied the capabilities of Antarctic endophytic fungi recovered from *D. antarctica* to produce bioactive secondary compounds against neglected tropical diseases and tumour cells. They discovered that extracts of the endophytic fungus *Phaeosphaeria herpotrichoides* showed selective leishmanicidal activity with an IC_{50} value equivalent to that of amphotericin B. Additionally, *Microdochium phragmitis* extracts had specific cytotoxic activity against the UACC-62 human cancer cell line. In addition, extracts from solid fermentation processes derived from cultures of *Pseudogymnoascus* strains displayed selective antifungal activities against *Candida albicans*, *Candida krusei*, and *C. sphaerospermum* (Furbino et al. 2014). An extract of *Purpureocillium lilacinum*, isolated from Antarctic soil, exhibited high trypanocidal, antifungal, and antibacterial activities, with moderate toxicity over normal human cells. Proton nuclear magnetic resonance (^{1}H NMR) spectral analysis indicated the presence of compounds containing a highly functionalised aromatic ring system (Gonçalves et al. 2015) (Fig. 1.4).

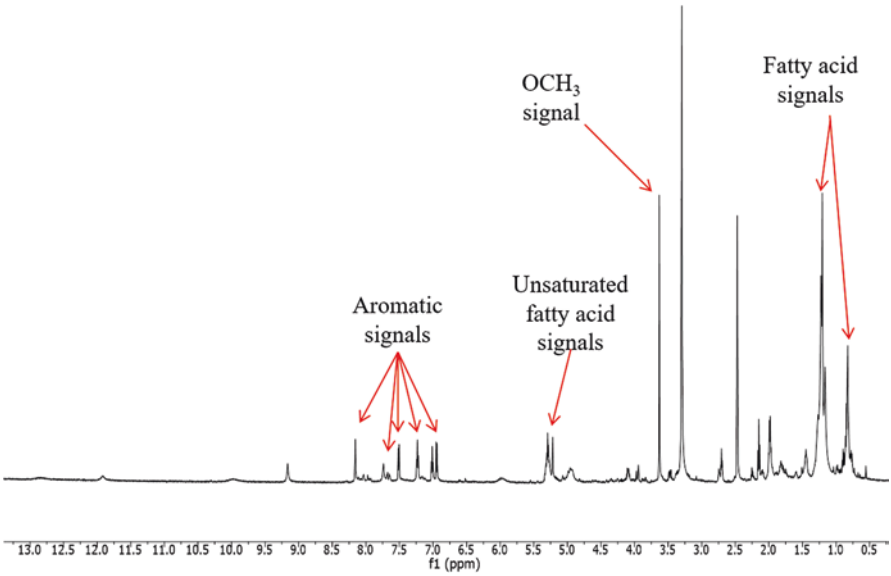

Fig. 1.4 ^{1}H NMR spectrum (600 MHz, DMSO-d_6) of methylene chloride bioactive extracts from freeze-dried culture medium of the Antarctic fungus *Purpureocillium lilacinum*, isolated from soil of the Antarctic Peninsula. Regions of interest are labelled above the corresponding signals

Gomes et al. (2018) assayed 218 fungal extracts to detect the presence of antiviral activity against dengue and Zika viruses, antiparasitic activity against *Trypanosoma cruzi* and *Leishmania amazonensis*, and herbicidal activity. Among them, extracts of *P. destructans*, *Mortierella parvispora*, and *P. chrysogenum* inhibited the growth of the trypomastigote and amastigote forms of *T. cruzi* to a similar degree as the control drug. Extract of *P. destructans* and *P. tardochrysogenum* showed strong and selective herbicidal activity against *Allium schoenoprasum* and *Lactuca sativa*. *Penicillium tardochrysogenum* (Fig. 1.5) is an endemic species of Antarctica producing penicillin, secalonic acids D and F (Fig. 1.6) (Houbraken et al. 2012). Different fungal strains of *Pseudogymnoascus*, *Penicillium*, *Cadophora*, *Paraconiothyrium*, and *Toxicocladosporium*, obtained from soil and marine sediments of Antarctica, could produce antimicrobial inhibitory compounds against the phytopathogen bacteria *Xanthomonas citri* (Vieira et al. 2018).

Among the fungi living in Antarctica, *Penicillium* are likely the most abundant and widespread in different environments and substrates in Antarctica. *Penicillium* are producers of bioactive compounds, but few species found in Antarctica have been investigated at the chemical level for this potential application. Brunati et al. (2009) showed that *P. chrysogenum*, obtained from Antarctic lakes, produces selective antimicrobial activities against *Staphylococcus aureus*, *Enterococcus faecium*, and *Escherichia coli*. Extracts of *P. chrysogenum* recovered from the endemic Antarctic macroalgae *Palmaria decipiens* displayed high and selective antifungal and/or trypanocidal activities (Godinho et al. 2013). *Penicillium steckii*, obtained

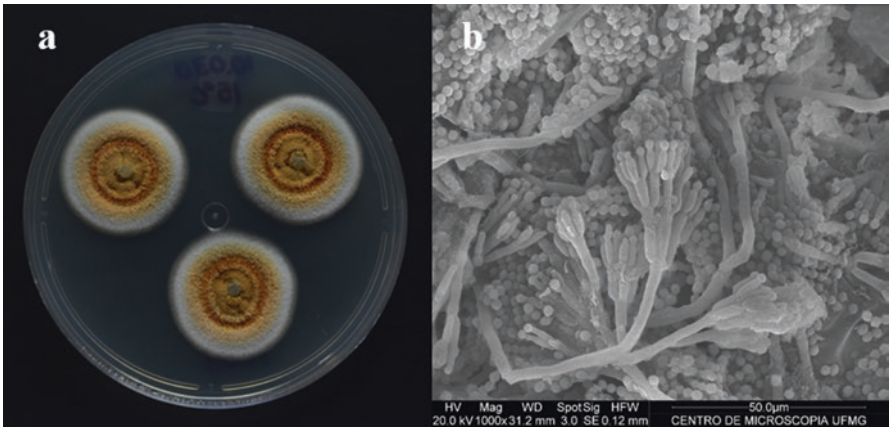

Fig. 1.5 (**a**) Colonies and (**b**) scanning electron microscopic images of the asexual reproductive structures of *Penicillium* sp. isolated from Antarctica. (Photos Credits: LH Rosa)

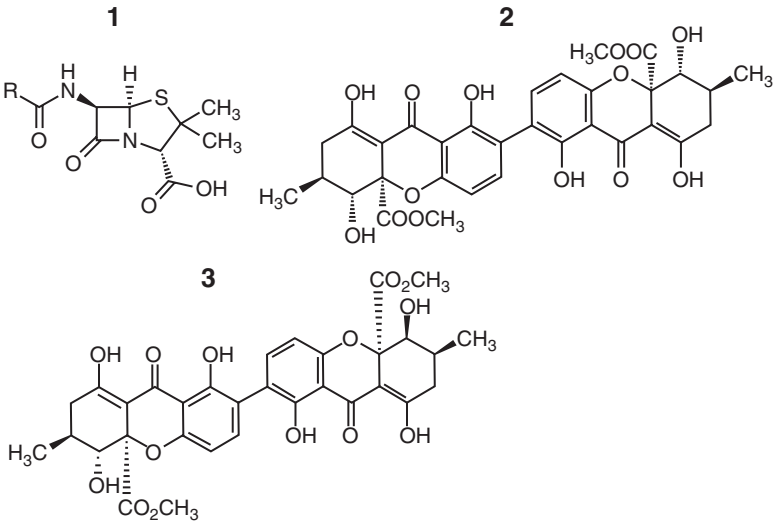

Fig. 1.6 Chemical structures of (**1**) penicillin, (**2**) secalonic acid D, and (**3**) secalonic acid

from the Antarctic macroalgae *Monostroma hariotti*, was reported as a producer of compounds with antiviral activity against yellow fever virus (Furbino et al. 2014). Extracts of different wild and pristine isolates of *A. sydowii*, *P. allii-sativi*, *P. brevi-compactum*, *P. chrysogenum*, and *P. rubens* displayed antiviral, antimicrobial (antibacterial and antifungal), anticancer, antiprotozoal, and herbicidal activities, with equal or greater activity compared to control drugs (Godinho et al. 2015). The bioactive extracts of these fungi were examined by ^{1}H NMR spectroscopy, and the presence of highly functionalised secondary metabolites was found as indicated by

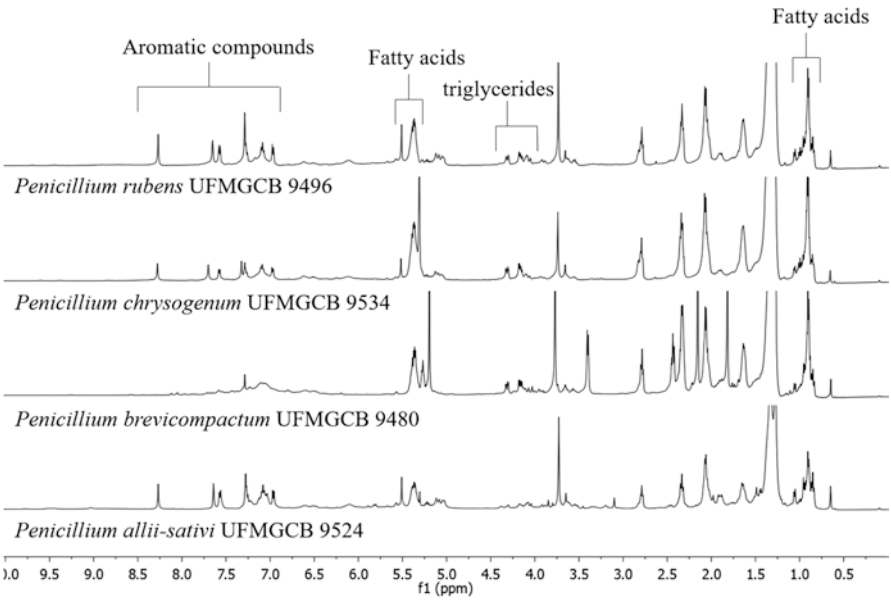

Fig. 1.7 ^{1}H NMR spectrum (500 MHz in $CDCl_3$) of representative crude extracts from wild *Penicillium* sp. isolated from soil of continental Antarctica. Labels indicate regions indicative of protons belonging to specific compounds

the presence of protons in the aromatic and olefinic regions (Fig. 1.7). *Penicillium* spp. are well known to be cosmopolitan, and some of them are abundant and adapted to the extreme conditions of Antarctica. *Penicillium* species are known to produce important bioactive compounds; however, few Antarctica *Penicillium* have been investigated at chemical level.

The number of bioactive compounds identified from strains of Antarctic fungi is increasing. Li et al. (2008) isolated geomycins B and C from *Pseudogymnoascus* sp., obtained from Antarctic soil that displayed antifungal activity against *A. fumigatus* and antibacterial activity against *S. aureus*, *Escherichia coli*, and *Streptococcus pneumonia*. *Pseudogymnoascus pannorum* from leaf litter produces pannomycin (Fig. 1.8), a cis-decalin secondary metabolite with potential antibacterial activity against *S. aureus* (Parish et al. 2009).

The compounds (pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)) produced by *Mortierella alpina* strains and recovered from the Antarctic moss *Schistidium antarctici* displayed strong antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* with low MIC values (Melo et al. 2014). The Antarctic soil fungus *Aspergillus ochraceopetaliformis* produced the antiviral secondary metabolites ochraceopone A, isoasteltoxin, and asteltoxin (Fig. 1.9) with activities against the H1N1 and H3N2 influenza viruses (Wang et al. 2016). Liu et al. (2019) isolated a secondary compound from *Penicillium crus-*

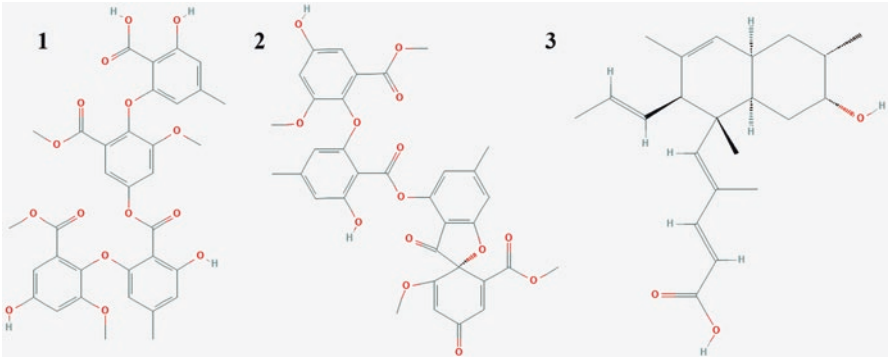

Fig. 1.8 Bioactive compounds (**1**) geomycin B, (**2**) geomycin C, and (**3**) pannomycin produced by *Pseudogymnoascus* species from Antarctica

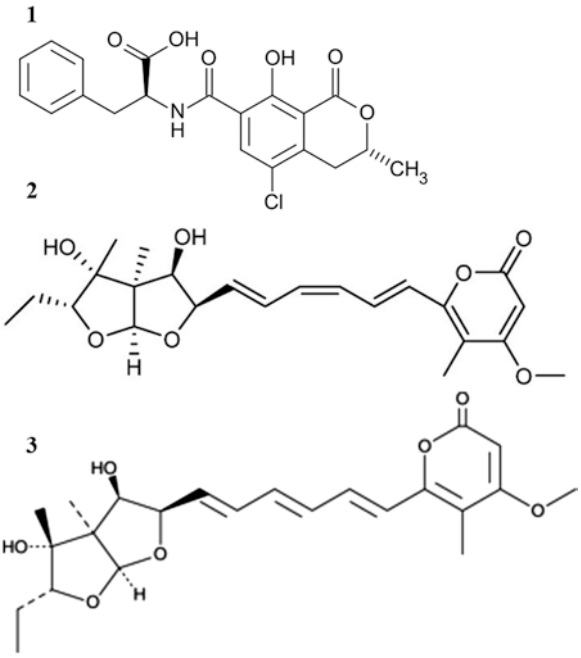

Fig. 1.9 Antiviral secondary metabolites (**1**) ochraceopone A, (**2**) isoasteltoxin, and (**3**) asteltoxin produced by the Antarctic soil fungus *Aspergillus ochraceopetaliformis*

tosum, found in Antarctic marine sediments, that was a new diketopiperazine showing cytotoxicity towards K562 human cancer cells. Lin et al. (2014) purified, from a crude extract of Antarctic deep-sea fungus *Penicillium* sp., the cytotoxic compounds eremophilane-type sesquiterpene and eremofortine C (Fig. 1.10), which exhibited cytotoxicity activities against HL-60 human cells with IC_{50} values of 45.8 and, 28.3 μM, respectively.

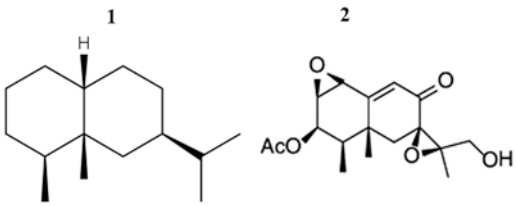

Fig. 1.10 Eremophilane-type (**1**) sesquiterpene and (**2**) eremofortine C cytotoxic compounds produced by *Penicillium* sp.

1.4 Conclusions and Perspectives

Recently, interest in studying the fungal communities from different Antarctic environments has increased. Published data have revealed the presence of fungi in all of the studied substrate/host microhabitats, demonstrating that fungi may occur in virtually all regions of Antarctica. Additionally, all of the studies conducted to date demonstrate that Antarctic fungal diversity is relatively high, which is remarkable considering the different extreme conditions to which they are constantly exposed. Antarctic fungal assemblages are predominantly composed of cosmopolitan cold-adapted taxa, but many endemic species have been newly described, suggesting that Antarctica remains yet to be fully characterised source of fungal and microbial diversity. Different Antarctic fungal assemblages fit a variety of ecological niches and roles, such as mutualists, decomposers, or pathogens of plants and animals. Additionally, several Antarctic fungi can produce secondary metabolites with various biological activities. These fungi represent potential biological "factories" that can produce compounds with great potential for direct use in medicine and agriculture or as prototypical molecules that can be chemically modified for pharmaceutical and agrochemical applications.

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Chapter 2
Uncultivated Fungi from Antarctica

Alysson Wagner Fernandes Duarte, Victor Borin Centurion, and Valéria Maia Oliveira

2.1 Introduction

Antarctica is a remote and inhospitable continent that harbours the coldest and driest climate known on Earth (Rogers et al. 2007). This continent holds ~90% of the ice on Earth, which is equivalent to ~60–70% of the available freshwater on the planet. Only 1–3% of the Antarctic surface is free of ice in summer months, and most of these areas are rocky deserts and rocky ice-covered mountains (Yergeau et al. 2007; Singh et al. 2018).

Antarctica is located below parallel 60°S. Antarctic regions can be differentiated according to parameters that include soil type, geology, and glaciology (Rignota et al. 2019). From a biogeographic point of view, the most accepted classification scheme divides Antarctica into two regions—continental Antarctica and maritime Antarctica (Singh et al. 2015). The former region is located in the innermost part of the continent, characterized by high latitude and a layer covered with ice reaching more than 4250 m, which results in the most rigorous living conditions on the continent. The latter region includes a clearly defined maritime region that encompasses the Antarctic Peninsula and has longer and warmer summers than the mainland, as well as marine influences (Bolter et al. 2002).

One of the most inhospitable characteristics of the Antarctic environment is low temperatures, which can drop to −50 °C to −20 °C in the winter in the McMurdo Dry Valleys, and the monthly average temperature is below 0 °C. Additionally, frequent

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freeze-thaw cycles in marine Antarctica, strong winds, oligotrophy, aridity, high sublimation and evaporation, and periods of high incidence of UV radiation followed by prolonged dark periods represent major factors that limit the development of any form of life in the harsh Antarctic environment (Bolter et al. 2002; Onofri et al. 2007). These environmental conditions are not homogeneous and vary in different niches of the Antarctic continent, which include diversity in the types of soils, sediments, rocks, snow, and ice that can each vary in thermal properties, nutrient availability, hydration, and salinity (Ruisi et al. 2007; Koo et al. 2018).

2.2 Antarctic Microbial Diversity: Cultivation-Dependent and Cultivation-Independent Approaches

For many years, assessments of the biological diversity in Antarctica have focussed on organisms such as fish, birds, and marine mammals, while microorganisms were rarely considered (Vicent 2000). Recently, as a consequence of marked advances in molecular techniques for the study of microbial diversity in nature, knowledge about evolution, endemism, invasion, and microbial selection in the Antarctic environment has accumulated (Richter et al. 2014; Bendia et al. 2018; Coleine et al. 2018a).

Biogeochemical cycles and food chains in environments with restrictive characteristics, such as the ones found in Antarctica, often neglect the role of microorganisms, which play fundamental roles in energy transport, organic matter recycling, and mineralization of nutrients that are necessary for different ecological niches to function (Ruisi et al. 2007; Yergeau and Kowalchuk 2008; Bridge and Spooner 2012; Selbmann et al. 2017; Koo et al. 2018). Antarctic fungi are decomposers of organic matter and also represent a major proportion of the microbial biomass in samples introduced to Antarctica, such as historic woods and surrounding soils (Ludley and Robinson 2008; Blanchette et al. 2010; Arenz et al. 2006, 2011; Farrell et al. 2011; Held and Blanchette 2017). Cultivation-independent studies of fungal diversity have been carried out with both exotic samples, such as wood, and indigenous samples from natural Antarctic environments (Fig. 2.1).

Fungi that inhabit Antarctica have developed a huge metabolic diversity that has allowed them to colonize different available niches, including different types of desert, oligotrophic and ornithogenic soils, sediments, rocks of different typologies (such as sandstone, granite, dolerite, quartz, and lava-dike), snow, ice, permafrost, and vegetation (such as mosses, lichens, bryophyte, and flowering plants, including *Colobanthus quitensis* and *Deschampsia antarctica*) (Ruisi et al. 2007; Coleine et al. 2018a).

The majority of studies of Antarctic fungi have been described using cultivation-dependent methods (Rosa et al. 2009, 2010; Arenz and Blanchette 2011; Godinho et al. 2013; Connell et al. 2018; Duarte et al. 2018). In this sense, Antarctic fungal strains have been studied with a focus on taxonomy and biotechnology and have been reported in literature as producers of a wide range of biomolecules, including

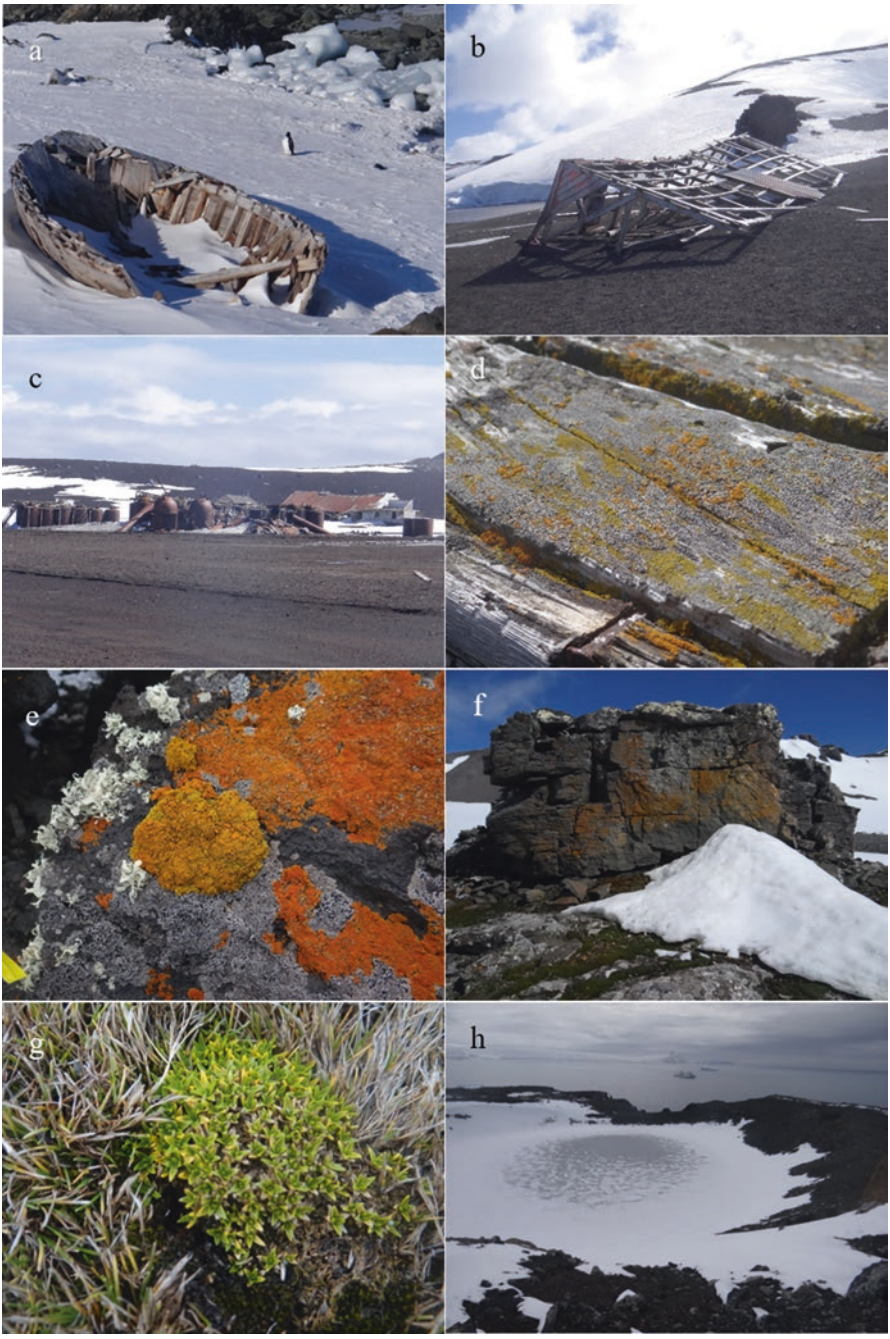

Fig. 2.1 Exotic (**a**–**d**) and natural (**e**–**h**) substrates in Antarctic environments. (**a**) Wood from boats on point *Hennequin*, King George Island; (**b**–**d**) Wood and metal structures from historic expedition huts on Deception Island; (**e**) Crustaceous lichens from Penguin Island; (**f**) Rocks from Nelson Island; (**g**) *Colobanthus quitensis* in Arctowski station, King George Island; and (**h**) an Antarctic lake under ice in Penguin Island. (Photos Credits: AWF Duarte)

those with antibacterial and antifungal (Gonçalves et al. 2015; Svahn et al. 2015), antiviral, and antituberculosis (Wang et al. 2015), antioxidant (Abrashev et al. 2016), photoprotector (Barahona et al. 2016), antiprotozoal (Gonçalves et al. 2015), insecticidal (Edgington et al. 2014), and cytotoxic (Brunati et al. 2009) activities. This promising biotechnological arsenal recovered by cultivation-dependent methods highlights the astonishing potential of Antarctic fungi for industrial applications and suggests an uncultured diversity that is possibly even greater.

The Antarctic fungi that have been characterized by cultivation-dependent methods are cosmopolitan, possibly because the region continuously receives microbial propagules from outside Antarctic regions by routes that include oceanic and wind currents. The dispersal of fungi may occur through a combination of favourable climatic factors such as winds and storms, in addition to dissemination by oceanic currents and vectors, such as dust, plant seeds, and birds (Margesin and Miteva 2011). Alternatively, some authors have argued that the great fungal diversity in Antarctica remains poorly understood, and there is evidence of a large number of endemic fungi (Bridge and Spooner 2012), a viewpoint that has been more recently reinforced by studies employing cultivation-independent methods (Pudasaini et al. 2017; Coleine et al. 2018a, b).

Although microbial isolation and cultivation techniques have improved in recent years, such as by the combined use of medium with high or low concentrations of nutrients (Ferrari et al. 2011) and cultivation with long-term incubation periods (Pulschen et al. 2017), our understanding of microbial ecology remains insufficient to recover and cultivate most microbes (Vester et al. 2015; Pulschen et al. 2017). Only 0.01–1% of the microorganisms in an environmental sample can be recovered through cultivation (Amann et al. 1995; Rappe and Giovannoni 2003). By fluorescence microscopy using DAPI (4′,6-diamidino-2-phenylindole, dihydrochloride), a fluorescent marker that binds DNA, direct counting of bacteria in a soil sample was estimated at 4×10^{10} cells per gram of soil. However, after the enrichment and cultivation of this soil, only 1×10^{6} colony-forming units, or 0.01% of bacteria, were recovered (Handelsman 2004).

Although values for cultivable fungi are different, they are still low. Approximately 70–90% of fungi in different soil environments are uncultivable (Magnuson and Lasure 2002). Some years ago, the entire Fungi kingdom on Earth was estimated to include 5.1 million species based on high-throughput molecular sequencing analysis (Blackwell 2011), and this estimate was recently increased to at least six million species (Taylor et al. 2014). Based on these estimates, only 2% of fungi species are currently known (Vitorino and Bessa 2018). These points raise the question, "where do most of these uncharacterized fungi exist?" In Antarctica, the relationship between fungi existing in the environment and those that are known is even more pronounced, considering the extreme conditions of that continent and difficulty in faithfully cultivating them.

Among the main limitations imposed by cultivation-dependent methods is the fact that most microorganisms occur as symbiotic consortia, performing their own functions while often contributing to the activities or survival of other microorgan-

isms, making their isolation and cultivation a virtually impossible task (Woyke et al. 2006).

By contrast, cultivation-independent analysis of an environmental sample is considered to be an alternative way to understand more precisely the diversity and function of a microbial community, including the mycobiota, as well as allowing the full exploitation of its metabolic potential. This approach does not involve any selection step, such as the cultivation or enrichment of samples (Pearce et al. 2012; Su et al. 2012; Newsham et al. 2015; Baeza et al. 2017; Borruso et al. 2018).

Antarctic fungal diversity has been accessed by various cultivation-independent methods, including in situ direct observations using magnifying lens (Coleine et al. 2018a), scanning electron microscopy of pieces of rocks (Yung et al. 2014), scanning electron microscopy with backscattered electron imaging of rocks (Archer et al. 2017), fluorescence microscopy (Wierzchos et al. 2004; D'elia et al. 2009; Marfenina et al. 2016), and microarray analysis (Chan et al. 2013; Wei et al. 2016). Additionally, ergosterol, quantified using HPLC (UV detection at 282 nm), has been used as biochemical marker of higher fungal active biomass in Antarctic permafrost samples, as described by Velázquez et al. (2016).

Microscopy techniques have been used to assess Antarctic fungal diversity, mainly using samples that include rocks colonized by endolithic microorganisms (de los Rios et al. 2004; Wierzchos et al. 2004), historic wood samples such as pine and birch (Blanchette et al. 2004, 2010), accretion ice (D'elia et al. 2009), and soil (Marfenina et al. 2016). The FUN-1 fluorescence viability probe that targets yeasts and filamentous fungi was used to observe metabolically active microorganisms by confocal laser scanning microscopy in granite rocks collected in McMurdo Dry Valleys (Wierzchos et al. 2004) and through fluorescence microscopy of soil samples for the detection of spores, mycelium, live cells, and dead cells (Marfenina et al. 2016). In addition to detecting fungi in Antarctic environmental samples, some studies have evaluated the impact of fungal growth in wood samples by light microscopy to detect soft rot cavities (Blanchette et al. 2004).

In recent decades, the development of new molecular techniques in clinical microbiology, such as the PCR amplification of rRNA gene regions combined with fingerprint methods (Lawley et al. 2004; Yergeau et al. 2007; Rao et al. 2012; Kochkina et al. 2012; Dreesens et al. 2014; Selbmann et al. 2017), clone libraries (Lawley et al. 2004; Antony et al. 2016), quantitative PCR (Ji et al. 2016), and RNA extraction followed by cDNA sequencing (Rao et al. 2012), has been shown to be more informative, reproducible, and faster compared with previous methods. Thus, these approaches have been widely employed to describe fungal communities (Fig. 2.2).

More recently, next-generation sequencing (NGS) approaches have become more affordable and widely used in studies of Antarctic fungal communities (Dreesens et al. 2014; Newsham et al. 2015; Baeza et al. 2017; Borruso et al. 2018). NGS-based studies of fungal diversity have been performed using different sequencing platforms, such as the 454 FLX titanium (Dreesens et al. 2014; Newsham et al. 2015; Park et al. 2015; Ji et al. 2016; Pudasaini et al. 2017; Brady et al. 2018),

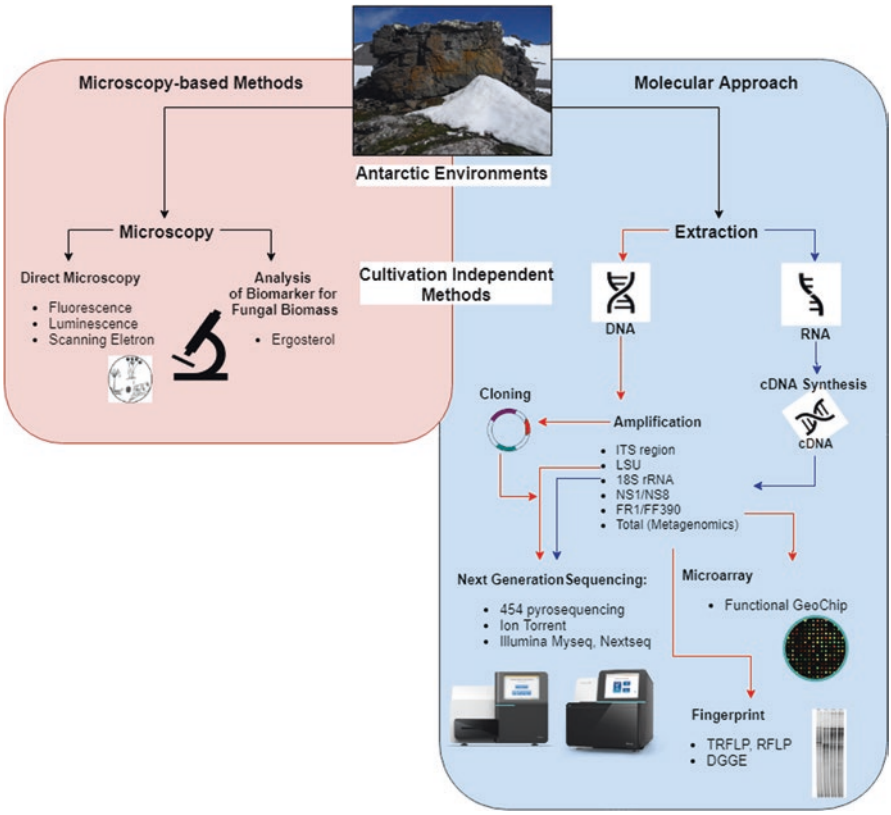

Fig. 2.2 Diversity analysis of Antarctic fungi by cultivation-independent methods

Illumina MiSeq (Czechowski et al. 2016; Rojas-Jimenez et al. 2017; Borruso et al. 2018; Coleine et al. 2018a, b), and Ion Torrent (Baeza et al. 2017) platforms.

The concept of "metagenomics" was initially proposed by Handelsman et al. (1998), which was defined as the application of modern genomic techniques to the study of microbial communities obtained directly from the natural environment, without the need for isolation or cultivation of individual species. This technique consists of extracting DNA from all organisms present in a given sample, such as snow or soil. This DNA can be directly evaluated by whole-genome shotgun metagenome (WGSM) sequencing to understand the structure, composition, and interactions of microbial communities, which represents an ecological approach (Schloss and Handelsman 2003). Alternatively, environmental DNA can be used to construct metagenomic libraries, followed by screening the resulting clones to search for a specific biological activity. This approach is often known as functional metagenomics and allows the metabolic potential of the microbial community in a given environment to be explored.

In these types of analyses, the starting point is a DNA of good quality for both purity and fragment size (Ekkers et al. 2012). Then, large fragments of DNA are ligated into vectors, such as BACs (bacterial artificial chromosome), fosmids, or cosmids, depending on the size of the DNA fragment to be analysed. This material is inserted into laboratory-cultured host cells, yielding a collection of clones known as a metagenomic library. Finally, these clones are subjected to subsequent screening for a biological activity of interest (Gillespie et al. 2002). Metagenomics allows for the identification of functional genes and a picture of the complexity of microbial communities, unveiling the richness, diversity, and dynamics of a community and enabling the recovery of complete genomes of hitherto uncultivated organisms (Alves et al. 2018).

Essentially, all metagenomic studies of Antarctic fungi are based on WGSM sequencing, and only one functional metagenomics-based study was found in literature (Ferrés et al. 2015). In that study, a metagenomic fosmid library was constructed from DNA extracted from glacial meltwater collected in the Antarctic Peninsula, yielding 52,000 clones (35–40 kb average insert size and almost 2 Gb of genetic information). This library was screened for bacterial genes that encoded cold-adapted enzymes, such as lipase/esterase, cellulase, and manganese peroxidase, and bioinformatics analysis revealed that eukaryotic DNA was less than 0.1% of total sequences.

In most studies of general microbial diversity based on cultivation-independent analysis from an Antarctic environment, bacteria are the most abundant group, followed by fungi or archaea depending on the environmental sample that is evaluated (Rogers et al. 2013; Pudasaini et al. 2017). Since recent advances in molecular methods, cultivation-independent methods have been broadly applied to study microbial communities of different environments including Antarctica.

Analyses of Antarctic fungal diversity based on cultivation-independent methods have been mainly performed on soil samples, including cold arid soil (Rao et al. 2012; Goordial et al. 2016), soil covered with vegetation (such as the mosses *Chorisodontium aciphyllum* and *Sanionia uncinata* and the lichen *Usnea antarctica*) (Yergeau et al. 2007), and maritime Antarctic soil (Lawley et al. 2004; Bridge and Newsham 2009). In addition to soils, other samples have been studied from samples that include wooden structures at historic sites (Blanchette et al. 2004, 2010), rocks colonized by endolithic microorganisms (de los Rios et al. 2004, 2005; Wierzchos et al. 2004; Selbmann et al. 2017; Coleine et al. 2018a, b), lichens (Park et al. 2015), permafrost (Kochkina et al. 2012, 2014), accretion ice from lakes (Rogers et al. 2013), hypoliths (Wei et al. 2016), ice-covered lakes (Rojas-Jimenez et al. 2017; Connell et al. 2018), hypersaline unfrozen brines found within a perennial frozen lake (Borruso et al. 2018), rock surface niches (Chan et al. 2013), and snow (Antony et al. 2016).

In general, the Antarctic samples analysed in fungal diversity studies can be divided into two main groups: (1) samples obtained from Antarctic natural environments, namely, natural or autochthonous samples, such as permafrost, lakes, desert rocks, or stones, and (2) samples introduced in Antarctica by anthropic activity, namely, exotic or allochthonous samples, such as woods and other structures left

behind by Antarctic explorers during expeditions, as shown in Fig. 2.1. In this chapter, we will cover the main types of samples that have been evaluated in cultivation-independent studies of fungal diversity (Table 2.1).

Table 2.1 Samples and methods used in studies of Antarctic fungal diversity by cultivation-independent approaches

Methods	Antarctic sample	Origin of sample	Technique	References
Microscopy-based				
	Accretion ice	Lake Vostok	Fluorescence microscopy with live/dead BacLightT	D'elia et al. (2009)
	Granite rocks colonized by endolithic microorganisms	Taylor Valley, Canadian Glacier (McMurdo Dry Valleys)	Confocal microscopy and fluorescent probe (FUN-1)	Wierzchos et al. (2004)
	Granite rocks colonized by endolithic microorganisms	McMurdo Dry Valleys	Confocal microscopy and fluorescent probe (FUN-1)	de los Rios et al. (2004)
	Granite rocks colonized by endolithic microorganisms	Canadian Glacier, Taylor Valley (Dry Valleys) and Cape Geology, Granite Harbour (Ross Sea)	Confocal laser scanning microscopy and fluorescence microscopy	de los Rios et al. (2005)
	Granite rocks colonized by endolithic and chasmoendolithic microorganisms	McMurdo Dry Valley	Scanning electron microscopy with backscattered electron imaging	Archer et al. (2017)
	Microbial mats from blighted and unaffected mats	Byers Peninsula (Livingston Island)	Scanning electronic microscopy and biomarker quantification (ergosterol)	Velázquez et al. (2016)
	Soil	Progress Station (valleys of the Larsemann Hills oasis) and West Russkaya Station (Marie Byrd Land)	Luminescence microscopy using calcofluor white, ethidium bromide, and fluorescein diacetate	Marfenina et al. (2016)
	Surface of rocks	Miers Valley (McMurdo Dry Valley)	Scanning electron microscopy	Yung et al. (2014)
	Wood samples including pine, spruce, and birch	Cape Royds and Cape Evans huts (Ross Island)	Scanning electron microscopy	Blanchette et al. (2004)
	Wood samples as pine and birch	Ross Sea Region	Scanning electron microscopy	Blanchette et al. (2010)
Molecular-based				

Table 2.1 (continued)

Methods	Antarctic sample	Origin of sample	Technique	References
	Accretion ice	Lake Vostok	DNA and RNA extraction followed by cDNA synthesis and 454 pyrosequencing	Rogers et al. (2013)
	Permafrost soil	Next to Bellingshausen, Novolazarevskaya, Progress, Russkaya stations and Banger Oasis and the Beacon Valley (Dry Valley)	DNA extraction, ITS region amplification, clone libraries, and sequencing	Kochkina et al. (2012)
	Permafrost (active layer)	Stations Novolazarevskaya (Schirmacher Oasis), Druzhnaya (Sandefjord Bay), Leningradskaya (Wilson Hills), and the Mirny observatory (Oates Coast).	DNA extraction, ITS region amplification, clone libraries, and sequencing	Kochkina et al. (2014)
	Permafrost soil	*University Valley*, McMurdo Dry *Valleys*	DNA extraction, ITS region-targeted pyrosequencing using the Roche 454 platform	Goordial et al. (2016)
	Surface of rocks	Miers Valley (McMurdo Dry Valley)	DNA extraction, PCR, T-RFLP and sequencing	Yung et al. (2014)
	Rocks of different typologies (sandstone, granite, dolerite, quartz, and lava-dike)	Along a latitudinal transect ranging from 73°S (Chisholm Hills, Cosmonaut Glacier) to 76°S (Battleship Promontory), and an altitudinal gradient from sea level (Kay Island)	DNA extraction, PCR and Denaturing Gradient Gel Electrophoresis (DGGE)	Selbmann et al. (2017)
	Sandstones with cryptoendoliths	University Valley (Upper Dry Valleys)	DNA extraction, ITS region amplification, and 454 pyrosequencing	Brady et al. (2018)
	Rocks of different typologies (sandstone, granite, dolerite, quartz, and lava-dike)	Along a latitudinal transect ranging from 73°S to 76°S (Victoria Land)	DNA extraction, ITS region amplification sequencing by Illumina MiSeq	Coleine et al. (2018a)

(continued)

Table 2.1 (continued)

Methods	Antarctic sample	Origin of sample	Technique	References
	Rocks (endolithic)	Along an altitudinal gradient from 834 to 3100 m a.s.l.—74°S–77°S (Victoria Land)	DNA extraction, ITS region amplification of ITS1 with ITS1F and ITS2 primers and sequence by Illumina MiSeq	Coleine et al. (2018b)
	Hypersaline unfrozen brines found within a perennial frozen lake	Tarn Flat (Northern Victoria Land)	DNA extraction, ITS region amplification and sequencing by Illumina MiSeq	Borruso et al. (2018)
	Lichens	Barton and Weaver Peninsulas (King George Island)	DNA extraction, amplification of LSU (eukaryotic large subunit), and 454 pyrosequencing	Park et al. (2015)
	Microbial mats from blighted and unaffected mats	Byers Peninsula (Livingston Island)	DNA extraction, amplification of 18S rRNA gene, and 454 pyrosequencing	Velázquez et al. (2016)
	Snow	Princess Elizabeth Land	DNA extraction, amplification with primers NS1/NS8, clone libraries, and sequencing	Antony et al. (2016)
	Soils covered or not with vegetation, such as mosses (*Chorisodontium aciphyllum* and *Sanionia uncinata*) and lichen (*Usnea antarctica*)	Anchorage, Signy, Fossil Bluff and Coal Nunatak	DGGE, real-time PCR, and microarray hybridization	Yergeau et al. (2007)
	Soil (maritime Antarctica)	Transect across Jane Col, Signy Island; Rothera Point, Adelaide Island; Mars Oasis and Coal Nunatak, Alexander Island, Sky-Hi Nunataks, Ellsworth Land and the LaGorce Mountains	DNA extraction, amplification, clone libraries, RFLP, and sequencing	Lawley et al. (2004)

(continued)

Table 2.1 (continued)

Methods	Antarctic sample	Origin of sample	Technique	References
	Soil (maritime Antarctica)	Mars Oasis (Alexander Island)	DNA extraction, amplification, clone libraries, and sequencing	Bridge and Newsham (2009)
	Soil (continental Antarctica)	McKelvey Valley (McMurdo Dry Valley)	DNA and RNA total extraction, followed by cDNA synthesis, amplification, clone libraries, T-RFLP, and sequencing	Rao et al. (2012)
	Soil	Miers Valley, Beacon Valley, Battleship Valley, Wright Valley, Alatna Valley, University Valley (McMurdo Dry Valleys)	DNA extraction, T-RFLP, and 454 pyrosequencing	Dreesens et al. (2014)
	Soil	Along a 1650 km gradient between 72 °S and 60 °S (Alexander, Jenny, Lagoon, Adelaide, Blaiklock, Detaille, Wiencke, Goudier, Seymour, Alectoria, Spert, James Ross, Deception, Livingston, Robert, Greenwish, King George and Signy Islands and Cape Evensen)	DNA extraction and 454 pyrosequencing	Newsham et al. (2015)
	Soil	Mitchell Peninsula (Windmill Islands)	DNA extraction, ITS region amplification, 454 pyrosequencing, and quantitative PCR (qPCR)	Ji et al. (2016)
	Soil	Mount Menzies, Mawson Escarpment and Lake Terrasovoje	DNA extraction, 18S rRNA amplification, and sequencing by Illumina MiSeq	Czechowski et al. (2016)
	Soil	King George, Deception, Snow, Dee, Livingstone, Greenwich, Robert, Nelson, Litchfield and Lagotellerie Islands, and Union Glacier	DNA extraction and sequencing using Ion Torrent technologies	Baeza et al. (2017)

(continued)

Table 2.1 (continued)

Methods	Antarctic sample	Origin of sample	Technique	References
	Soil	Browning Peninsula (Windmill Islands)	DNA extraction, ITS region amplification, and 454 pyrosequencing	Pudasaini et al. (2017)
	Soil under of *Colobanthus quitensis* and *Deschampsia antarctica* (vascular plants that occur in Antarctica)	Bird, Signy, and Léonie Islands	DNA extraction, ITS region amplification, and 454 pyrosequencing	Cox et al. (2016)
	Soil and hypoliths	Miers Valley (McMurdo Dry)	DNA extraction, GeoChip functional microarray	Wei et al. (2016)
	Soil and rock surface niches	McKelvey Valley	Microarray with GeoChip-based functional genes	Chan et al. (2013)
	Water from ice-covered lakes	Five lakes in the Taylor and Miers Valleys (McMurdo Dry)	DNA and RNA extraction, cDNA synthesis, 18S rRNA amplification, and sequencing by Illumina MiSeq	Rojas-Jimenez et al. (2017)
	Wooden structures and surrounding soils	South Pole, Robert F. Scott and Ernest Shackleton (Ross Island)	DNA extraction and DGGE using the internal transcribed spacer (ITS) regions	Arenz et al. (2006)

Although fungal biomass has been found to be relatively low in the various samples collected in Antarctica, there is a considerable fungal richness, with a higher abundance of fungi of the phylum Ascomycota, followed by Basidiomycota; this pattern is similar to that observed in cultivation-dependent studies. Additionally, the occurrence of *Zygomycota* and *Chytridiomycota* in soils (Cox et al. 2016) and *Zygomycota*, *Cryptomycota*, and *Blastocladiomycota* in ice-covered lakes (Rojas-Jimenez et al. 2017) has been reported. Some authors have observed that Antarctica is characterized by a low frequency of endemic fungi, with the majority of them belonging to the class *Eurotiomycetes* (Cox et al. 2016).

Despite the high fungal richness in Antarctica, a limited literature addresses the functional diversity of these microorganisms by a cultivation-independent approach. However, it is possible to predict that these organisms are crucial for the maintenance of the Antarctic ecosystem, as demonstrated by Wei et al. (2016) who observed

via microarray hybridizations to a functional GeoChip that a high frequency of fungal organisms is related to the carbon and nitrogen cycle, in addition to expressing genes related to environmental stress responses.

2.3 Antarctic Samples Analysed by Cultivation-Independent Approaches

2.3.1 Soil

Antarctic soil is distinct from other biomes as consequence of its long-term persistence under harsh environmental conditions, in addition to its long history of isolation, resulting in a high degree of endemism (Convey 2010). Today, anthropic influences increasingly threaten the unique Antarctic soil communities through human-mediated climate change, increasing pollution, and the introduction of exogenous organisms by exploration activities, which may outcompete the endemic diversity in some environmental sites.

Antarctic soils have distinct characteristics, ranging from continental arid soils that are characterized by the low availability of nutrients and low moisture content to ornithogenic soils from penguin regions that receive a high content of faeces and are consequently characterized by high levels of carbon, nitrogen, phosphorus, silicon, and moisture (Rao et al. 2012; Guo et al. 2018). Recently, Guo et al. (2018) observed that the expansion of penguin activity in maritime Antarctica has been associated with a change in the soil geochemistry, which consequently affects the composition of the soil microbiome.

In a study of eukaryotic diversity in Antarctic soil from the Prince Charles Mountains (East Antarctica) that used an 18S rDNA amplicon sequencing and metataxonomic approach, the main phylotypes were fungal species, followed by nonalgal protists (Czechowski et al. 2016). This study revealed that sequences related to the extremophilic fungus Ascomycota *Cryomyces antarcticus* were among the most abundant. Other sequences were mainly related to lichenized fungi (a mycobiont), highlighting the importance of lichen diversity, even for the microbial composition present in the Antarctic soil (Czechowski et al. 2016).

Marfenina et al. (2016) reported that the fungal biomass in Antarctic soils was 0.3 and 0.6 mg/g of soil in arid soil and soil covered with vegetation (i.e., mosses and lichens), respectively. These were both mainly composed of small spores (2.5 μm), as observed by luminescence microscopy. The same study recovered 38 species of cultivated microscopic fungi, with *Penicillium* and *Phoma* being the most abundant genera in soil (Marfenina et al. 2016). The presence of vegetation and birds are two parameters that were found to strongly influence microbial diversity in Antarctic soils (Teixeira et al. 2013).

The McMurdo Dry Valleys are characterized by arid desert and mineral soil; different studies have reported that this environment harbours a restricted fungal diver-

sity (Rao et al. 2012; Pudasaini et al. 2017). Environmental DNA and RNA (cDNA) from soil collected in the McMurdo Dry Valleys have been used to infer total fungal diversity and putative metabolically active assemblages, respectively (Rao et al. 2012). Additionally, the authors employed cultivation-based approaches using a variety of laboratory growth conditions. ITS sequence analysis revealed a highly restricted diversity, with sequences affiliated with to two known genera, *Helicodendron* and *Zalerion*. Moulds and mitosporic genera commonly found in marine Antarctic samples were not found in this study, and a comparison of diversity estimates by DNA and RNA (cDNA) sequences suggested that taxa active metabolically can be present in different abundances than indicated by DNA libraries. *Debaryomyces hansenii* was recovered using plating techniques, but it was not detected by the cultivation-independent approach, which might reflect the inherently low abundance of this yeast in soil samples when compared to other yeasts (Rao et al. 2012). These findings indicate that these two approaches are complementary, and each accesses a distinct microbial fraction of total diversity in nature (Arenz et al. 2006; Rao et al. 2012; Antony et al. 2016).

In another study, Dreesens et al. (2014) observed that soil samples from the McMurdo Dry Valleys were quite heterogeneous in physicochemical properties, with salinity ranging from 107 to 3920 μS and moisture content ranging from 1% to 3%, which was correlated with the fungal diversity associated with individual samples. Fingerprinting by T-RFLP and 454 pyrosequencing analysis revealed different levels of heterogeneity in fungal diversity, with a significant number of OTUs annotated as derived from *Chytridiomycota* species, especially in the Miers Valley sampling point. Similar to the other two sampling points (Alatna Valley and Battleship Promontory), most OTUs were annotated as Ascomycota, although *Chytridiomycota* was also highly abundant. Notably, these findings are quite interesting because previous studies suggested that Antarctic Dry Valley soils were dominated by Ascomycota and *Basidiomycota* based on both cultivation-dependent (Arenz and Blanchette 2011) and -independent methods (Rao et al. 2012).

In a study that used pyrosequencing to assess fungal diversity in 29 soils sampled from a 1650 km-transect in maritime Antarctica, Newsham et al. (2015) showed that the most abundant fungal OTUs were related to *Ascomycota*, followed by *Basidiomycota*. Moreover, redundancy analysis based on presence/absence data indicated that changes in the frequency of the lichenized fungi *Verrucaria* largely accounted for increased fungal diversity in warmer soils (Newsham et al. 2015). These authors reported that surface air temperature is an important factor that shapes fungal diversity in Antarctic soil. Moreover, warmer soil in maritime Antarctica showed more water availability and enhanced metabolic activity, which extended the period for which fungi are active and enabling a switch from survival to growth and dispersal strategies.

One of the distinguishing characteristics of maritime Antarctic soils is frequent freezing and thawing cycles. Besides being a limiting factor to microbial development, this environmental phenomenon also affects the physical properties of such soils, as fine mineral particles are surrounded by other elements, such as coarser material, stones, and rocks (Lawley et al. 2004).

A reduction in microbial richness and abundance correlated with increases in latitude was proposed by Yergeau et al. (2007). However, some authors have shown that lower diversity in Antarctic soil samples was not related to increases in latitude, but it was instead associated with the local environmental conditions of the sampling site (Lawley et al. 2004; Ji et al. 2016).

Denaturing gradient gel electrophoresis (DGGE) and real-time PCR analyses revealed that fungal 18S rRNA gene abundance in soil samples covered with mosses and lichens was not influenced by vegetation cover per se but, instead, was affected by location and interactions between location and vegetation cover (Yergeau et al. 2007). The authors showed that fungal communities can respond differently to changes in organic input levels and quality depending on the environmental conditions. Additionally, estimates of fungal biomass differed when using cultivation-dependent (CFU counts) versus -independent (real-time PCR) approaches, and a high correlation was found between values obtained by real-time PCR and phospholipid fatty acids (PFLA). In general, CFU counts are thought to provide a biased view of the microbial abundance in an environment.

Ji et al. (2016) studied 93 soil samples from Mitchell Peninsula that were characterized by an acidic pH (ranging from 4.82 to 6.8), moisture ranging between 1% and 10%, and low total carbon (< 0.6%). These authors found 374,996 fungal ITS gene sequences after read-quality filtering; many of the fungal OTUs could not be taxonomically assigned because of poor sequence alignment (<50% of nucleotides aligned), which represented 13% of the total filtered sequences. Additionally, 17%, 18%, and 22% of all fungal ITS sequences could not be classified at the class, order, or family level, respectively. Fungal communities were dominated by *Ascomycota* (77.1%), followed by *Basidiomycota* (9.7%) and *Chytridiomycota* (0.01%), with more than 46% of fungal sequences classified as *Lecanoromycetes*, one of the largest classes of lichenized fungi.

Wei et al. (2016) investigated the microbial functional diversity of soil and hypolith samples from Miers Valley in the McMurdo Dry Valleys by GeoChip microarray. The GeoChip microarray (with 84,000 50-mer oligonucleotide probes covering 152,000 gene variants) primarily targets bacterial genes, but some fungal genes are also represented. Among the functional gene categories analysed, fungal genes were identified associated with the carbon transformation (carbohydrate catabolism and aromatic compound catabolism), nitrogen transformation (nitrification, denitrification, ammonification, and assimilatory nitrogen reduction), and stress response (heat shock, nitrogen limitation, phosphate limitation, and oxygen stress) categories. Agaricomycetes, Leotiomycetes, Saccharomycetes, and Sordariomycetes showed the highest intensity of hybridization to genes related to stress responses. Intriguingly, *Ustilaginomycetes* and unidentified fungi showed the highest expression of genes related to nitrogen transformation. Finally, no difference was observed among the fungal groups for genes involved in carbon transformation.

2.3.2 Permafrost

Some studies suggest that the combination of deep freeze, aridity, and oligotrophy in Antarctic permafrost severely limits microbial activity and survival, resulting in very low levels of total carbon (0.01–0.05%) and total N nitrogen (undetectable to 0.09%) in samples collected from the McMurdo Dry Valleys (Goordial et al. 2016). Goordial and colleagues evaluated microbial diversity by 18S ITS pyrosequencing and RNA analyses and, even using relatively high soil amounts for RNA extraction, they could not detect RNA. They hypothesized that biomass was extremely low in their samples and suggested that the extracted DNA in these samples was mainly derived from dormant or nonviable fungi. Fungal sequences were dominated by the *Ascomycetes* fungi *Dothideomycetes* and *Eurotiomycetes*; the latter was only found on the surface of permafrost samples.

Based on cultivation-dependent and -independent methods, Kochkina et al. (2012) also reported low fungal biomass in permafrost from sites near Russian research stations. They found that the sequences of some organisms were only recovered by cultivation-independent methods, whereas some strains of *Penicillium* and *Cladosporium* were only obtained by isolation and cultivation methods, highlighting the complementarity of both approaches.

2.3.3 Lichen

Antarctic terrestrial ecosystems are dominated by lichen-symbiotic organisms that pair a mycobiont (lichenized fungi) and a photobiont (algae and/or cyanobacteria). These relationships have a certain level of specificity, as one species of mycobiont and one species of photobiont can form a symbiotic relationship in a thallus (Domaschke et al. 2012).

Taxonomic affiliations of the Antarctic lichen-associated fungi from *Cladonia borealis*, *Cladonia gracilis*, *Umbilicaria antarctica*, *Usnea aurantiaco-atra*, *Buellia granulosa*, *Amandinea coniops*, and *Ochrolechia parella* showed that they belonged to the *Ascomycota* (*Arthoniomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, *Leotiomycetes*, and *Sordariomycetes*) and *Basidiomycota* (*Cystobasidiomycetes* and *Tremellomycetes*) groups (Park et al. 2015). *Lecanoromycetes* has been the most dominant fungal class identified in Antarctic lichens, including those isolated from rock substrates (Coleine et al. 2018a).

Moreover, lichenized fungal species are important components of Antarctic soil biodiversity and may have a widespread distribution across Antarctic sites, as reported by Czechowski et al. (2016) for a study of samples from Prince Charles Mountain analysed using high-throughput sequencing. Notably, global warming mainly affects the maritime Antarctic and, in this sense, can also affect the fungal diversity of lichens in Antarctic soils.

2.3.4 Lake and Ice-Covered Lake Water

Lake Vostok is the largest of the nearly 400 subglacial Antarctic lakes and has been continuously buried by glacial ice for 15 million years. Metagenomic and metatranscriptomic analyses of Lake Vostok accretion ice revealed sequences that belong to the three domains of life (94% Bacteria, 6% Eukarya, and only two sequences of Archaea). The predominant eukaryotic sequences were similar to those from *Ascomycota*, followed by uncultivated fungi, *Basidiomycota* and *Mucorales* fungi (Rogers et al. 2013).

Among such pristine environments, analyses of ice-covered lake and water from five lakes showed that fungal taxa represented between 0.93% and 60.32% of the eukaryotic sequences (by 18S rRNA), with *Cryptomycota* and *Chytridiomycota* as the most abundant phyla of the fungal communities in all lakes, followed by members of *Ascomycota*, *Basidiomycota*, *Zygomycota*, and *Blastocladiomycota* (Rojas-Jimenez et al. 2017).

2.3.5 Snow

Antarctic snow pack harbours diverse, active, and viable microbial communities that represent almost all of the major phylogenetic groups, as described by Antony et al. (2016). Antony et al. found that sequences related to Ascomycota (Aspergillaceae), including *Aspergillus* and *Penicillium* strains, accounted for 82% of fungal communities in snow collected in Princess Elizabeth Land (East Antarctica). The remaining 18% of sequences were affiliated with Cryptomycota, which is a distinct fungal group that lacks a chitin-rich cell wall. Additionally, the cultivation-dependent approach used allowed the isolation of 17 fungi belonging to the genus *Cryptococcus*.

2.3.6 Wood and Historic Structures

Many structures were introduced into Antarctica by explorers, including woods and other organic structures, such as roof planks, fragmented wooden boxes, and wooden boats (Fig. 2.1). This material, which naturally favours the growth of fungi, has been widely used in studies of fungal diversity and was a likely source of exogenous microorganism introduction into Antarctica (Ludley and Robinson 2008; Arenz et al. 2006, 2011; Blanchette et al. 2010; Arenz and Blanchette 2011). In this sense, fungal strains have been long recovered from sites of human activity, as Antarctic soils have been contaminated with petroleum and discarded wood at sites near Antarctic stations, as described by Kerry (1990).

Wood samples from Lake Fryxell Basin (Dry Valley) and remote sites at Mt. Fleming and Allan Hills were subjected to DGGE using the ITS regions of ribosomal DNA for further identification. A total of 48 samples analysed by DGGE revealed 100 ITS sequences belonging to 71 fungal profiles and 28 taxa that were not detected by cultivation. Conversely, 25 fungal taxa were only detected using cultivation-dependent methods. Among filamentous fungi, most sequences showed high similarity with Ascomycota, followed by *Zygomycota*. For yeasts, most sequences were related to *Basidiomycota*, followed by Ascomycota. The most abundant taxa were *Cadophora*, *Cladosporium*, *Cryptococcus, Epicoccum*, and *Hormonema* (Arenz et al. 2006). Representatives of the genera *Cladosporium*, *Hormonema*, *Penicillium*, and *Lecythophora* were isolated from wood samples at the historic expedition huts on Ross Island (Held et al. 2005).

2.4 Conclusions and Perspectives

Although Antarctic fungal diversity remains incompletely characterized, recent studies have been illuminating. Although the preponderance of studies has focussed on understanding Antarctic bacterial diversity rather than fungal diversity, this might simply reflect the markedly greater abundance of bacteria at most environmental sites.

Knowledge of Antarctic fungal communities has been mainly gained through cultivation-dependent techniques and, more recently, cultivation-independent approaches that employ either direct microscopic analysis or NGS strategies. Notably, studies of Antarctic fungal diversity are essential to understand the impact of global warming and how the introduction of structures by explorers could affect biodiversity in such a remote and pristine place. Additionally, they may help to inform how the transport and dissemination of species can occur in Antarctica, as there is a continuum of environments that is often ignored. For example, fungal species can share similar lichens and be found in various types of soil samples.

Future strategies to cultivate Antarctic fungi that are currently considered to be noncultivable are promising. If successful, they may add up to our knowledge of endemism, dispersal, and fungal colonization in Antarctica and drive discovery of new taxa and metabolic routes. Additionally, many of these fungi possess unique properties and an array of putative new molecules to be used in biotechnological processes with the potential to improve the quality of life in modern society.

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Chapter 3
Fungi Present in Soils of Antarctica

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3.1 Introduction

Despite extreme conditions, the different Antarctic ecosystems and their substrata present themselves as natural habitats, occupied and colonised by several fungal species that range from the endemic to the cold-adapted cosmopolitan fungi. Among the substrates/microhabitats present in Antarctica, to date, soils have been the most studied ecosystem regarding the richness and diversity of fungi present (Tubaki and Asano 1965; Boyd et al. 1966; Heal et al. 1967; Sun et al. 1978; Bailey and Wynn-Williams 1982; Baublis et al. 1991; Vishniac 1996; Onofri et al. 2004; Adams et al. 2006; Fell et al. 2006; Ruisi et al. 2007; Bridge and Spooner 2012; Rao et al. 2012; Godinho et al. 2015; Gomes et al. 2018). Various studies of mycology in Antarctic soils have been conducted in the last decades with the objective of knowing the communities of fungi present and understanding their interactions and importance in the different terrestrial ecosystems of the region (Sun et al. 1978; Fletcher et al. 1985; Kerry 1990; Vishniac 1996; Marshall 1998; Onofri et al. 2000; de Hoog et al. 2005; Fell et al. 2006; Malosso et al. 2006; Arenz and Blanchette 2009).

Different types of soils are found in Antarctica; diversity is basically associated with the role of climatic variability, lithology, and biological colonisation (French

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2007). In this region, different microclimates reflect environmental differences between its maritime and continental portions (French 2007). The Antarctic sea forms a climatic zone that surrounds the continent, covering archipelagos and part of the Antarctic Peninsula, and presents less severe climatic conditions, higher temperatures, and greater precipitation in water (Campbell and Claridge 1987; Simas et al. 2007, 2008). These conditions allow the development of deeper soil and greater vegetation cover (Campbell and Claridge 1987). In continental Antarctica, the climatic conditions are more severe, exemplified by the lower temperatures in relation to the peninsular region, and characterised by almost no precipitation (Green et al. 1999; Pannewitz et al. 2003). Therefore, the soils of the continental area are less developed and more stony and may have accumulation of salts as a marked characteristic (Delpupo et al. 2017).

The major fungal communities found in Antarctic soils include species belonging to phyla *Ascomycota*, *Basidiomycota*, *Glomeromycota*, traditional *Zygomycota*, and *Chytridiomycota* (Lawley et al. 2004; Onofri et al. 2004; Malosso et al. 2006; Fell et al. 2006; Ruisi et al. 2007; Arenz and Blanchette 2009; Arenz and Blanchette 2011; Bridge and Spooner 2012; Arenz et al. 2014; Pudasaini et al. 2017; Gomes et al. 2018).

The most represented orders of fungi in the soils are *Onygenales*, *Eurotiales*, *Mortierellales*, *Mucorales*, *Saccharomycetales*, *Thelebolales*, and *Helotiales* (Newsham et al. 2018). The resident fungi already identified in the Antarctic soil have different essential ecological roles as decomposers, pathogens, parasites, and mutualists (Swift et al. 1979; Yergeau et al. 2007; Upson et al. 2009; Lindo and Gonzalez 2010; Tedersoo et al. 2014). According to Newsham et al. (2015), with potential warming of the Antarctic regions, especially the peninsula areas, the Antarctic soils may be heavily colonised by fungi, suggesting a tendency towards an increase of 20–27% richness of various species in the southernmost soils by the end of the twenty-first century. Thus, in the coming years, there is a propensity for the discovery of new species and expansion of the geographical distribution of fungi along Antarctica, as well as for a more detailed understanding of their ecological relationships because of possible climatic changes in the region.

Besides the significant ecological importance, the fungi present in the Antarctic soils have also been studied as a source of bioproducts for biotechnological use. According to Santiago et al. (2012), because much of Antarctica still represents a preserved, primitive, and geographically isolated natural environment, some species of fungi inhabiting the region may have new and/or unique metabolic pathways capable of generating useful substances in different biotechnological processes with potential applications in the food, medicine, and agricultural industries.

3.2 The Antarctic Soils

Only 0.35% (45,000 km^2) of Antarctica possesses ice-free areas, in which conditions for soil formation are verified (Bockheim 2015). These ice-free areas occur in continental and maritime Antarctica, with emphasis on the South Shetland

archipelago and the Antarctic Peninsula, as well as the dry valleys of the Transantarctic Mountains (Bockheim and Ugolini 2008; Bockheim 2015). In maritime Antarctica, the sea has higher temperatures and precipitation than the continental Antarctica, resulting in the formation of deeper and more developed soils. Physical weathering is favoured in both the regions by the action of freezing and thawing cycles, whereas the presence of water in a liquid (unfrozen) state in austral summers as well as biological activity is predominant in the maritime region, which favours chemical weathering (Simas et al. 2006, 2007). Among the main processes of soil formation in Antarctica are the translocation (movement) of clays, cryoturbation, sulphurisation, podzolisation, phosphatisation, and salinisation. The pedological diversity is related mainly to the diversity of the material of origin, to different types of rocks and sediments, to the biological processes, and to the occurrence and distribution of the permafrost (Simas et al. 2008; Moura et al. 2012). Permafrost is defined as a thermal condition wherein the substrate temperature is below 0 °C for two or more consecutive years (Muller 1943; Van Everdingen 1998; French 2007). Associated with permafrost, an active layer can be termed as a layer that undergoes freezing and thawing and represents the highest expression of cryoturbation processes.

The main soil classes occurring in Antarctica are arenosols/neosols, cryossolos/gelisols, leptosols, gleysols, and cambisols. The relationship among these different classes is limited, and the terrain in which they occur is narrow, mainly owing to the dynamics and recent exposure of the source material. The cryossolos is characterised by the presence of permafrost up to 1 or 2 m deep when gellic features (vertical orientation of gravels, buried horizons, soils with patterns) are present. They occur in diverse environments, notably in moraines, geoforms related to cryoturbation, and gelifluction because of raised marine platforms, presenting a well-developed structure. Recent exposure and constant reworking of the source material favours the formation of leptosols (shallow or stony) with an incipient structure, these being more common in raised platforms and are closely related to residual landforms. The gleysols are found close to thaw channels and in depressions with slopes, being commonly present in the gleanings of the subsurface horizons in cryossolos because of the impediment of drainage caused by the presence of permafrost. The neosols (often sandy) occur at low altitudes, especially on the sea terraces, smooth slopes, and deposits (tills and flood plains), and show little or no structural development, small horizon differentiation, absent cryoturbation, and no diagnostic horizon. Cambisols are characterised by a finer texture and moderate structure; they do not present permafrost or cryoturbation and occur at low altitudes, in marine platforms, and in erosive features or deposits.

Some soils of extremely important scientific and environmental interest occur in Antarctica and are testimonies of the pedodiversity of this region of the earth. Among these soils, we highlight the ornithogenic soils, soils with patterns, sulphide soils and the desert soils. Ornithogenic soils are particularly abundant in maritime Antarctica, associated with phosphatisation process that comprises interaction between the substrate (rocks and sediments) and guano deposited by birds (Tatur and Myrcha 1984; Myrcha et al. 1985; Tatur and Barczuk 1985; Tatur 1989; Myrcha and Tatur 1991; Schaefer et al. 2004; Simas et al. 2007; Pereira et al. 2013). These

soils are among the most developed soils in Antarctica and represent very important areas for biological dissemination and fixation (Fig. 3.1). In fact, on some of these soils, the development of an oasis with extensive vegetation cover shows a very high microbiological activity, comparable to that observed in a temperate zone (Myrcha et al. 1985). The development of vegetation in areas more distant from the coast (near nests of birds) indicates the importance of the fertilisation of soils by these faunae in establishing more complex vegetal communities and with greater capacity of fixation of C (Michel et al. 2006).

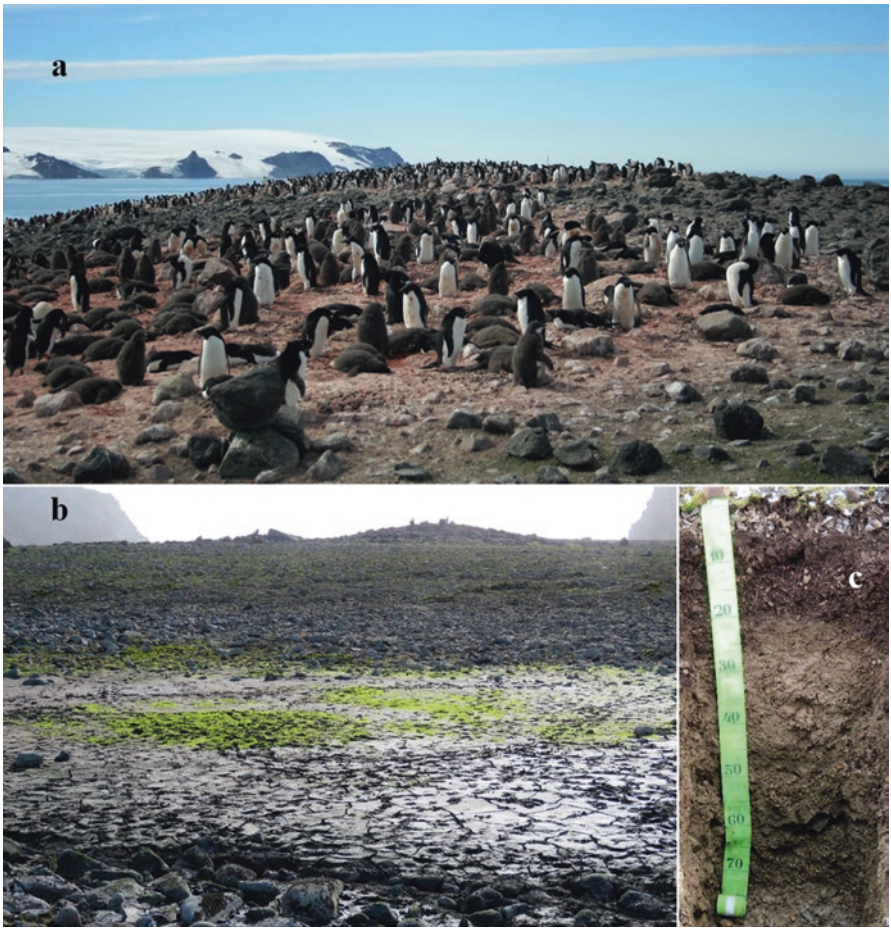

Fig. 3.1 Ornithogenic soils. (**a**) *Pygoscelis adeliae* colonies, (**b**) guano pool formed by the accumulation of penguin droppings at the base of the snow accumulation, and (**c**) ornithogenic soil profile enriched in phosphorus. The photographs were taken on Harmony Point peninsula, Nelson Island, South Shetland Archipelago, maritime Antarctica. (Photo credits, LH Rosa and FS de Oliveira)

Patterned ground is one of the most distinct formations in permafrost areas of the polar regions (Hallet et al. 2011). Patterned ground (or polygonal soils) occurs in ice-free areas by periglacial processes (French 2007). Patterned ground is a general term for any soil surface exhibiting a symmetric, discernible, and ordered pattern of the morphology of the terrain and, when present, of the vegetation (Fig. 3.2). Although not limited to permafrost regions, patterned ground formations develop best in areas affected by permafrost, either in recent times or in the bygone years. Such soils are direct products of cryoturbation processes. The process of the occurrence of high concentration of fine grains in the soils of periglacial environments (besides ornithogenic soils) is identified. The upward displacement of the thin material at the centre is caused by freezing and thawing (frost heave) of the ice lenses at the top and bottom of the active layer; the downward displacement at the borders is a gravity-induced movement (Mackay 1979).

Sulphated soils are characterised by a genesis related to the presence of rocks rich in sulphides (Francelino et al. 2011). The oxidation of these minerals is related to their exposure to the atmosphere, generating particular geochemical conditions. For example, a change from sulphide to sulphate leads to an increase in Eh-pH causing acidity, which in turn leads to the formation of sulphated minerals (by sulphurisation/thiomorphism) (Krauskopf 1979). These minerals, such as jarosite, give a

Fig. 3.2 Soils with pattern. (**a**) rock garlands with strong orientation of rock fragments, (**b**, **c**) mud boil pattern, showing the separation between coarse rock fragments (garland) and fine sediments (in the centre of the circle), and (**d**, **e**) abrupt contact between the stone garland and the fine particle concentration zone. The photographs were taken on the Harmony Point peninsula, Nelson Island, South Shetland Archipelago, maritime Antarctica. (Photo credits, FS de Oliveira)

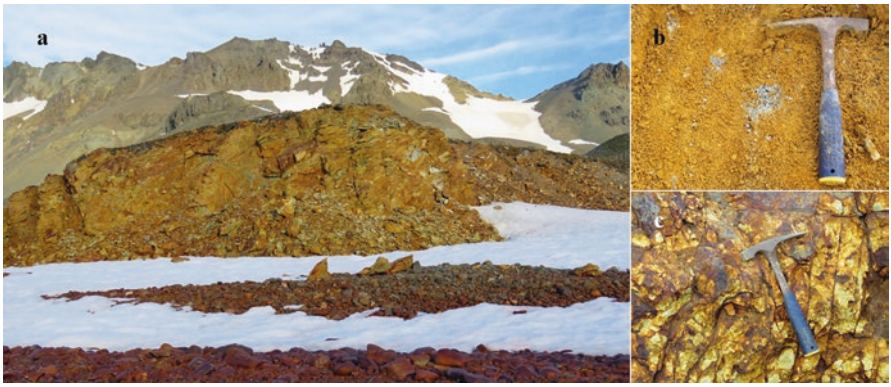

Fig. 3.3 Sulphated acids alone. (**a**) Outcropping of a pyritised andesite shaft with oxidised surface (yellow colour) enveloped by beach pebbles capped by ferruginous film (red colour), (**b**) saprolite (pyrrhotite andesite rock), and (**c**) acid soil profile of sulphated soil, in an area known as Yellow Point. The photographs were taken off the Keller Peninsula, King George Island, South Shetland Archipelago, maritime Antarctica. (Photo credits, d FS de Oliveira)

yellowish colour to the soils (Fig. 3.3), and therefore the areas of maritime Antarctica in which they occur are known as Yellow Points. Although the presence of sulphide rocks has also been verified in continental Antarctica, the sulphurisation was observed in an incipient way according to Delpupo et al. (2017).

As a pedological marker of areas of continental Antarctica with extreme aridity and even more intense cold, soils have been designated as polar desert soils (Bockheim 1990; Bockheim and Ugolini 1990). The soils of this region are skeletal to shallow, with poorly developed structures and lighter colours (Fig. 3.4). Salinisation is a remarkable process and is related not only to the climatic conditions but also to the drainage conditions of the slopes and the role of the winds, which commonly act in the formation of stony pavements by wind erosion of surface fines.

3.3 Diversity of Fungi in Antarctic Soils

There are records that suggest the presence of fungi in Antarctica in the Permian (Paleozoic), Triassic, and Jurassic (Mesozoic) periods as demonstrated by the studies of Stubblefield and Taylor (1983), Taylor and White (1989), Taylor and Osborne (1996), Harper et al. (2012), and Arenz et al. (2014). Most of the species of fungi found in Antarctic soils are cold-adapted cosmopolitan fungi and also those that are considered endemic (Arenz et al. 2014), among which many have a high potential for colonisation and dispersion (Marshall 1998). In the last decades, there has been an increase in the understanding of the microbial diversity present in the Antarctic soils. Satellite images showed that 0.35% of Antarctica's 45,000 km^2 area is free of

Fig. 3.4 Polar desert soils. (**a**) Polar desert environment with stony desert pavement, (**b**) details of rock fragments of a stony desert pavement, and (**c**) profile of polar desert enriched in salt. The photographs were taken in the dry valleys of Edson Hills, Ellsworth Mountains (Delpupo et al. 2017), and continental Antarctica. (Photo credits, FS de Oliveira)

ice and has different types of exposed soils (Cowan et al. 2014), which are potential microhabitats for different communities of resident fungi.

The different types of Antarctic soils seem to harbour different communities of fungi, according to their physicochemical characteristics. Studies of fungi on Antarctic soils have uncovered the presence of diversified assemblies, which is demonstrated by the diversity, richness, and dominance indexes of the taxa identified (Table 3.1). In addition, according to Cowan et al. (2014), certain taxa dominate

Table 3.1 Examples of fungal diversity present in soils from different regions of the Antarctic Peninsula and continental Antarctica

Region	Diversity indices[a]			References
	Fisher α	Margalef	Simpson	
Ross Island	21.57	7.96	0.94	Arenz et al. (2006)
Deception Island	10.89	4.80	0.90	Arenz and Blanchette (2009)
Dry Valleys	1.43	0.97	0.73	Arenz and Blanchette (2011)
Antarctic Peninsula	26.87	9.36	0.95	Arenz and Blanchette (2011)
Ross Sea region	35.06	14.26	0.96	Arenz and Blanchette (2011)
Browning Peninsula	–	34.68	0.89	Pudasaini et al. (2017)
Ellsworth Mountains	1.42	1.25	0.80	Godinho et al. (2015)
King George Island	4.07	2.18	0.75	Gonçalves et al. (2015)
Ornithogenic soil, King George Island	7.82	2.06	0.78	Gonçalves et al. (2015)
Rhizosphere soil, King George Island	6.82	3.26	0.87	Gonçalves et al. (2015)
Deception Island	13.19	2.92	0.86	Abneuf et al. (2016)
King George Island	3.98	1.74	0.60	Krishnan et al. (2011)
King George Island	1.24	1.07	0.86	Gomes et al. (2018)
Penguin Island	0.41	0.31	0.61	Gomes et al. (2018)
Nelson Island	1.63	1.4	0.9	Gomes et al. (2018)
Robert Island	1.57	1.37	0.88	Gomes et al. (2018)
King George Island	6.53	4.44	0.88	Wentzel et al. (2018)

[a]For calculation of the diversity index, the number of isolates or CFU g^{-1} available in published articles was used (not reported)

the communities of the different Antarctic habitats, such as *Pseudogymnoascus destructans*, *Pseudogymnoascus appendiculatus*, *Penicillium tardochrysogenum*, *Penicillium verrucosum*, *Mortierella antarctica*, *Mortierella alpina*, *Rhodotorula mucilaginosa*, *Mortierella amoeboidea*, *Antarctomyces pellizariae*, *Aspergillus flavus*, *Aspergillus niger*, *Mrakia frigida*, and *Thelebolus globosus* (Arenz and Blanchette 2011; Alias et al. 2013; Marfenina et al. 2016; Gomes et al. 2018; Kochkina et al. 2019).

3.4 Major Genera of Fungi Present in Antarctic Soils

Different species and genera of fungi have been reported in the Antarctic soils (Table 3.2), among which are the cosmopolitan and endemic taxa. Among the genera most found in Antarctic soils are *Penicillium*, *Aspergillus*, *Cladosporium*,

Table 3.2 Species of fungi present in Antarctic soils

Fungal taxa	Site of recovery	Reference
Acremonium alternatum	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
A. berkeleyanum	Bellingshausen (Russian research station), Antarctica	Kochkina et al. (2019)
A. implicatum	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Alternaria alternate	Mount Erebus, Antarctica	Connell and Staudigel (2013)
A. corda	Victoria Land, Antarctica	Onofri and Tosi (1989)
A. maritime	Livingston Island, Antarctic Peninsula	Litova et al. (2014)
A. robusta	Signy Island, South Orkney Islands, Antarctica	Duddington et al. (1973)
Antarctomyces pelizarie	Penguin Island and Robert Island	Gomes et al. (2018)
A. psychrotrophicus	Yankee Bay, Antarctic Peninsula	Abneuf et al. (2016)
Arthrobotrys ferox	Schirmacher Oasis, Victoria Land	Onofri and Tosi (1992)
Aspergillus flavus	Oasis (Russian research station), Antarctica	Kochkina et al. (2019)
A. fumigatus	Livingston Island, Antarctic Peninsula	Litova et al. (2014)
A. glaucus	Livingston Island, Antarctic Peninsula	Kostadinova et al. (2009)
A. niger	Russkaya station, Antarctica	Marfenina et al. (2016)
A. penicillioides	Mount Erebus, Antarctica	Connell and Staudigel (2013)
A. rubrobrunneus	Southern Victoria Land	Sugiyama (1970)
A. sydowii	Ellworsth Mountain, Continental Antarctica	Godinho et al. (2015)
A. ustus	Southern Victoria Land	Baublis et al. (1991)
A. versicolor	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
Aureobasidium pullulans	Mount Erebus, Antarctica	Connell and Staudigel (2013)
A. melanogenum	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
Beauveria bassiana	Demay point, Antarctic Peninsula	Gonçalves et al. (2015)
Bensingtonia yamatoana	Chabrie Rock and Ulmann point, Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
Bjerkandera adusta	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
Bulleromyces albus	Cape Royds Hut, Ross Sea	Arenz et al. (2006)
Cadophora fastigiata	Various Russian research stations, Antarctica	Kochkina et al. (2019)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
C. luteoolivacea	Various Russian research stations, Antarctica	Kochkina et al. (2019)
C. malorum	Various Russian research stations, Antarctica	Kochkina et al. (2019)
C. melinii	Druzhnaya-4 and Bellingshausen (Russian research stations), Antarctica	Kochkina et al. (2019)
Caloplaca saxicola	Dry Valleys, Victoria Land	Fell et al. (2006)
Candida glaebosa	Demay Point, Chabrie Rock and Henryk Arctowisky Station; Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
C. parapisilosis	Cape Royds Hut, Ross Sea; Lake Fryxell Basin, Dry Valleys	Arenz et al. (2006)
C. zeylanoides	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Ceriporiopsis subvermispora	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Chaetomium globosum	Browning Peninsula, Windmill Islands, Eastern Antarctica	Pudasaini et al. (2017)
Cladosporium cladosporioides	Russkaya station, Antarctica	Marfenina et al. (2016)
C. herbarum	Various Russian research stations, Antarctica	Kochkina et al. (2019)
C. oxysporum	Livingston Island, Antarctic Peninsula	Kostadinova et al. (2009)
C. sphaerospermum	Druzhnaya-4 and Oasis (Russian research stations), Antarctica	Kochkina et al. (2019)
Clavispora lusitaniae	Southern Victoria Land	Connell et al. (2008)
Cochliobolus heliconiae	Dry Valleys, Victoria Land	Fell et al. (2006)
Coniochaeta lignaria	Dry Valleys, Victoria Land	Fell et al. (2006)
Cordyceps bassiana	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
Cosmospora vilior	Cape Evans Hut and Cape Royds Hut, Ross Sea	Arenz et al. (2006)
Cryptococcus albidus	Livingston Island, Antarctic Peninsula	Pavlova et al. (2001)
C. albidosimilis	Southern Victoria Land	Connell et al. (2008)
C. antarcticus	University Valley, Continental Antarctica	Vishniac and Kurtzman (1992)
C. arrabidensis	Taylor Valley, McMurdo Land	Fell et al. (2006)
C. carnescens	Southern Victoria Land	Connell et al. (2008)
C. curvatus	Taylor Valley, McMurdo Land	Arenz et al. (2006)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
C. friedmannii	Cape Royds Hut,Ross Sea; Allan Hills, Dry Valley	Arenz et al. (2006)
C. grevilleae	Mount Erebus, Antarctica	Connell and Staudigel (2013)
C. laurentii	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
C. luteolus	Dry Valleys, Victoria Land	Sugiyama et al. (1967)
C. macerans	Dry Valleys, Victoria Land	Sugiyama et al. (1967)
C. nyarrowii	Southern Victoria Land	Connell et al. (2008)
C. saitoi	Southern Victoria Land	Connell et al. (2008)
C. sphaerospermum	Mount Erebus, Antarctica	Connell and Staudigel (2013)
C. stepposus	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
C. tephrensis	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
C. victoriae	Rip Point, Nelson Island	Gomes et al. (2018)
C. vishniacii	Southern Victoria Land	Connell et al. (2008)
C. wieringae	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Clavispora lusitaniae	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Cochliobolus lunatus	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Cyphellophora laciniata	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Cystobasidium laryngis	King George Island, Antarctica	Rovati et al. (2013)
Cystofilobasidium macerans	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Debaryomyces hansenii	Ellworsth Mountain, Continental Antarctica	Godinho et al. (2015)
Dioszegia hungarica	King George Island, Antarctica	Rovati et al. (2013)
Dipodascus australiensis	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
Doratomyces microsporus	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
Emericella nidulans	Progress station, Antarctica	Marfenina et al. (2016)
Engyodontium album	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
Epicoccum nigrum	Mount Erebus, Antarctica	Connell and Staudigel (2013)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
Erysiphe polygoni	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Eurotium amstelodami	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
Exidia glandulosa	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Exophiala spinifera	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
E. xenobiotica	Various Russian research stations, Antarctica	Kochkina et al. (2019)
Filobasidium floriforme	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Fomitopsis pinicola	Oasis (Russian research station), Antarctica	Kochkina et al. (2019)
Fusarium oxosporum	Cape Evans Hut and Cape Rouds Hut, Ross Sea	Arenz et al. (2006)
Fusarium solani	Progress station, Antarctica	Marfenina et al. (2016)
Fusicladium peltigericola	Progress-2 (Russian research station), Antarctica	Kochkina et al. (2019)
Fusicladium radiosum var. *radiosum*	Oasis (Russian research station), Antarctica	Kochkina et al. (2019)
Ganoderma applanatum	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Gibellulopsis nigrescens	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Glaciozyma watsonii	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Goffeauzyma gilvescens	King George Island, Antarctica	Rovati et al. (2013)
Holtermanniella watticus	Browning Peninsula, Windmill Islands, Eastern Antarctica	Pudasaini et al. (2017)
Hormonema dematioides	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
Hyphodontia rimosissima	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Isaria farinosa	Progress-2 (Russian research station), Antarctica	Kochkina et al. (2019)
Kabatiella zeae	Deception Island, Antarctic Peninsula	Abneuf et al. (2016)
Lecanicillium muscarium	Livingston Island, Antarctic Peninsula	Kostadinova et al. (2009)
Leucosporidiella creatinivora	Ulmann Point, Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
L. fragaria	Chabrie Rock, Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
Leucosporidium creatinivorum	King George Island, Antarctica	Rovati et al. (2013)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
L. scottii	Botany Point, Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
Lewia infectoria	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Malassezia globosa	Mount Erebus, Antarctica	Connell and Staudigel (2013)
M. restricta	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Mammaria echinobotryoides	Bellingshausen (Russian research station), Antarctica	Kochkina et al. (2019)
Monodictys austrina	Livingston Island, Antarctic Peninsula	Kostadinova et al. (2009)
Mortierella alpine	Antarctic Peninsula	Arenz and Blanchette (2011)
M. amoeboidea	Yellow Point, King George Island	Gomes et al. (2018)
M. antarctica	Rip Point, Nelson Island	Gomes et al. (2018)
M. elongate	Progress station, Antarctica	Marfenina et al. (2016)
M. minutissima	Windmill Islands	McRae and Seppelt (1999)
M. parvispora	Yellow Point, King George Island	Gomes et al. (2018)
M. polycephala	Signy Island, South Orkney Islands, Antarctica	Hughes et al. (2007)
M. turficola	Signy Island, South Orkney Islands, Antarctica	Bailey and Wynn-Williams (1982)
Mrakia blollopsi	Syowa station, East Antarctica	Tsuji (2016)
M. frigida	Rip Point, Nelson Island	Gomes et al. (2018)
M. psychrophila	Fildes Peninsula, Antarctica	Xin and Zhou (2007)
M. stokesii	Southern Victoria Land	Connell et al. (2008)
Mucor circinelloides	Progress station, Antarctica	Marfenina et al. (2016)
M. hiemalis	Russkaya station, Antarctica	Marfenina et al. (2016)
Myrothecium verrucaria	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Nadsonia commutate	Chabrie Rock, Admiralty Bay, Antarctic Peninsula	Vaz et al. (2011)
Paecilomyces variotii	Oasis (Russian research station), Antarctica	Kochkina et al. (2019)
Paraphoma fimeti	Various Russian research stations, Antarctica	Kochkina et al. (2019)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
Penicillium allii-sativi	Ellworsth Mountain, Continental Antarctica	Godinho et al. (2015)
P. amphipolaria	Quartemain Mountains, Dry Valleys	Visagie et al. (2016)
P. antarcticum	Schirmacher Oasis, East Antarctica	Singh et al. (2006)
P. aurantiogriseum	Progress station, Antarctica	Marfenina et al. (2016)
P. brevicompactum	Macquarie Island and Casey	Kerry (1990)
P. chrysogenum	Rip Point, Nelson Island	Gomes et al. (2018)
P. citreonigrum	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
P. commune	Livingston Island, Antarctic Peninsula	Litova et al. (2014)
P. coprobioum	Livingston Island, Antarctic Peninsula	Litova et al. (2014)
P. corylophilum	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
P. expansum	Oasis (Russian research station), Antarctica	Kochkina et al. (2019)
P. funiculosum	Continental Antarctica	Greenfield (1981)
P. glabrum	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. glandicola	Russkaya station, Antarctica	Marfenina et al. (2016)
P. implicatum	Russkaya station, Antarctica	Marfenina et al. (2016)
P. italicum	Livingston Island, Antarctic Peninsula	Kostadinova et al. (2009)
P. janczewskii	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. janthinellum	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. lividum	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. miczynskii	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. montanense	Russkaya station, Antarctica	Marfenina et al. (2016)
P. palitans	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
P. oxalicum	Mount Erebus, Antarctica	Connell and Staudigel (2013)
P. restrictum	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
P. roqueforti	Ellworsth Mountain, Continental Antarctica	Godinho et al. (2015)
P. rubens	Ellworsth Mountain, Continental Antarctica	Godinho et al. (2015)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
P. rugolosum	Livingston Island, Peninsula Antarctica	Litova et al. (2014)
P. solitum	Russkaya station, Antarctica	Marfenina et al. (2016)
P. tardochrysogenum	McMurdo Dry Valley, Antarctica	Houbraken et al. (2012)
P. variabile	Novolazarevskaya and Bellingshausen (Russian research stations), Antarctica	Kochkina et al. (2019)
P. verrucosum	Robert Island, Coppermine Peninsula	Gomes et al. (2018)
P. viridicatum	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
P. vulpinum	Russkaya station, Antarctica	Marfenina et al. (2016)
P. waksmanii	Russkaya and Progress stations, Antarctica	Marfenina et al. (2016)
Peniophora lycii	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Phaeococcomyces nigricans	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Phenoliferia glacialis	Rip Point, Nelson Island	Gomes et al. (2018)
Phialophora Alba	Various Russian research stations, Antarctica	Kochkina et al. (2019)
Phoma herbarum	Druzhnaya-4 and Molodezhnaya (Russian research stations), Antarctica	Kochkina et al. (2019)
Preussia flanaganii	Rip Point, Nelson Island	Gomes et al. (2018)
Pseudeurotium bakeri	Port Lockroy and Detaille Island, Antarctic Peninsula	Arenz and Blanchette (2009)
P. desertorum	View Point, Duse Bay, Antarctic Peninsula	Arenz and Blanchette (2009)
P. hygrophilum	Bellingshausen (Russian research station), Antarctica	Kochkina et al. (2019)
Pseudogymnoascus appendiculatus	Penguin Island, Antarctic Peninsula	Gomes et al. (2018)
P. cretaceous	Signy Island, South Orkney Islands, Antarctica	Bailey and Wynn-Williams (1982)
P. destructans	Rip Point, Nelson Island	Gomes et al. (2018)
P. gilvescens	Robert Island, Coppermine Peninsula	Gomes et al. (2018)
P. pannorum	Various Russian research stations, Antarctica	Kochkina et al. (2019)
P. verrucosus	Penguim Island, Antarctic Peninsula	Gomes et al. (2018)
P. vinaceus	Bellingshausen (Russian research station), Antarctica	Kochkina et al. (2019)
P. vulgare	Signy Island, South Orkney Islands, Antarctica	Bailey and Wynn-Williams (1982)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
Purpureocillium lilacinum	Pendulum cove, Antarctic Peninsula	Gonçalves et al. (2015)
Resinicium bicolor	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Rhizopus oryzae	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
Rhizopus stolonifer	Progress station, Antarctica	Marfenina et al. (2016)
Rhodosporidium kratochvilovae	Southern Victoria Land	Connell et al. (2008)
Rhodotorula laryngis	Southern Victoria Land	Connell et al. (2008)
R. mucilaginosa	Robert Island, Coppermine Peninsula	Gomes et al. (2018)
Saccharomyces cerevisiae	Rip Point, Nelson Island	Gomes et al. (2018)
Sarea difformis	Ross Sea, Antarctica	Arenz et al. (2006)
Sistotrema brinkmanii	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Skeletocutis chrysella	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Sporidiobolus metaroseus	McMurdo Dry Valleys, Antarctica	Zucconi et al. (2012)
S. salmonicolor	Ross Sea, Antarctica	Arenz et al. (2006)
Sporobolomyces symmetricus	Cape Evans Hut, Ross Sea	Arenz et al. (2006)
Stereum sanguinolentum	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Thelebolus caninus	Cape Royds Hut, Ross Sea	Arenz et al. (2006)
T. globosus	Beaufort Island; Continental Antarctica	Alias et al. (2013)
T. microspore	Beaufort Island; Continental Antarctica	Alias et al. (2013)
Torulopsis psychrophila	Schirmacher Oasis, East Antarctica	Singh et al. (2006)
Toxicocladosporium irritans	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Trametes cubensis	Mount Erebus, Antarctica	Connell and Staudigel (2013)
T. villosa	Henry Arctowisky station, Antarctic Peninsula	Gonçalves et al. (2015)
Trichoderma harzianum	Druzhnaya-4 (Russian research station), Antarctica	Kochkina et al. (2019)
T. reesei	Windmill Islands, continental Antarctica	Bradner et al. (1999)
Trichosporiella cerebriformis	Bellingshausen (Russian research station), Antarctica	Kochkina et al. (2019)
Trichosporon asteroides	Henry Arctowisky station, Antarctic Peninsula	Gonçalves et al. (2015)
T. domesticum	Dry Valleys, Victoria Land	Fell et al. (2006)

(continued)

Table 3.2 (continued)

Fungal taxa	Site of recovery	Reference
T. loubieri	Dry Valleys, Victoria Land	Fell et al. (2006)
T. ovoides	Dry Valleys, Victoria Land	Fell et al. (2006)
Trichurus spirales	Progress Station, Antarctica	Marfenina et al. (2016)
Verticillium dahliae	Mount Erebus, Antarctica	Connell and Staudigel (2013)
Volutella colletotrichoides	Mount Erebus, Antarctica	Connell and Staudigel (2013)

Mortierella, *Antarctomyces*, *Pseudogymnoascus* (synonymous *Geomyces*), *Rodothorula*, and *Cryptococcus* (Wicklow 1968; Mercantini et al. 1989; Stchigel et al. 2001; Arenz et al. 2006; Margesin et al. 2007; Bensch et al. 2010; Melo et al. 2014; de Menezes et al. 2017; Gomes et al. 2018).

Aspergillus is a cosmopolitan genus, being commonly isolated from soil and plants (Arenz et al. 2014; Godinho et al. 2015). In Antarctica, species of *Aspergillus* were isolated from ornithogenic soil (Wicklow 1968). *Cladosporium* is one of the biggest genera having a worldwide distribution and includes saprobic and parasitic species (Bensch et al. 2010). Although some species of *Cladosporium* are known to be present only in specific hosts or have a restricted geographic distribution (Meyer et al. 1967), they are one of the dominant genera found in the dry valley soils of Antarctica (Arenz et al. 2006).

Mortierella is a genus that occurs in different types of substrates (Kirk et al. 2008). Species of *Mortierella* isolated from Antarctic soils correlated with moss (Frate and Carreta 1990; Tosi et al. 2002; Melo et al. 2014), rhizosphere *D. antarctica*, and *C. quitensis*, found in varied soils of the Antarctic Peninsula (Bridge and Newsham 2009; Gomes et al. 2018; Wentzel et al. 2018). The species *Mortierella antarctica* has already been isolated from samples of different soils (Frate and Carreta 1990; Zucconi et al. 1996; Adams et al. 2006; Gomes et al. 2018). According to Onofri et al. (2004) and Melo et al. (2014), *M. antarctica* has an acid (linoleic acid and arachidonic acid) production capacity, which is important for its development at low temperatures; therefore, it is able to grow and sporulate at 0 °C (Onofri et al. 2004). The genus *Antarctomyces*, which is considered to be a psychrophilic and endemic species of *Thelebolales*, was isolated from the Antarctic soil (Stchigel et al. 2001) and represents the first species of the genus described.

Pseudogymnoascus has a wide geographical distribution in cold ecosystems (www.mycobank.org), including Antarctic soils (Mercantini et al. 1989; Arenz and Blanchette 2011; Godinho et al. 2015; Gonçalves et al. 2015; Gomes et al. 2018). Mercantini et al. (1989) and Arenz et al. (2006) have reported that this genus plays an important role in the decomposition and recycling of organic matter in Antarctica. Among the species of *Pseudogymnoascus* found in Antarctica, *Pseudogymnoascus destructans* is phylogenetically close to those species that attack bats in North

America and Europe/Palearctic Asia. It is obtained from several soils of different islands of Antarctica as reported by Gomes et al. (2018), suggesting that Antarctic soil may be a natural habitat for this species.

The genus *Rhodotorula* comprises basidiomycetous yeasts isolated from different substrates (Nagahama et al. 2001; Libkind et al. 2003; Butinar et al. 2005; Margesin et al. 2007; Sampaio 2011; de Garcia et al. 2012). *Rhodotorula mucilaginosa* is a cosmopolitan species that occurs in aquatic soils and habitats (Kurtzman et al. 2011). In Antarctica, *R. mucilaginosa* was already reported to be present in the soil (Ray et al. 1989; Vishniac, 1996; Pavlova et al. 2001). According to Shivaji and Prasad (2009), *Cryptococcus*, which had some of its species reclassified as *Naganishia*, *Torula*, and *Vishniacozyma* by Liu et al. (2006), is the genus of yeast most abundant on the Antarctic continent and is frequently distributed in different places and substrates. The species of *Cryptococcus* that have already been isolated from different Antarctic soils are *Torula laurentii* (*Cryptococcus laurentii*) and *Vishniacozyma victoriae* (*Cryptococcus victoriae*) (Vaz et al. 2011; Sousa et al. 2017).

3.5 Correlation of the Physicochemical Characteristics of Fungi with Antarctic Soils

Fungi perform a function of early colonisation of sites, develop soil structure and transform nutrients into bioavailable forms. In addition, fungi seem to be one of the main agents in the driest Antarctic soils to synthesise sterols required by invertebrates present in the soil (Connell et al. 2006). Soil biology plays a key role in determining soil carbon; the composition of species of the primary carbon-regulating communities can affect the entry of carbon into terrestrial ecosystems (Newsham et al. 2018).

Antarctic soils have the capacity of autotrophic carbon fixation and nitrogen fixation (Hopkins et al. 2006; Cowan et al. 2011; Cameron et al. 2012). The competition between fungi for carbon sources can influence the efflux of CO_2 in the soil, leading to a decrease in efficiency of the use of carbon, which can further affect the carbon rate present in soils (Newsham et al. 2018). It is possible that these fungi mineralise the soil and consume the generated nutrients. The abundance of cultivable fungi in the soil can be correlated with carbon and nitrogen, suggesting that nutritional limitations in highly oligotrophic environments are prime factors in determining the distribution and abundance of native fungi (Connell et al. 2006). The results obtained by Newsham et al. (2018) suggest that the carbon rate found in warm Antarctic soils has increased, which may be caused by taxa of different microorganisms, including fungi. Different organic compounds have different fixation times in the soil and are degraded by specific taxa of saprophytic fungi (Newsham et al. 2018).

3.6 Conclusion and Perspectives

Despite the different and extreme conditions, the Antarctic soils shelter various genera and species of fungi, whether be they are cold-adapted cosmopolitan fungi or endemic. Studies on Antarctic soils have increased in recent years, and these demonstrate that there is a great diversity of genera and species present in different types of soils, many of which may be new to science. Further research is needed to increase knowledge about the fungal diversity associated with different types of soils in Antarctic environments, especially in areas where there are progressive thaws in the Antarctic Peninsula, leading to exposing of soils not yet studied along with their resident fungal communities. An interesting point that needs to be investigated in the future is the correlation of physicochemical characteristics found in soils with diversity, ecological function, and potential biotechnological applications of Antarctic fungi, which could ultimately elucidate the intrinsic characteristics of each genus and species.

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Chapter 4
Diversity and Ecology of Fungal Assemblages Present in Lakes of Antarctica

Mayara Baptistucci Ogaki, Rosemary Vieira, Juan Manuel Lírio, Carlos Augusto Rosa, and Luiz Henrique Rosa

4.1 Introduction

The aquatic ecosystems of Antarctica comprise a range of habitats covered by temporary or perennial water bodies, such as saline and freshwater lakes, swamps, streams, rivers, estuaries, melting ice areas, sea, and ocean. Usually, the freshwater aquatic zones of Antarctica are in direct contact with rocks, soil, and mud and are bordered by vegetation like *Deschampsia antarctica*, *Colobanthus quitensis*, mosses, macroalgae, and lichens. In addition, the Antarctic lakes have an interface with air, named as extra-aquatic zone; consequently, these lakes represent important sites for the study of microbial diversity and ecology.

Almost the entire land surface of Antarctica is covered by a vast ice cap (more than 70% of the world's freshwater). Nevertheless, water bodies that contain water in a liquid state (unfrozen water) for at least part of a year are a common feature of the Antarctic landscape. Most of subaerial Antarctic lakes are formed when ice recedes and because of exposed depressions in the terrain, formed either by glacial erosion or by the deposition of terminal moraines, or because of folds and depressions in the underlying geological topography (Hodgson 2012).

Seasonal ice-free areas occur in the marginal ice zones, some containing lakes, ponds, streams, and wetlands. The majority of lakes are found in the islands and in the coastal zones of McMurdo Dry Valleys, Vestfold Hills, Larsemann Hills, Bunger Hills, and Schirmacher in continental Antarctica (Vicent et al. 2008; Sokratova

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2011; Phartiyal et al. 2011). However, maritime and subantarctic islands in Antarctica, such as Signy and King George Islands, also have ice-free lakes (Pienitz et al. 2008) and are the most intensively studied areas.

All major fungal phyla identified within freshwater systems, including water and lake sediments, are *Ascomycota*, *Basidiomycota*, traditional *Zygomycota*, and *Chytridiomycota* (Shearer et al. 2007; Monchy et al. 2011; Wurzbacher et al. 2011). Additionally, some *Oomycetes* taxa (Stramenopila) have been described (Shearer et al. 2007; Gonçalves et al. 2012). Moreover, some fungi are largely spread in different Antarctic freshwater environments and are represented by decomposer and parasitic taxa that actively participate in the cycling of organic matter and nutrients (Knox and Peterson 1973; McInnes 2003; Hao et al. 2005). As decomposers, the major functional role of fungi in freshwater ecosystems is breakdown and mineralisation of allochthonous and autochthonous organic matter (Kuehn 2015).

The mycota living in the Antarctic lakes is under the influence of various adverse factors, such as extreme low temperatures, frequent freeze-thaw cycles, high salinity, alkaline and acidic pH values, high UV radiation, and low nutrient availability (Gonçalves et al. 2012). Researches conducted on fungi in Antarctic lakes include studies of different microhabitats, such as water bodies, biofilms composed of plants and microalgae, and sediments (Baublis et al. 1991; de Hoog et al. 2005; Tsuji et al. 2013; Conell et al. 2018), which, despite the extreme conditions, shelter rich and diverse fungal assemblages.

4.2 Lakes of Maritime Antarctica

The maritime Antarctic region includes the west coast of the Antarctic Peninsula and the associated islands of the Scotia Arc and extends from the South Sandwich Islands through South Orkney and South Shetland Islands and down the western side of the Antarctic Peninsula to approximately 72°S (Camacho 2006). In Antarctica, the highest increases in temperature were observed in the Antarctic Peninsula, especially on its west coast. Between 2000 and 2009, the intensity of the Antarctic Peninsula melt accounted for more than 50% of the total intensity of Antarctic surface melt, a finding supported by regional climate modelling of surface meltwater production over a period of 1979–2010 (Abram et al. 2013).

Maritime Antarctica presents lakes and ponds formed in the rocky substratum, as well as coastal lagoons influenced by marine water intrusions (Camacho et al. 2012). The lacustrine system is located in areas, climatically less extreme than the interior of the continent. Therefore, although temperatures less than 0 °C predominate, in summer, the events of fusion and liquid precipitation are common, owing to a more active hydrological cycle with sediment and nutrient circulation throughout the lacustrine basin and in the glaciomarine environment (Quayle et al. 2002; Mckay et al. 2009; Camacho et al. 2012). These climatic conditions favour a large number of freshwater ecosystems that melt out and become ice-free in summer (Toro et al. 2006).

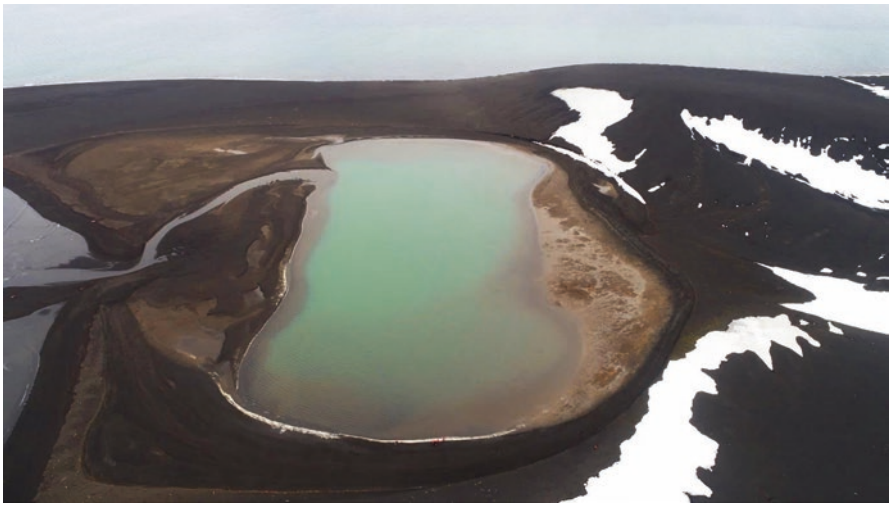

Fig. 4.1 Aerial photo of Kroner Lake, Deception Island (maritime Antarctica). The photo shows the connection between the sea (on the left) and the lake (on the right). (Photo credits, LH Rosa)

Most of the Antarctic lakes have arisen because of glacial retreat, and a few have been formed by tectonic activity; in addition, some lakes may have been formed by volcanic activity (Priddle and Heywood 1980). Volcanoes are still active in some areas of Antarctica such as the South Shetland Islands. Kroner Lake (Fig. 4.1) on Deception Island (located in the South Shetland Islands), occupies a shallow, circular depression in a lava plain; the fumarole activity supplies heat to the lake, which makes it a unique geothermally heated lake in Antarctica (Priddle and Heywood 1980).

Kroner Lake is an example of a lake with marine water intrusion, which has a vast environmental and biological heterogeneity because of its connection with the sea on the margins of the lagoon (Izagirre et al. 2006). Besides, some types of lakes are formed in volcano areas or volcanic depressions, which contain deposits of pyroclastic material on South Shetlands Islands, such as the Crater Lake at Deception Island (Fig. 4.2b) and a smaller Crater Lake at the Penguin Island (Fig. 4.2a).

Two of the largest ice-free areas of Southern Shetland Islands are found on the Byers Peninsula, on the Livingston Island (Oliva et al. 2017), and Fildes Peninsula, King George Island (Peter et al. 2008). In these areas, permafrost dynamics control the interaction between lacustrine and terrestrial ecosystems (Izaguirre et al. 2012). Limnological processes are influenced by a complex set of feedback mechanisms, driven by climatic conditions (e.g. biological productivity or lake ice cover) and other factors directly controlled by permafrost (e.g. hydrological, geomorphological, and sedimentological processes). Therefore, the permafrost controls geomorphic processes, which in turn influence the limnological processes and patterns of lake sedimentation (Oliva et al. 2017). The permafrost may also contain considerable stocks of ancient organic matter that is released during melting (Quesada et al. 2006).

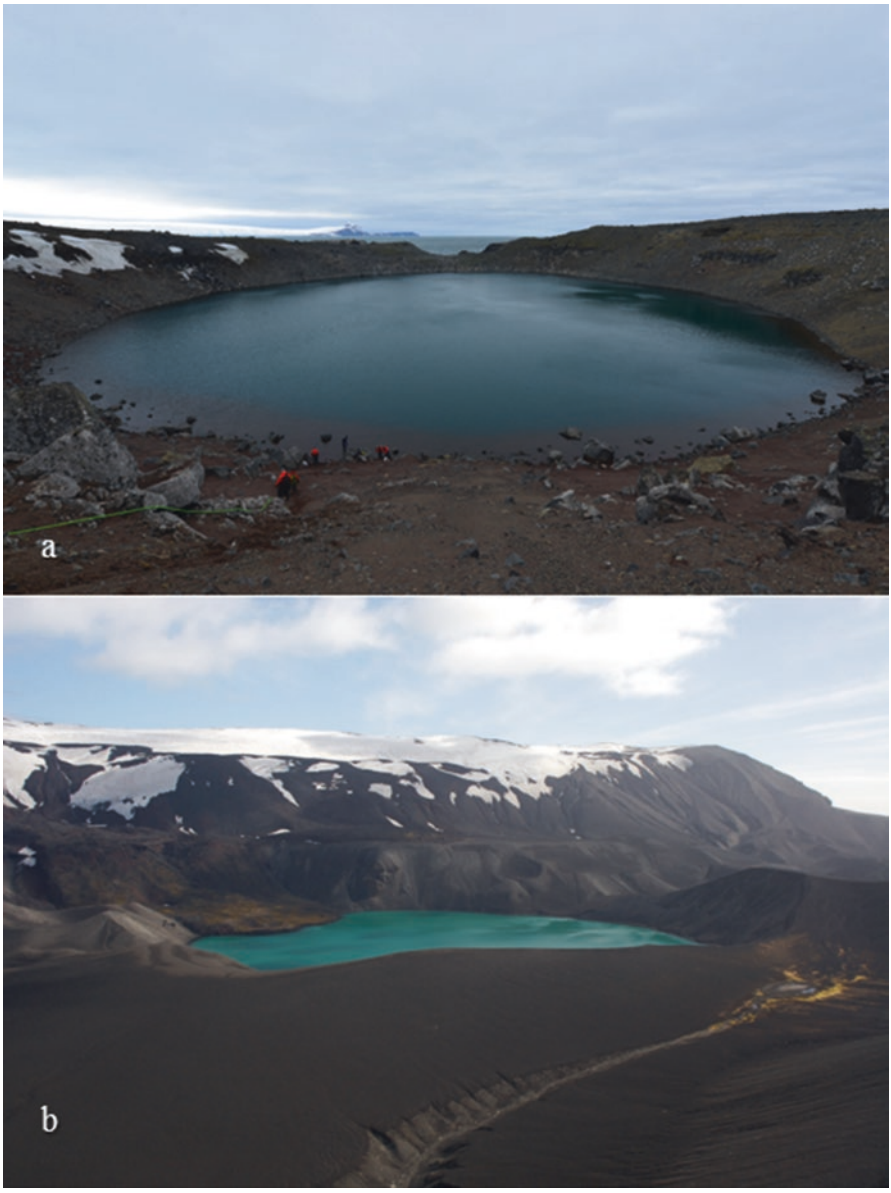

Fig. 4.2 Lakes formed in volcano depressions (called Crater lakes) on South Shetlands Islands. (**a**) depicts a small Crater lake at Penguin Island (area affected by the last volcanic eruption in 1905) and (**b**) Crater lake on Deception Island (area affected by the last eruption in 1969). (Photo credits, LH Rosa)

The Byers Peninsula on Livingston Island holds a high number of lakes, ponds, and flooded or wet areas. Lakes are found both in the central part of the peninsula, in a plateau of 100 m high, and in coastal areas, the latter being accessible to marine animals. The shallow lagoons located in coastal areas are found on low relief lands, and their surrounding areas may vary from sandy and dry terrains to those that are largely covered by moss cushions and plants (Toro et al. 2006; Camacho et al. 2012).

Regarding King George Island, about 60 freshwater lakes of different types and origins are located on the Fildes Peninsula. Most of the lakes are small and shallow endorheic ponds located in depressions formed by deglaciation. However, large and deep lakes also exist. During summer, the lakes are mostly ice-free, and snowmelt water is transported by a local streamflow network (Shevnina and Kourzeneva 2017). The peninsula's vast ice-free area experiences different conditions, transitioning from paraglacial to periglacial, as one moves away from the Collins Glacier. Periglacial landforms occupy approximately 70% of the Fildes Peninsula, and the remaining 30% are composed of structural landforms and rock outcrops shaped by glacial erosion (Michel et al. 2014).

The basins of most lakes are over-deepened glacial basins, and the valleys of the largest streams are glacial troughs (Michel et al. 2014). Solids and particulate matter from catchment areas are carried by water and deposited in lake sediments, contributing to the lake biogeochemical processes (Alfonso et al. 2015); consequently, the microbial community may be affected by such constant changes in the solute composition of lake habitats.

4.3 Lakes of Continental Antarctica

Continental aquatic habitats differ from each other; the ice-free areas have larger lakes. Most of them have a typical concentration of ions and solutes, which make them saline or hypersaline. The differences in salinity and ionic composition of the lakes are related, in part, to how the lakes have responded to temperature changes. These lakes acquire a significant fraction of salt content when glacial meltwaters come in contact with soils and sources that surround them. Furthermore the concentrations of all the ions increase with the distance that the meltwater travels to these lakes before finding slots for percolation (Green and Canfiel 1984; Priscu and Foreman 2009).

The Antarctic oases are ice-free regions with distinct weather patterns and are located in relief depressions or old marine lagoons (Laybourn-Parry and Wadham 2014; Shevnina and Kourzeneva 2017). The lakes and ponds found in these regions are shallow and relatively warm, but there are also some large, deep, and cold-water lakes, which occur in tectonic faults or in proximity to glaciers (Sokratova 2011; Shevnina and Kourzeneva 2017). Till date, many of these lakes show high-density stratification with hypersaline bottom water, thought to have formed during glacial

lowstands. Water levels in this hyperarid environment are controlled by the balance between surface inflow and sublimation of the perennial ice cover (Hall et al. 2010).

The presence of unfrozen water (in the form of a system of seasonal streams and non-freezing lakes) makes the Antarctic oases unique landscapes (Sokratova 2011). The perennial ice can have a profound impact on the lake dynamics. The thick perennial ice cover can minimise wind-generated currents, limit lake circulation, restrict light penetration, increase dissolved gas concentrations, and cause heterogeneous sedimentation on the lake bottom (Doran et al. 2000; Phartiyal et al. 2011).

Many lakes and ponds having various salt contents are scattered in the depressions of valleys and are generally covered with ice, 3–5 m thick, throughout the year (McKay et al. 1985; Matsumoto 1993; Doran et al. 1996). The salt composition of lake waters of the Antarctic oases is formed because of the transport of marine aerosols by precipitation, salt freezing, and ion inflow from the upper layers of the soils of lake basins (Sokratova 2011).

The distribution of total salts and chloride ion concentrations corresponds to freshwater and saline lakes, reflecting the presence and absence of outflows, respectively. The local arid condition enables subsurface waters to contain salts, possibly resulting from the weathering of rocks and evaporation of waters. Nutrients are concentrated in the anoxic bottom waters because of the lack of circulation, producing an oligotrophic status of the lakes and ponds (Bishop et al. 1996, 2014). However, these extreme habitats host active biosystems: microbial mat communities that can flourish in the lake bottom sediments, owing to the absence of a significant foraging fauna (Bird et al. 1991; Bishop et al. 1996, 2014).

The McMurdo Dry Valleys consist of three major valleys, namely, Victoria, Wright, and Taylor, and these comprise the largest ice-free area (2500 km^2) in Antarctica (Vocke and Hanson 1981). The Vestfold Hills on Princess Elizabeth Land are at the margin of the East Antarctic ice sheet, and they have more than 150 hypersaline and low-salinity lakes, because of the retreat of the ice from ice sheets (Bird et al. 1991). In contrast, in the East Antarctic Shield, about 100 km southwest of the Vestfold Hills, are the Larsemann Hills that consist of several ice-free peninsulas and islands along the coast, with more than 100 freshwater lakes, ranging from small ponds to deep lakes (Stüwe et al. 1989; Shevnina and Kourzeneva 2017). Furthermore, the Bunger Hills are one of the largest ice-free areas on the Antarctic continent, located near the coast of East Antarctica at about 100″ longitude, covering an area of about 300 km^2 and consisting of low rocky hills and glacially deepened valleys (Sheraton et al. 1993). In this area, there are a few perennially ice-covered lakes at the oasis edge, and these lakes are largely in contact with glacier ice (Doran et al. 2000). Most of the lakes are in the centre of the oasis, and thus become ice-free in the summer months, while the lakes at the edge of the oasis, in contact with glacier ice, mostly retain their ice covers around the whole year (Doran et al. 1996).

In general, continental Antarctica presents epiglacial lakes found on the surface of the ice sheets, glaciers, and ice shelves. Since 1970s, subglacial lakes, rivers, and

wetlands were discovered using airborne radio echo, deep beneath the Antarctic ice cap (Robin et al. 1977). According to Wright and Siegert (2012), satellite observations and radar measurements revealed that there are active and partially interconnected subglacial hydrological systems connecting more than 379 lakes, existing extensively beneath the Antarctic ice sheet.

All these lakes are subject to high pressure (approximately 350 atmosphere), low temperatures (about −3 °C), and permanent darkness. However, dissolved oxygen is available on the surface of the lake because of an equilibrium with the air hydrates released by melting basal layers of glacier ice (Siegert et al. 2001). They lie up to 4200 m under the Antarctic ice sheet and range in sizes from 1 to 241 km long (Hodgson et al. 2004). These lakes have been isolated from the outside world for thousands of years, and combined with these characteristics are some of the most extreme environments on Earth. Lake Vostok, for example, was continually buried under glacial ice for 15 million years (Rogers et al. 2013); therefore Lake Vostok and others subglacial lakes may be an ecosystem still awaiting to be explored.

Lake Vostok is the best-known subglacial lake in Antarctica; it is the largest and deepest lake in East Antarctica (240 km long, 50 km wide) and lies between 3750 m (at the south of the lake) and 4150 m (at the north) beneath the central-east Antarctic ice sheet (Siegert et al. 2001). The freshwater in Lake Vostok is kept in a liquid state by the pressure of the ice overburden (equivalent to −350 atmosphere) and, perhaps, by geothermal heating (Karl et al. 1999). The water residence time of this lake is estimated to be around 50,000 years, and there is speculation that the lake may contain microbes, which have remained isolated from the rest of the biological world for thousands of years (Hodgson et al. 2004). In spite of its isolation from the ice surface, Lake Vostok and similar lakes may contain previously undescribed relic populations of microorganisms that are adapted to life in these presumably oligotrophic (low-nutrient, low-biomass, and low-energy flux) habitats (Karl et al. 1999).

4.4 Diversity of Fungi in Antarctica Lakes

There are few detailed reports of fungal communities in freshwater lakes in the Antarctic continent, most of them in maritime Antarctica as compared to those in continental Antarctica (Ellis-Evans 1996; Brunatti et al. 2009; Gonçalves et al. 2012). Regarding the diversity of lake microbiota, there is variation in the communities of microorganisms according to the temperature gradient that occurs latitudinally in the continent. A summary of the main research involving the mycobiota of lakes of the Antarctica with respect to the regions of study is given in Table 4.1.

Table 4.1 List of fungal species isolated from different lakes of Antarctica

Region	Lake	Substrate	Fungal taxa	Reference
Maritime Antarctica				
Deception Island	Kroner, Relict, and two unnamed lakes	Surface and depth water	*Rhodotorula*, *Candida* and one unknown filamentous fungus	Stanley and Rose (1967)
	Lake in Port Foster	Sediment	*Cystobasidium laryngis*	Vaz et al. (2011)
	Crater lake	Water	*Pseudogymnoascus pannorum*, *Mortierella* sp., *Cladosporium* cf. *cladosporioides*, *Penicillium* sp., *Davidiella tassiana*, *Cladosporium* sp., and *Trichoderma longibrachiatum*	Gonçalves et al. (2012)
King George Island	Lake in Agat point	Water	*P. pannorum*, *Phaeosphaeria* sp., *Cadophora malorum*, *D. tassiana*, *Helotiales* sp., *Gibberella moniliformis*, *Penicillium paneum*, and *Penicillium* cf. *verrucosum*	Gonçalves et al. (2012)
	Lake next to the Brazilian Refuge II	Water	*Antarctomyces psychrotrophicus*, *Cladosporium cladosporioides*, *D. tassiana*, *Helgardia* sp., *P. pannorum*, *Microdochium* sp., *Microdochium nivale*, *Mortierella* sp., *Pleosporales* sp., *Saprolegniaceae* sp., and *Thelebolus* sp.	Gonçalves et al. (2012)
	Lake next to the Brazilian Station	Sediment	*Helotiales* sp. and *Schizophyllum commune*	Gonçalves et al. (2015)
	Lake next to Copacabana USA Refuge	Freshwater and sediment	*Candida glaebosa*, *Nadsonia commutate* (freshwater); *Issatchenkia* (*Pichia*) *orientalis*, *Kodamaea ohmeri*, *Meyerozyma guilliermondii*, *Rhodotorula mucilaginosa*, and *Vishniacozyma victoriae* (sediment)	Vaz et al. (2011)
	Lake in Jardew point	Sediment	*Annulohypoxylon* sp. and *Cosmospora* sp.	Gonçalves et al. (2015)

	Two lakes next to Machu Picchu Station	Freshwater	*Aureobasidium pullulans*, *Exophiala xenobiotica*, *Leucosporidium creatinivorum*, *Microglossum* sp., *Rhodotorula mucilaginosa*, *Sporidiobolus salmonicolor*, *V. victoriae*	Vaz et al. (2011)
	One lake next to Machu Picchu Station	Freshwater	*Cladosporium* sp., *C. malorum*, *Fontanospora* sp., *P. pannorum*, *Helgardia* sp., *Mortierella* cf. *alpina*, *Phoma* cf. *paspali*, *Phaeosphaeria* sp., and *Thelebolus* sp.	Gonçalves et al. (2012)
	Three lakes next to Machu Picchu Station	Sediment	*Aspergillus* sp., *C. malorum*, *Pseudogymnoascus* sp., *Penicillium* sp., *Pleosporaceae* sp., and *Sordariomycetidae* sp.	Gonçalves et al. (2015)
	Lake next to Stain House glacier	Water	*A. psychrotrophicus*, *C. cladosporioides*, *Cosmospora* cf. *vilior*, *Cadophora* cf. *luteo-olivacea*,*P. pannorum*, *Helotiales* sp., *Heydenia* sp., *Microdochium* sp., *Mortierella* cf. *alpina*, *Phoma herbarum*, *Phoma fimeti*, and *Thelebolus microsporus*	Gonçalves et al. (2012)
	Lake next to Wanda glacier	Water	*Cladosporium* sp., *P. pannorum*, *Mortierella* sp., *Pseudeurotium* sp., *Phoma herbarum*, *Penicillium paneum*, and *Thelebolus* sp.	Gonçalves et al. (2012)
Signy Island (South Orkney Islands)	Signy lakes	Freshwater	*Chytriomyces* sp., *Chytriomyces willoughbyi*, and aquatic "phycomycetes" *Aphanomyces* sp.	Willoughby (1971)
		Freshwater	*Lagenidium giganteum*, *Hyphochytrium catenoides*, *Aphanomyces* sp. (*Saprolegniaceae*), 6 basidiomycetous forms including *Rhodotorula* sp., and *Leucosporidium* sp.	Ellis-Evans (1985)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
		Benthic cyanobacterial mat and sediments	*Lecophagus antarcticus*	McInnes (2003)
Ice-free areas (continental Antarctica)				
McMurdo Dry Valleys (Southern Victoria Land)	McMurdo oasis next to McMurdo Station	Freshwater, soil, and algae	*Scherffelliomyces appendiculatus*, *Chytridium versatile*, *Rhizophlyctis rosea*, *Rhizophydium proliferum*, *Phlyctochytrium recurvastomum*, *Catenophlyctis variabilis*, *Aphanomyces* (*Saprolegniales*), *Pythium tenue*, and *Pythium* sp.	Knox and Paterson (1973)
	Bonney Lake	Soil in lake side and inflow stream	*Candida australis*	Goto et al. (1969)
		Bottom water	*Dendryphiella* sp. and *Diheterospora catenulata*	Waguri et al. (1975), Waguri (1976)
	Basins Lake located in the Taylor and Miers Valleys (including two samples from Bonney Lake)	Water	*Cryptomycota* sp., *Chytridiomycota* sp., *Ascomycota* sp., *Zygomycota* sp., *Blastocladiomycota* sp., *Glaciozyma* sp., and *Mrakia* sp.	Rojas-Jimenez et al. (2017)
	Fryxell Lake	Algae in lake side	*Candida scottii*	Goto et al. (1969)
		Bottom water	*Aureobasidium foliicolum*	Waguri et al. (1975), Waguri (1976)
		Biomats	*Thelebolus ellipsoideus* and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Embellisia* sp., *Onychophora* sp., *Leucosporidium antarcticum* (*Glaciozyma antarctica*), and *Mrakia frigida*	Brunati et al. (2009)

		Water	*Acremonium* sp., *Aureobasidium pullulans*, *Cladosporium cladosporoides*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Pseudogymnoascus* sp., *Heydenia alpina*, *Penicillium commune*, *Penicillium dipodomyicola*, *Thelebolus ellipsoideus*, *Thelebolus globosus*, *Toxicocladosporium strelitziae*, *Filobasidium magnus*, *Glaciozyma antarctica*, *Glaciozyma watsonii*, *Holtermanniella yarrowii*, *Mrakiella aquatica*, *Naganishia albidosimilis*, *Naganishia globosa*, *Rhodotorula mucilaginosa*, and *Vishniacozyma victoriae*	Conell et al. (2018)
	Hoare Lake	Meltwater foam, microbial mat from benthos and from meltwater	*Candida ciferrii*, *Cephalosporium acremonium*, *Aureohasidium pullulans*, *Chrysosporium pannorum*, *Geotrichum candidum*, and *Penicillium notatum*	Baublis et al. (1991)
		Biomats	*Thelebolus ellipsoideus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Leucosporidium antarcticum*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Miers Lake	Water and an outlet stream	*Candida diffluens*, *R. texensis* (in water), and *Rhodotorula rubra* var. *miersensis* (in an outlet stream)	Goto et al. (1969)
	Vanda Lake	Water and sediment	*Cryptococcus albidus*, *Candida diffluens*, *Candida humicola*, *Trichosporon cutaneum* var. *antarcticum*, *Rhodotorula glutinis* var. *rufusa*, and *Rhodotorula texensis* (water); *Sporobolomyces antarcticus*, *C. diffluens*, *C. scottii*, and *Rhodotorula rubra* (sediment)	Goto et al. (1969)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
		Deep water (68 m)	*Aspergillus*, *Penicillium*, *Stachybotrys*, and *Trichoderma*	Kriss et al. (1976)
		Water from several depths (5–69 m)	*Candida* sp.	Nagashima et al. (1990)
Vestfold Hills	Ace Lakes	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Cladosporium* sp., *Pseudogymnoascus* sp., unidentified filamentous fungus; *Cryptococcus albidus*, *Cryptococcus infirmo-miniatus*, *Cryptococcus laurentii*, *Leucosporidium antarcticum*, *Leucosporidium scottii*, *Mrakia frigida*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Druzby Lake	Biomats	*Thelebolus ellipsoideus*, *Thelebolus globosus*, and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., and *Leucosporidium scottii*	Brunati et al. (2009)
	Highway Lakes	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., and unidentified filamentous fungus	Brunati et al. (2009)
	Organic Lake	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp. and *Penicillium* sp.	Brunati et al. (2009)

	Pendant Lake	Biomats	*Acremonium* sp., *Alternaria* sp., *Arthrinium* sp., *Aspergillus* sp., *Beauveria* sp., *Botrytis* sp., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Pseudogymnoascus* sp., and unidentified filamentous fungus	Brunati et al. (2009)
	Watts Lake	Biomats	*Thelebolus globosus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Beauveria* sp., *Phialophora* sp., and unidentified filamentous fungus	Brunati et al. (2009)
Larsemann Hills (Princess Elizabeth)	Manning Lake	Biomats	*Thelebolus ellipsoideus*, *Thelebolus microsporus*, and *Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., *Curvularia* sp., and *Rhodotorula minuta*	Brunati et al. (2009)
	Reid Lake	Biomats	*Thelebolus microsporus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., unidentified filamentous fungus, *Candida lipolytica*, *Cryptococcus albidus*, *Debaryomyces hansenii* var. *hansenii*, *Leucosporidium scottii*, and *Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Sarah Tarn Lake	Biomats	*Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Aspergillus* sp.	Brunati et al. (2009)

(continued)

Table 4.1 (continued)

Region	Lake	Substrate	Fungal taxa	Reference
Skarvsnes (Lutzow-Holm Bay, East Antarctica)	Abi-ike, Ageha-ike, Bosatsu-ike, Ebi-numa, Hyoutan-ike, Jizou-ike, Kuwai-ike, Kumogata-ike, Magoike, Naga-ike, Nisehyoutan-ike, Nyorai-ike, Ohgi-ike, Oyako-ike, Shimai-ike, and Tokkuri-ike Lakes	Surface soil around lakes and sediments	*Embellisia* sp. and *Phoma* sp., *Pseudogymnoascus* sp., *Tetracladium* sp., *Thelebolus* sp., *Mrakia* sp., *Cryptococcus* sp., *Dioszegia* sp., *Rhodotorula gracialis*, and *Leucosporidium antarcticum*	Tsuji et al. (2013)
Subglacial lakes				
Vostok Station (under surface of the central East Antarctic ice sheet)	Vostok Lake	Accretion ice	*Cystofilobasidium* sp., *Cryptococcus* sp., *Pseudozyma* sp., *Penicillium* sp., *Aeurobasidium* sp., and *Aspergillus* sp.	D'Elia et al. (2009)
		Accretion ice and surface of the southern main basin	*Ascomycota* sp., *Basidiomycota* sp., *Zygomycota* sp. (*Mucorales*), and unknown uncultured fungi	Rogers et al. (2013)

4.5 Fungi from Lakes in Maritime Antarctica

The islands of maritime Antarctica are formed by different archipelagos (located at South Shetland and South Orkney Islands) or active volcanoes (at Deception, Ross, and South Sandwich Islands) (Hodgson 2012). The maritime areas of the Antarctic Peninsula have higher temperatures around the year compared to the rest of the continental Antarctica and, consequently, influence the microbial communities of the water bodies present in the region.

The taxa of fungi found in maritime Antarctica, where there are warmer temperatures and more variations of humidity and water availability, are already described. The main fungal phyla are *Ascomycota*, *Basidiomycota*, traditional *Zygomycot*a, and *Chytridiomycota*, as well as allied species from *Oomycota*.

Different studies have described the presence of freshwater fungi, mainly on Signy Island (Willoughby 1971; Ellis-Evans 1985; McInnes 2003; Rogers et al. 2013) in the South Orkney Islands, located on the east of the South Shetland Islands (northern extremity of maritime Antarctica). The most detailed studies about the mycobiota of the South Orkney Islands were carried out in the last decades by Ellis-Evans [Ellis-Evans (1981, 1985, 1996) and McInnes (2003)], which showed that aquatic fungi of the classes *Hyphomycetes* and *Chytridiomycetes* (fungi) as well as *Oomycetes* (Stramenopila) were observed to be either associated with cyanobacterial mats or to have a predation relationship with algae and aquatic invertebrates.

Ellis-Evans (1985) described six psychrotolerant basidiomycetous taxa, including those of the genera *Rhodotorula* and *Leucosporidium*. According to Ellis-Evans (1981), yeasts can compete with bacteria in the water columns and, therefore, are considered as transient propagules, whereas in the sediments, yeasts are present in comparatively low numbers but may play some roles in nutrient cycling. Nevertheless, the ecological role of yeasts in these lakes remains unclear. The *Oomycetes* represented by *Saprolegniaceae* sp., *Aphanomyces* sp., and six species of *Chytridiomycetes* were described by Ellis-Evans (1985). In addition, an account of freshwater fungi at the Signy Island was given by Willoughby (1971), including two species, *Chytriomyces* and *Chytriomyces willoughbyi*. Isolates of *Saprolegniaceae* was also found in the Keller Peninsula, South Shetlands Islands (Gonçalves et al. 2012), and the genus *Aphanomyces* in McMurdo oasis in Ross Island (Knox and Peterson 1973), thus indicating that the order *Saprolegniales* is representative of the Antarctica lakes. In an evaluation of shallow lake margins on Signy Island, McInnes (2003) described a new species, *Lecophagus antarcticus*, a predaceous fungus of rotifers and tardigrades, collected from benthic cyanobacterial mats and sediments. However, there is little information about aquatic *Hyphomycetes* in Antarctica lakes, which mainly possess such type of an ecological relationship.

A large number of freshwater lakes with varying complexity occur in the South Shetland Islands. Stanley and Rose (1967) reported yeasts and a filamentous fungus from five lakes located on the Deception Island (Kroner Relict, and two unnamed Lakes). They obtained only one unknown filamentous fungus and four yeasts, but because of the rudimentary identification techniques available at that period of time,

only two yeasts were categorised as belonging to the genera *Rhodotorula* and *Candida*. Their growth profiles at different temperatures indicated that most of the yeasts grew optimally below 20 °C. Years later, by means of molecular identification techniques, more detailed studies about fungi in the South Shetland freshwater were published.

Vaz et al. (2011) isolated fungi from different substrates of Antarctica, including water and lake sediment. The samples were collected in areas of anthropic influence near research stations (Machu Picchu and the Copacabana United States Refuge, in King George Island) and the sealing history area – the first exploratory industry in the Antarctic (Port Foster on Deception Island). The authors reported the presence of species *Candida glaebosa*, *Nadsonia commutate*, and *Cystobasidium laryngis* in samples of the lake sediment and nine genera from the lake freshwater, of which *Exophiala xenobiotica* and *Microglossum* sp. were the most isolated taxa. All fungal taxa were evaluated for biotechnological applicability and *E. xenobiotica*, *C. laryngis*, *R. mucilaginosa*, and *Microglossum* sp. were observed to produce mycosporines and/or carotenoids, UV-protective compounds, and other pigments. In addition, most of the yeast isolates had extracellular enzymatic activities at different temperatures, indicating that the fungi would have been metabolically active in the substrate.

In the King George and Deception Islands, Gonçalves et al. (2012) isolated 128 fungi from freshwater, and the most common taxon isolated was the genus *Pseudogymnoascus*, which has been reported in other cold ecosystems, such as arctic, temperate and alpine regions (Kobayashi et al. 1967; Semenova et al. 2015; Wang et al. 2015b; Zukal et al. 2016). The endemic genera *Antarctomyces*, as well as *Mortierella* (*Zygomycota*), and one zoosporic fungus (*Oomycota*) were also identified by Gonçalves et al. (2012). The other taxa identified were only at the order or family levels, such as *Helotiales* sp. and *Pleosporales* sp., as they did not have matches with any of the species deposited in GenBank.

Gonçalves et al. (2015) isolated fungi from different substrates, including sediment from lakes situated next to the Ferraz (Brazilian) and Machu Picchu (Peru) stations, as well as Jardew point, both located in King George Island. In the freshwater of these lakes, the authors identified species such as *Schizophyllum commune* and the genera *Pseudogymnoascus*, *Penicillium*, *Cosmospora*, *Annulohypoxylon*, and *Aspergillus*. In addition, another taxon *Incertae sedis* belonging to the order *Helotiales* was obtained by Gonçalves et al. (2015), suggesting that Antarctic lakes provide a unique environment to study the origin and activities of aquatic fungal communities and trophic webs, the importance of which remains unknown. Wang et al. (2015a) described some records about psychrotolerant species in *Helotiales* (principally *Letiomicetes* class), which are fungi associated with glacier soil from China and Antarctic soil from the Great Wall (Chinese) Station in King George Island, including the new species described by the authors as *Psychrophila antarctica*.

According to Bridge et al. (2008), there are 25 fungal taxa belonging to the order *Helotiales* cited in different regions of Antarctica, including Bird Island, Signy Island, and King George Island in maritime Antarctica. From this list of non-lichenised fungi from Antarctica, most of the fungi were associated with substrates such as lichens, different species of bryophyte, plants such as *D. antarctica* and *C.*

quitensis, and soil; however, the association with the lake substrates for the order *Helotiales* is not cited in their study.

4.6 Fungi from Ice-Free Lakes of Continental Antarctica

Among the most extensively studied Antarctic lakes are those that occur in the ice-free oases on the edge of the continent region, such as the Vestfold Hills, Larsemann Hills, and McMurdo Dry Valleys (Hodgson 2012). Most of the mycobiota studies of these types of lakes are concentrated in these areas of Antarctica. In addition, research on fungi of saline lakes, situated on Victoria Land, such as Lake Bonney and Lake Vanda, has been reported.

Antarctic saline lakes have many factors that limit the species diversity and growth rates of organisms inhabiting this ecosystem. According to Wright and Burton (1981), high salinity produces a rise in osmotic strength and opacity of ice, decline in the freezing point of water, and a reduction in the ice cover. In addition, in summer, these lakes have a high exposure to light. Therefore, as a lake freezes, salts eliminated from the ice increase the salinity of the water, which remains in the water layers at the bottom, making the environment even more hostile. Most of these factors also affect organisms inhabiting the alpine lakes and Antarctic oceans. Consequently, microbial communities that constitute saline lakes in Antarctica may vary from those present in cold freshwater ecosystems.

The Taylor Valley is the southernmost valley of the three large Dry Valleys in the Transantarctic Mountains, in Victoria Land, where the three major lakes – Lake Bonney, Lake Fryxell, and Lake Hoare – are located. The permanently ice-covered lakes in the valleys vary dramatically in their geochemistry. Lake Bonney contains hypersaline deep waters where sodium chloride and magnesium chloride are predominantly present, while sulphides and methane are absent. Lake Fryxell has weakly saline deep waters and sediments that are both highly sulphidic and methanogenic, whereas Lake Hoare is essentially a freshwater system (Green and Lyons 2009; Tregoning et al. 2015). Detailed reports of the fungi obtained from these Taylor Valley saline lakes and other saline lakes from ice-free areas are given below.

Lake Vanda is a meromictic and hypersaline lake which lies about 30 km northwest of Lake Bonney in the Wright Valley (Tregoning et al. 2015). In the Lake Vanda, Goto et al. (1969) reported the presence of genera *Candida*, *Rhodotorula*, and *Cryptococcus* from the water and sediments. They also identified a new variety of *Trichosporon cutaneum* var. *antarcticum* from freshwater and a new species *Sporobolomyces antarcticus* (synonym *Pseudozyma antarctica*) from the sediment; the species was subsequently transferred to the genus *Moesziomyces* (teleomorphic genera) by Wang et al. (2015b).

Microbiological studies conducted by Sugiyama (1970) also revealed fungal species from other genera in Lake Vanda such as *Eurotium*, *Geotrichum*, *Stachybotrys*, and *Trichoderma.* Kriss et al. (1976) reported the genera *Stachybotrys* and *Trichoderma* in the same lake and other cosmopolitan fungi, such as *Aspergillus* and *Penicillium*. Other psychrophilic and non-halotolerant yeasts, *Candida* species,

were isolated at a depth of 5 m in Lake Vanda by Nagashima et al. (1990). None or very few yeast species were discovered in samples of lake water or sediment collected from Lakes Bonney and Fryxell by Goto et al. (1969). However, *Dendryphiella* sp. and *Diheterospora catenulata* were obtained from the water at the bottom of Lake Bonney, and *Aerobasidium foliicolum* and an unidentified species were recovered from Lake Fryxell (Waguri et al. 1975; Waguri 1976).

Baublis et al. (1991) demonstrated the richness of diversity of fungi from several selected microhabitats in the McMurdo Dry Valleys, including samples from Lake Hoare and Victoria Land. The highest number of fungi was found during the fall season compared to that in spring, and *Candida ciferrii*, *Cephalosporium acremonium*, *Aureohasidium pullulans*, and *Pseudogymnoascus pannorum* were identified on the meltwater foam. *P. pannorum* and *Fusarium fusavioides* were obtained from a microbial mat from the benthos, while a microbial mat from meltwater contained species, such as *Geotrichum candidum* and *Penicillium notatum*. All these species indicate that Taylor Valley contains a diverse mycobiota and occasionally high population levels, despite the severe climate and limited substrates.

A more detailed study by Brunati et al. (2009), involving fungal diversity and bioprospecting, which also covered a larger area, was carried out in the Victoria Land region. They isolated fungi associated with biomats from lakes in the Larsemann Hills (Manning and Rei Lakes), Vestfold Hills (Ace and Druzby Lakes), and McMurdo Dry Valleys (Fryxell and Hoare Lakes). Genera of psychrophilic fungi such as *Thelebolus* and *Pseudogymnoascus* were identified, as well as the cold-adapted cosmopolitan species, namely, *Aspergillus*, *Penicillium*, *Cladosporium*, and *Acremonium*. In addition, the *Beauveria* and *Curvularia* genera were also identified. *Basidiomycetes* yeasts were recurrently isolated from the biomats; however, only a single genus of ascomycetous yeasts, *Debaromyces hansenii*, was identified. Furthermore, de Hoog et al. (2005) identified some *Thelebolus* species (*T. ellipsoideus*, *T. microsporus*, and a new species, *T. globosus*) also associated with biomats in the same lakes situated in the McMurdo Dry Valleys and Vestfold Hills. According to the authors, the high frequency of members of *Thelebolus* obtained from Antarctic biomats in the diverse types of lakes was unexpected, but more recent articles have shown that the genera is widely spread in samples obtained from such lakes.

Conell et al. (2018) explored the biodiversity of culturable microfungi from the water column of Lake Fryxell; the most abundant taxa isolated were *Glaciozyma watsonii* (59%) and *Penicillium* spp. (10%). In this study, the authors reported a sharp decline in the fungal abundance in the water at 9 m below the lake with a concurrent shift in diversity; the fungi *Toxicocladosporium strelitziae*, *Clavispora lusitaniae*, and *Holtermanniella nyarrowii* were found at this depth. The genera *Aureobasidium*, *Cladosporium*, *Pseudogymnoascus* sp., *Heydenia*, *Thelebolus*, *Filobasidium*, *Mrakiella*, *Naganishia*, and *Rhodotorula* were obtained both on the surface and in the deep water. In contrast, the cosmopolitan filamentous genera *Acremonium*, *Pseudogymnoascus*, and *Penicillium* and the yeasts *Debaryomyces*, *Vishniacozyma*, and *Glaciozyma* were found only on the surface.

Rojas-Jimenez et al. (2017) using a culture-independent method of analysis explored the diversity and community composition of fungi in five permanently cov-

ered lake basins located in the Taylor and Miers Valleys (including two samples from Lake Bonney). Unculturable microorganisms represent a significant part of biodiversity of the Earth; therefore, culture-independent method of analysis makes it possible to unravel fundamental characteristics of the microbiology and ecology of microorganisms in a community (Ravin et al. 2015), including those present in lakes.

The fungal taxa found by the authors represented between 0.93% and 60.32% of the eukaryotic sequences. *Cryptomycota* and *Chytridiomycota* dominated in the fungal communities of all the lakes, and *Cryptomycota* was particularly dominant in Lakes Miers, Bonney West, and Fryxell, while *Chytridiomycota* was the dominant species in Bonney East and Hoare. In addition, members of *Ascomycota*, *Zygomycota*, *Blastocladiomycota*, and *Basidiomycota* were also present. *Basidiomycota* was dominant in the upper layer of Lake Hoare, and the main genera identified were *Glaciozyma* and *Mrakia*. Furthermore, Rojas-Jimenez et al. (2017) carried out a comparative study of the fungal composition (derived from RNA or DNA) obtained from Lake Bonney.

On the east of Antarctica, in Skarvness ice-free area, Tsuji et al. (2013) isolated fungi from lake sediments and identified fungi of the genera *Pseudogymnoascus*, *Thelebolus*, *Mrakia*, *Dioszegia*, and *Leucosporidium* (*Glaciozyma*). Some fungi species are able to secrete extracellular fatty acids and polysaccharides, to avoid getting frozen when exposed to extremely cold climatic conditions (Robinson 2001). These authors also evaluated the antifreeze activity of fungal isolates, wherein the species *G. antarctica* and *Rhodotorula glacialis* displayed antifreeze activities. Tsuji (2016) evaluated the ability of *Mrakia blollopis* isolates, obtained in a previous study, regarding their responses to cold stress. The author reported that one of the isolates was capable of accumulating high levels of tricarboxylic acid cycle metabolites, lactic acid, aromatic amino acids and polyamines, probably as an adaptation to resist cold shock. These data demonstrated the mechanisms by which fungi, isolated from frozen lakes, could remain active during periods of freezing, besides growing and decomposing organic compounds under subzero temperatures.

4.7 Fungi from Subglacial Lakes in Continental Antarctica

In 1998, Russian and French scientists completed drilling of Lake Vostok at a termination depth of 3623 m (Fig. 4.3), which represented the largest drilling ever made. Moreover, Abyzov et al. (1998) inferred that Lake Vostok could contain viable microorganisms. They analysed a portion of the ice core obtained in 1998 and samples extracted from core depths of 1500–2750 m (with corresponding ages ranging from 110,000 to 240,000 years) and demonstrated the existence of prokaryotic and eukaryotic microorganisms. Subsequently, analysis of these frozen ice cores led to inferences about the chemistry of lake water and revealed the existence of a small number of microbes (Karl et al. 1999; Siegert et al. 2001).

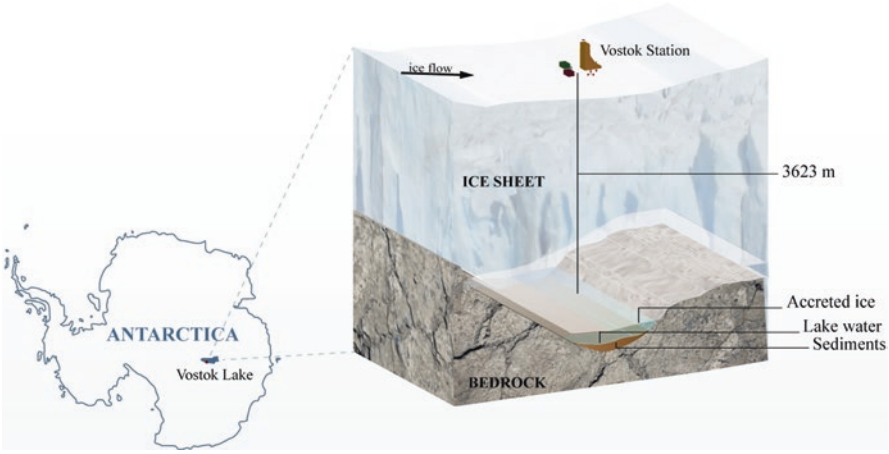

Fig. 4.3 Location of Lake Vostok in continental Antarctica and a scheme of the distance of the ice sheet drilling. Accumulation of accretion ice is depicted above the unfrozen lake water, and shown below is the accumulation of sediments. (Picture credits, M.B. Ogaki)

The most of the analyses of microbiota associated with Lake Vostok were performed with the ice found above the lake; this layer of accretion ice (frozen lake water collected above lake liquid surface) can retain linear and temporal contents of the upper surface of the lake. Karl et al. (1999) analysed a core section that contained frozen water derived from Lake Vostok (a body of liquid water located beneath glacial ice at about 4 km). They revealed viable bacterial cells (predominance of gram-negative bacteria) and low concentrations of potential growth nutrients. Other evaluations of the accretion ice revealed phylotypes closely related to extant members of the α-, β-, γ-, δ-, and *Proteobacteria* and the actinomycetes (Priscu et al. 1999; Christner et al. 2001, 2006); however, all these papers did not report the presence of fungi in the accretion ice.

D'Ellia et al. (2009) also isolated fungi from ten accretion ice sections (3300–5100 years old) from Lake Vostok. A total of 38 fungal cultures were analysed by ribosomal ITS region; the fungi belonged to four genera of *Basidiomycetes* and five of *Ascomycetes*. The species *Rhodotorula mucilaginosa* is the most frequently found, and other genera were also reported, including *Cystofilobasidium*, *Cryptococcus*, *Pseudozyma*, *Penicillium*, *Aeurobasidium*, and *Aspergillus*. The remaining taxa were classified as unknown. All the isolates identified by D'Elia et al. (2009) were related to polar taxa isolated from a variety of permanently cold environments. The presence of these types of fungi indicates that the ecological conditions within the regions of Lake Vostok are sufficient to support heterotrophic metabolism and a high diversity of microorganisms.

Shtarkman et al. (2013) evaluated two core sections that accreted in the vicinity of an embayment (at depths of 3563 and 3585 m) and two ice core sections that accreted over the southern main lake basin (at 3606 and 3621 m) using metagenomic/metatranscriptomic sequence analyses of mRNA. They obtained 3507 unique gene sequences. Among these, 1623 could be taxonomically classified from both

the core sections. Approximately 94% of the sequences obtained were from bacteria, and 6% were from eukaryotes, represented by Amoebozoa, Archaeplastida, Animalia, Chromalveolata, and Excavata. Fungi (represented by *Ascomycota*, *Basidiomycota*, *Zygomycota* taxa) represented 23% of the sequences obtained from the samples.

In the meltwater ice from Lake Vostok, Rogers et al. (2013), using metagenomic/metatranscriptomic analyses, noted the presence of thousands of species of organisms (94% *Bacteria*, 6% Eukaryota, and 2% Archaea). Two samples were analysed, one from the accretion ice over a shallow embayment and the other from the surface of the southern main basin. Only about 6% of the unique sequences were closest to eukaryotes. Of these, the phyla *Ascomycota*, *Basidiomycota*, and *Zygomycota* (*Mucorales*) and some sequences showing no matches with GenBank database (uncultured fungi) were identified. The data were very similar to those obtained by Shtarkman et al. (2013). These kinds of analyses allowed to gather information about the ecology, in a broader way, about organisms in Lake Vostok, since it was based on RNA sequences present in the samples collected, but precise identification of which types of fungi would be present in these lakes has not been evaluated.

Until now, the probability of subglacial lakes harbouring life remains poorly characterised (Kuhn et al. 2017), mainly because of potential contamination during sample collection, which are difficult to obtain. From the few samples obtained by penetration of subglacial lakes, acquiring pure samples to evaluate the taxa present would be hampered by contamination, because of the mixing of the lake water with well-drilling fluid (Lukin and Vasiliev 2014). This would result in an ambiguous analysis of the communities present. Because of these challenges in obtaining samples, practically no information is known about the fungi that inhabit the subglacial lakes of Antarctica.

According to Bulat (2016), only two studies of penetrations of Lake Vostok were performed with the viewpoint of a possible sampling of clean water. The results of these samples yielded only one new unknown bacterial phylotype, which indicates that a hitherto unrecorded microbial life could exist within water body of Lake Vostok. The search for lake inhabitants should aim to sample the cleanest lake water in order to ensure that the results are more robust. In addition, the use of clean laboratory facilities and the establishment of a library of contaminants are considered prerequisites for the research of Lake Vostok microorganisms (Bulat 2016). Therefore, further drilling is expected to be done in the future in order to analyse, more accurately, the microbial community in subglacial lakes.

4.8 Factors that Influence Fungal Communities in Antarctic Lakes

In Antarctica, lakes usually show low nutrient availability, constant low temperatures, short ice-free seasons, and different ranges of pH in comparison with other regions of the Earth; these factors influence biotic diversity in the lacustrine systems (Ellis-Evans 1996). In an evolutionary sequence report outlined, the

development of an Antarctic lake is initially seen as a function of catchment complexity and natural eutrophication (Priddle and Heywood 1980), wherein the biotic development is initiated with the colonisation of the lake bottom by algae, bacteria, and macroinvertebrates, followed by a rise in the dominance of benthic cyanobacterial mats and aquatic mosses, and finally switching to phytoplankton dominance. When nutrient inputs increase, the water column turbidity reduces light penetration (Ellis-Evans 1996).

It is known that the biological communities of the Antarctica lakes usually contain simplified and truncated food webs (Laybourn-Parry et al. 1997), absence of fish, low zooplankton biomass, and low floristic diversity at the lake borders. For these reasons, there are no plants of higher orders in the Antarctic lakes. Only benthic cyanobacterial mats are extremely common in these lakes, and annual algae and aquatic mosses can occur in some systems (Ellis-Evans 1996; Laybourn-Parry and Pearce 2007). The biota of the Antarctic lakes are primarily constituted by microorganisms (Vincent 2000) adapted to disperse in the water column or adhere to other lacustrine substrates that display supersaturated levels of dissolved oxygen, extremely low light conditions on an annual basis, and thick ice covers, which reduce or eliminate wind-induced internal water circulation (Simmons et al. 1992). All these characteristics limit colonisation and growth of many microbial species in these environments. The microbial communities in these types of extreme lakes have to survive hostile habitats and deal with high ultraviolet radiation loads, freeze-thaw cycles, and low organic resources and are dependent on long-term primary products produced by photosynthetic organisms (Tranter et al. 2004). Ice and snow covers are responsible for low levels of annual photosynthetically available radiation in the lakes; however, when the ice melts in summer, the high transparency of the water column can transmit a high intensity of light and can have an inhibitory effect on photosynthesis (Hodgson 2012). Therefore, these light-darkness and freeze-thaw cycles are decisive in establishing a trophic web in these lakes.

In the Antarctic lakes, nutrient cycling occurs because of the activity of benthic communities of cyanobacteria, bacteria, and fungi (Ellis-Evans 1996; Gonçalves et al. 2012). The low diversity of organisms limits the availability of nutrients, since most fungi in Antarctica are associated with the decomposition of organic matter (Arenz et al. 2006), as endophytes (Rosa et al. 2009) and parasites (McInnes 2003). According to Laybourn-Parry et al. (1997), the lakes of Antarctica are ultra-oligotrophic, unless they are enriched by bird droppings and plant debris. In addition, the continents and islands located in the ice-free areas support the well-known fauna of seals, penguins, and marine birds and a variety of plants and lichens (Hodgson 2012). These organisms can carry or harbour vectors and microbial propagules (Ellis-Evans and Walton 1990). In the Antarctica lakes, fungi were reported to be associated with different lacustrine substrates or were present in water columns. Among the Antarctic lacustrine substrates, fungi have been identified in the marginal microbial mats (Brunati et al. 2009), marginal soil and sediment (Vaz et al. 2011; Tsuji et al. 2013; Gonçalves et al. 2015), and water (de Hoog et al. 2005; Gonçalves et al. 2012; Rojas-Jimenez et al. 2017; Connell et al. 2018) associated with invertebrates (McInnes 2003) and macroalgae (Knox and Peterson 1973).

The colonisation of water bodies by fungi occurs mainly because of the dispersion of their propagules. Most of the terrestrial fungi disperse their spores carried by air and by precipitation or by floods, which are subsequently deposited in the water (Dix and Webster 1995). In addition, freshwater fungi produce spores adapted to dispersion in aquatic conditions. Fungi taxa reported from aquatic habitats range from those that are adapted to complete their life cycles in aquatic habitats and are not found outside the aquatic environment (indigenous or residents) to those present in different ecosystems and substrates (cosmopolitan) (Shearer et al. 2007). Most of residents are able to sporulate in water and keep their biomass at a constant level corresponding to the substrates and nutrients available (Dix and Webster 1995). In contrast, those fungi that inhabit the water randomly by being washed, leached, blown in, or dispersed by wind are present transiently. Some transient propagules enter the aquatic environment and can reach maturation but are unable to sporulate and colonise new substrates (Dix and Webster 1995; Shearer et al. 2007). In addition, transitional fungi can be immigrant or migratory. Among these, immigrants usually have an extra-aquatic habitat and their propagules, by dispersion, are deposited in the water. As for the migratory species, they alternate periodically between aquatic and extra-aquatic habitats (Dix and Webster 1995).

Antarctic lakes exhibit different physicochemical conditions and can be either freshwater or hypersaline (Laybourn-Parry et al. 1997). Chemically, these lakes range from some of the freshest lakes in the world to the most hypersaline lakes, with concentrations of salt exceeding eight times that of seawater, which prevent them from freezing over, even during winter (Hodgson 2012). Additionally, many saline lakes exhibit seasonal or permanent stratification of water columns because of temperature and salinity gradients (Hodgson 2012). The water composition depends on the material leached from the catchment areas and other intrinsic factors, such as a rocky composition and marine influence around the lakes in maritime Antarctica.

During the trajectory of the water in the formation of water bodies, there are marked changes in hydrologic, physical, chemical, and biological conditions (Townsend and Hildrew 1994). These changes of nutrients, which occur by leaching, contribute to a water composition that provides solutes essential to microbial community. In addition, oxygen availability, water temperature, and biological challenges, such as competition with other organisms, influence the occurrence and adaptations of fungi. Most of the Antarctic lakes exhibit unusual thermal profiles, and most are covered, to some degree, with perennial ice or annual ice cover (Simmons et al. 1992; Laybourn-Parry and Pearce 2007). Consequently, abiotic and biotic forces exert a high selective pressure on fungi. However, the constant cold temperature, freezing periods, and high UV radiation compel them to have adaptive mechanisms that include changes in the composition of the cell membrane, the production of intra- and extracellular antifreeze substances (trehalose, cryoprotectant sugars, proteins, polyols), and the ability to produce cold-active enzymes and protective UV compounds, such as mycosporines and pigments (Robinson et al. 2000; Vaz et al. 2011). Many of these characteristics allow mainly the psychrophilic and psychrotolerant fungi to survive. The production of such substances, as well as

other secondary metabolites, may have different applications in industrial and medical fields as well as be of great scientific interest.

4.9 Conclusions

Fungi are widely diffused by the diverse ecosystems that the Antarctic environment offers, including lake ecosystems and their range of associated substrates (biomats, invertebrates, sediment, and water columns). In this article, several studies regarding the fungal diversity among the taxa that characterise these lacustrine environments and variations between endemic species, such as *Antarctomyces psychrotrophicus*, *Thelebolus globosus*, and *Thelebolus ellipsoideus*, cosmopolitan species such as the genus *Penicillium*, and psychrotolerant fungi such as *Pseudogymnoascus* of Antarctica lakes were addressed. These fungi tend to be well adapted, since the Antarctic continent presents several adverse factors for fungal colonisation and growth, such as freezing and thawing cycles with low annual precipitation, temperature, water availability, strong winds, and high incidence of UV radiation. Therefore, the fungal communities of the lakes could have a great ecological importance in the Antarctica by playing a role in primary decomposition of organic materials, actively participating in nutrient cycling important for the balance of the micro- and macronutrients in lake systems, contributing to demonstrate the effects of the climatic global changes, and last but not the least, by their ability to produce bioproducts for further biotechnological applications.

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Chapter 5
Rock-Inhabiting Fungi in Antarctica: New Frontiers of the Edge of Life

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5.1 Introduction

Adverse conditions in several places on the Antarctic continent are life-limiting factors (Ruisi et al. 2007). However, microorganisms can colonise the cracks and cavities in different types of rocks in Antarctica. Within such microhabitats, temperature, light, and available water result in a micro-climate favourable for colonisation by specific microorganisms (Gorbushina 2007). However, the rock surface is considered an inhospitable environment because of extreme temperature conditions, rapid changes in water activity and high ultraviolet (UV) radiation found in the region (Staley et al. 1982). Despite extreme conditions, distinct microbial communities are found inside rocks (called endolithic organisms) and on their surface (called epilithic organisms) (Warscheid and Braams 2000).

The microbial community in rocks usually comprises cyanobacteria, chemoorganotrophic bacteria, lichenised fungi (lichens), and non-lichenised fungi (Gorbushina 2007). Fungi are easily dispersed and settle on and colonise different substrates in different environmental conditions. The fungal community in rocks is formed by taxa sharing common macro- and micro-morphological characteristics

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but having different phylogenetic origins, which are called melanised fungi and micro-colonial fungi (MCF) (de Hoog and Grube 2008), represented by endemic (Onofri et al. 1999; Øvstedal and Lewis Smith 2001; Selbmann et al. 2005) and cosmopolite species (Gonçalves et al. 2017).

Rock-inhabiting fungi use various strategies to withstand extreme conditions and establish themselves successfully in this microhabitat. Mycosporines found in some fungi isolates from different rocks (Ruisi et al. 2007) function as photoprotectors and are associated with the survival, restricted growth and longevity of these fungi (Gorbushina et al. 2003). Meristematic growth and the presence of melanin in some species minimise the exposure of the colony surface to solar radiation and protect them against UV radiation (Gorbushina 2007). The thick melanised wall in many of these fungi is presumably a stable and highly effective protective barrier against UV radiation (Sterflinger et al. 2012). In contrast, endolithic fungi found inside rocks are protected from UV radiation by the habitat in which they are isolated (Gorbushina 2007).

Biological communities in rocks can survive extreme changes in planetary surface conditions to maintain viability for up to 100 years in the absence of liquid water, energy, and nutrient sources to induce cellular damage or death and to survive direct exposure to high doses of solar energy and cosmic radiation on the rock surface (Gorbushina et al. 2002). In this context, melanised fungi associated with rocks represent unique eukaryotes that can be used as study models for astrobiology, since they inhabit ecosystems exposed to high UV radiation and extreme temperatures, with scarce availability of water and nutrients.

Earlier studies have characterised a community of endolithic fungi associated with rocks from extreme environments such as deserts (Sterflinger et al. 2012; Gonçalves et al. 2015) and historical monuments exposed to high incidences of UV radiation and having low availability of water and nutrients (Sterflinger 2010b; Ruibal et al. 2008). In contrast, few studies have characterised the fungal diversity in rocks of the Antarctic continent, even though these environments represent a promising source of extremophilic fungi and eukaryotic models to study the limits of life and its applications in astrobiology (Onofri et al. 2004). Recent studies on the in vitro pathogenic potential of fungi obtained from Antarctic rock samples have highlighted the importance of conducting further investigations to identify possible pathogens that may pose a risk for humans by widespread dispersion owing to climatic changes on our planet (Gonçalves et al. 2017; Alves et al. 2019).

5.2 Rocks as Fungal Micro-Habitats

Rocks are naturally occurring solid materials composed of varying proportions of one or more minerals, which, in turn, are crystalline solids with a defined chemical composition and may include organic remains (Plummer and McGeary 1996). Rocks can be classified into three types: igneous, metamorphic, and sedimentary. The corresponding rock-forming environments are also classified similarly.

Igneous rocks are formed by the cooling and consolidation of magma (or melt). Magma is a molten material whose chemical composition depends on whether it is formed on the mantle or the crust of the planet. The density of magma is lesser than that of the surrounding solid material (country rocks), and its temperature and chemical composition influence its viscosity. While moving, magma can get lodged inside the fractures of the lithosphere or extrude on its surface, becoming lava in the latter case. In both cases, magma can form different types of rocks. When magma cools internally, it forms intrusive or plutonic igneous rocks, which have a higher mineral growth time (e.g. granites and gabbros). Plutonic rocks are varied, and their shape depends on their relationship with country rocks, commonly forming dikes, sills and batholiths. When magma extrudes on the surface, it cools faster, forming extrusive or volcanic igneous rocks (e.g. basalts and andesites).

When exposed, rocks may get weathered on contact with surface environmental conditions. Weathering is the set of physical and chemical processes that lead to the disaggregation (mechanical fragmentation) and decomposition (geochemical alteration) of rocks (Fig. 5.1). The main chemical weathering agents are liquid solutions, which may be acid rain (H_2CO_3) or organic acids released by organisms that colonise the rock surface. The minerals originally present in the rock interact with these weathering solutions to form new minerals, which are more adapted to the surface conditions. A leaching solution containing the most soluble chemical elements is also formed. The weathering of rocks results in the formation of soils that constitute a terrestrial layer (pedosphere), which is a product of the interaction between the lithosphere, biosphere, atmosphere and hydrosphere.

The action of agents such as water, wind, ice and gravity can lead to soil erosion. These agents also carry the eroded materials (sediments) and deposit them near or far from the source area. In the places where they are deposited, sediments consolidate by forming cements or because of the pressure exerted by the weight of the sedimentary package between the grains. This process is called diagenesis and results in the formation of sedimentary rocks or clastic sedimentary rocks (e.g.

Fig. 5.1 Examples of rocks in Keller Peninsula, King George Island, and South Shetland Archipelago, Antarctica, subjected to physical (**a**) and chemical (**b**) weathering in Antarctica. A rock fragment disaggregated by cryoclasts, which consists of breakage promoted by the increase in water volume through the cycles of freezing and thawing. (**b**) Reddish portions indicate the oxidation and remobilisation of iron in sulphide-enriched rocks. (Photos Credits: FS Oliveira)

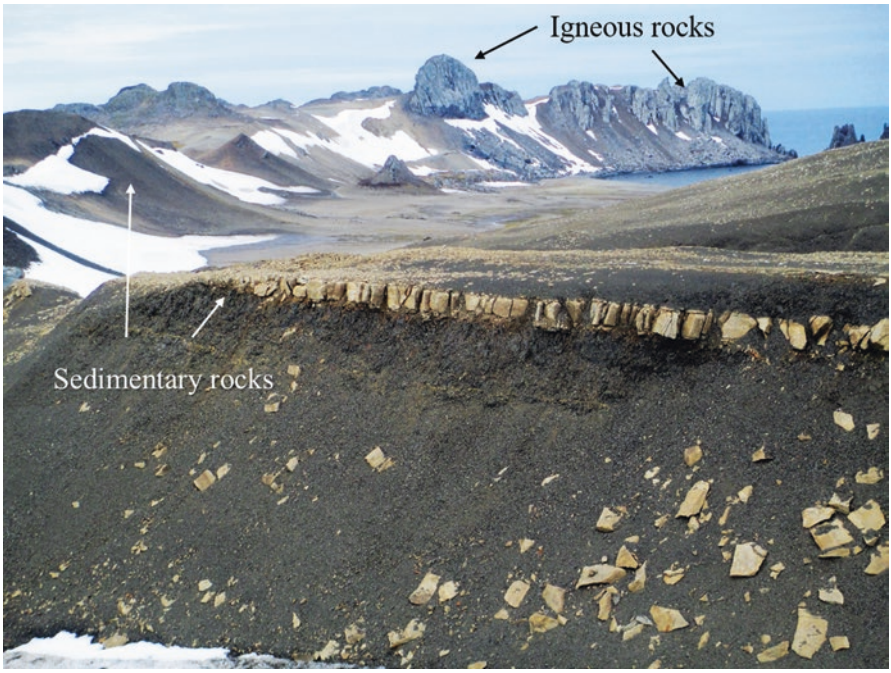

Fig. 5.2 Upper Jurassic-Lower Cretaceous sedimentary rocks and igneous intrusive rocks of Antarctic Peninsula Magmatic Arc in the President Head Peninsula, Snow Island, Antarctica. (Photos Credits: FS Oliveira)

sandstones, argillites, conglomerates and gaps). Chemical sedimentary rocks, such as some limestones, are formed by the precipitation of chemical compounds dissolved in water. The evolutionary history of several sedimentary rock-forming environments can be reconstituted by studying the features of these rocks, which usually have strata and layers produced by sedimentation processes (Fig. 5.2).

Metamorphic rocks are formed from the transformation of igneous and sedimentary rocks (Fig. 5.3). They can be created under conditions of high pressure and temperature in the interior of the planet. When a rock is tectonically positioned at a depth between 3 and 20 km, the pressure and the temperature conditions unsettle the original rock and transform its original minerals into new ones, whereas recrystallisation transforms its size and habit. Consequently, new texture and mineral assemblies are created. These transformations are called regional metamorphism, and represent one metamorphism type.

Igneous, metamorphic and sedimentary rocks can be transformed into one another. The spatial scale on which these transformations are manifested is global (called the rock cycle), and they are usually related to the lithosphere dynamics and the behaviour of the continental and oceanic plates. Correspondingly, the time scale also varies from millions to billions of years, and hence, processes that occur over a very long temporal scale, as opposed to those occurring over a shorter time scale

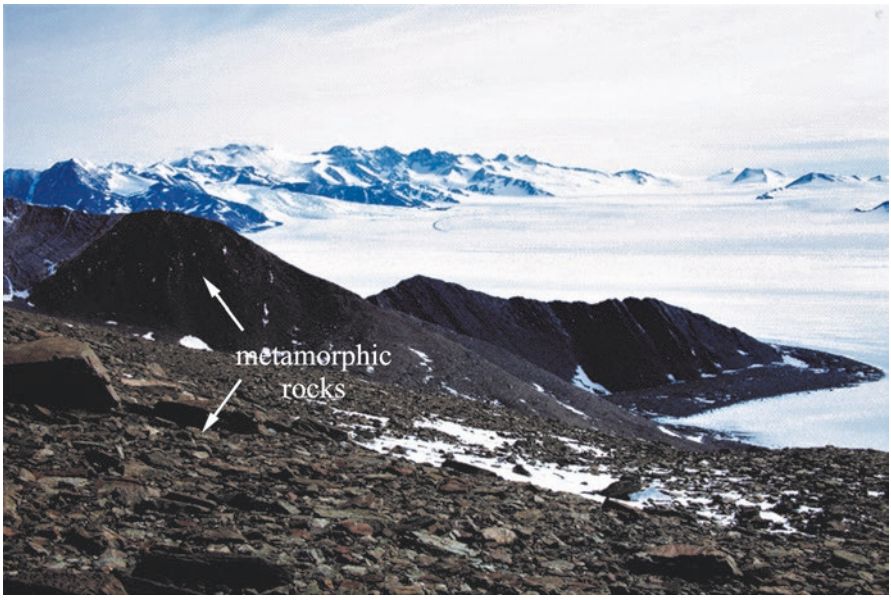

Fig. 5.3 Metamorphic rocks (phyllites and quartzites) from the Ellsworth Mountains, continental Antarctica. (Photos Credits: FS Oliveira)

(e.g. biological processes), are considered when studying the geological history of the planet. Nonetheless, rocks and geological events are strongly related to biotic systems and can influence the evolution of life. More importantly, they provide the habitat for various organisms.

For microorganisms, rocks may represent distinct microhabitats depending on the nature of the colonisation (Burford et al. 2003a). The characteristics of the rocks, their position in the landscape and the environmental conditions influence the type of organism inhabiting the rock and the form of microbial colonisation. Thus, understanding the fungal colonisation in rocks helps in understanding the interaction between the extrinsic and intrinsic factors of rocks. The extrinsic factors include the environmental aspects (climate, solar radiation, position in the landscape, wind and others) and biological aspects (types of colonising organisms and their adaptive capacity) (Gadd 2007). The intrinsic factors are related to the physical and chemical properties of rocks, in particular, their compositional, textural and structural aspects.

The compositional aspects are related to rock mineralogy and, consequently, the chemical composition of rocks (Wolfaardt et al. 1994; Rogers et al. 1998). The mineral composition of rocks can influence several microbial processes, such as energy generation, nutrient acquisition, cell adhesion and biofilm formation (Gadd et al. 2005; Gadd 2007). Rocks are composed of distinct mineral assemblies based on their forming environment. Some minerals may be rich in elements such as Fe, Mg, Ca, K, P, S and C, while others in Si and Al. Depending on the nutritional requirements of organisms, rocks containing certain minerals may be more suitable habitats

than others (Gleeson et al. 2006). Additionally, minerals are weathered on rock surfaces in a distinctive way, with some being more resistant than others (such as quartz). Consequently, microbial access to mineral nutrients is related to the degree of resistance to weathering that the minerals exhibit and to the capacity of the organism to promote this access, considering that some microorganisms play an important role as agents of bioweathering, as discussed later.

The textural aspects include mineral organisations and their physical properties. Rock-forming environments favour specific types of mineral assemblies that may influence microbiological communities. Igneous rocks, for example, when extruded, have thicker, randomly arranged minerals with more closer inter-grain contacts. In contrast, in metamorphic and sedimentary rocks, minerals are organised in specific textures, which can vary from very oriented mineral foliation (schistosity) to compositional bands (separated felsic and mafic minerals) and stratified planes. Some of these organisations may facilitate the penetration and/or storage of water, making the rock more humid, influencing rock temperature, or representing segregation between specific types of minerals, as observed in the case of dark and light bands in metamorphic rocks (Fig. 5.4). In addition, the minerals can be thick or thin, which

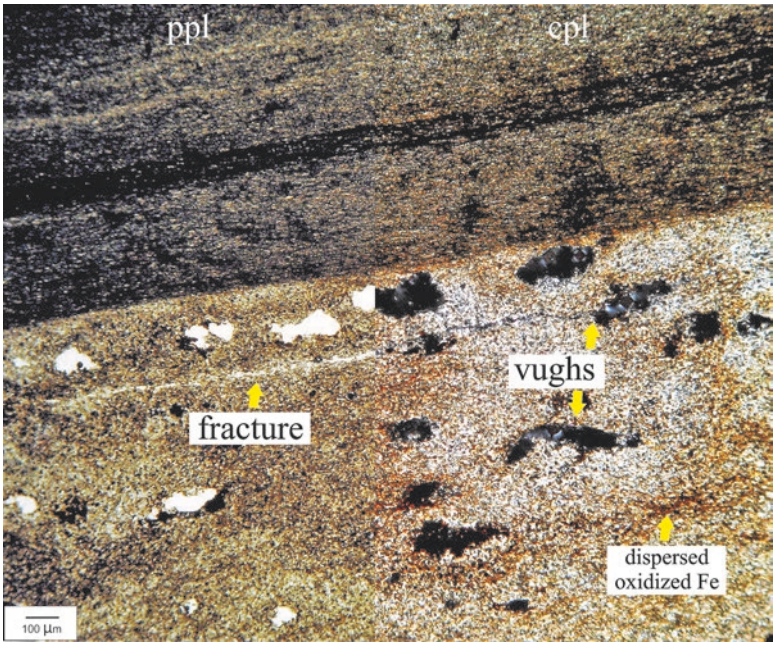

Fig. 5.4 Photomicrographs obtained through petrographic microscopy in polar polarised light (PPL) and crossed polarised light (CPL) of a sericitic phyllite from the Ellsworth Mountains, continental Antarctica, with alternation of dark and light bands. The lighter bands are more fractured than the dark bands, which may be related to the differential expansion of the rock. In addition, the light band has dissolution cavities, sometimes filled, and zones with dispersed oxidised iron, suggesting that they have undergone chemical weathering more efficiently. (Photos Credits: FS Oliveira)

can directly affect rock porosity and the degree of mineral alteration. Thus, not only mineral composition but also their physical and physicochemical properties such as microtopography, surface charge and hydrophobicity may influence microbial colonisation (Fredrickson et al. 1995; Bennett et al. 1996; Gadd 2007).

Structural aspects include rock features such as humidity, temperature variation and gaseous flow that influence their behaviour. Rock porosity, presence of fractures, surface roughness and the actual colour of the rock are the main features. The pores (inter-grain spaces) and fractures (grain fragmentation in linear features) are conductors of water and gas, creating microhabitats suitable for certain microorganisms. Depending on the type of rock, the porosity will be higher or lower, and there may be distinctions between the proportion of macropores and micropores. Highly porous rocks – such as thick sedimentary rocks (psamtics) – are more efficient in water infiltration, which in turn depends on pore size and connectivity: larger pores transport water more efficiently and smaller pores retain it. Some petrogenetic processes such as magmatic cooling form less porous rocks as the growth of the minerals in such rocks generates closed suture planes with well-defined grain contacts. However, the situation changes based on how this cooling occurs. In extrusive igneous rocks, faster cooling can generate fractures that compensate for the loss of volume in the liquid-solid transformation. Likewise, depending on the volatile content in the magma, the rock can be enriched with vesicle-like pores. Intrusive igneous rocks can also contain many fractures because these rocks are formed at great depths, and their presence on the surface indicates that the overlying material has been eroded. In this process, the rocky body tends to undergo decompression, which leads to the formation of fractures (also known as relief joints).

The colour of the rocks and their surface roughness are related to aspects such as temperature (absorption and propagation of heat) and surface moisture. The rock colour depends on the minerals present and their state of weathering. Darker or greenish rocks are typically enriched with mafic minerals, usually of iron-magnesium composition. Lighter rocks are composed of felsic minerals, usually quartz-feldspars. When weathered, iron-rich minerals become oxidised, which attributes a reddish to yellowish coloration to the rocks. Darker rocks tend to retain more heat and, therefore, are more easily heated. The dilation between dark and light minerals is distinct and can generate micro-fractures in the contacts between them. If the rock is composed of thick minerals, it will typically exhibit a more irregular exposed surface. This surface roughness directly influences water retention, facilitating colonisation by biofilms.

5.3 Geological Overview of Antarctica and the South Shetland Islands

The geological framework of the Antarctic continent comprises a set of tectonic landscapes, as reflected in the chemical and petrological characteristics of its rocks and sediments. Generally, in the active convergent margins, there are common

plutonic and volcanic associations as well as their metamorphic counterparts. The tholeiitic, calc-alkaline to alkaline chemical signature is a common characteristic of its rocks and is normally observed in their associated sediments. Divergent constructive margins such as mid-ocean ridges and back-arc basins mainly have a tholeiitic signature in their volcanic rocks. Within plate sceneries, the magmatism is normally associated with hot spots, having an alkaline to tholeiitic signature (Wilson 2007).

Several authors consider the term platform to be synonymous to craton, describing a tectonic stable part of the continent (Suess 1885; Kober 1921), normally with the oldest crust and rocks of the continent. Cratons are derived from a continuum set of tectonic processes in time and in different areas (from plate margin, within plate) (Condie 2013) and possess a great diversity of rocks with variable chemistry signatures, similar to an airplane black box.

Like other continents in the world, Antarctica possesses a set of tectonic sceneries with a Precambrian platform – the East Antarctic Shield and a Phanerozoic (post 540 My) mobile belt, and West Antarctica (including the Antarctic Peninsula). Both were sutured during the Ross Orogeny in the Cambro-Ordovician times (c. 540–480 My) (Fig. 5.5a).

The East Antarctic Shield is the oldest and biggest part of Antarctica, comprising a set of smaller Archaean cratonic cores (older than 2.5 Ga) surrounded by relatively younger Proterozoic mobile belts (2.5 Ga–540 My) and established in their current

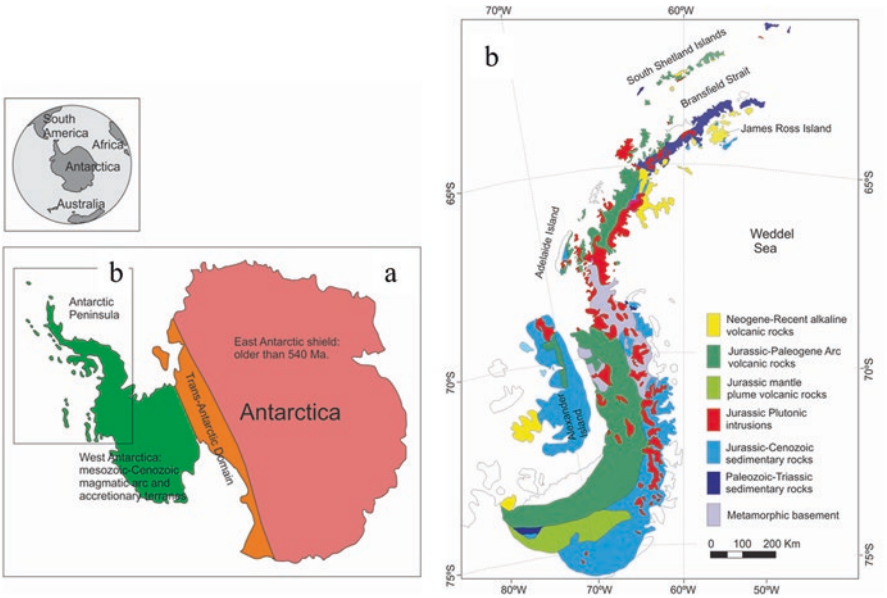

Fig. 5.5 (**a**) Tectonic domains in Antarctica. (Modified from Harley (2009)) and (**b**) geological sketch of the Antarctic Peninsula and South Shetlands Islands. (Modified from Burton-Johnson and Riley (2015))

configuration during the Pan-African Orogeny (c.700–500 My) (Vaughan et al. 2005; Fütterer et al. 2006).

The West Antarctic Shield is composed of five distinct terrenes: the Antarctic Peninsula, Thurston Island, Marie Bird Land, the Ellsworth-Whitmore Mountains (the southern region adjoining the Transantarctic Mountains) and the Haag Nunatak (Storey et al. 1988). Some of these terrenes were accreted in the western Pacific margin of Gondwanaland during the Ross Orogen (c. 540–480 My) (Ellsworth-Whitmore Mountains and Haag Nunatak), coeval with the East and West Gondwana collisional event (Tessensohn et al. 1999; Hervé et al. 2006). The other terrenes were structured in an orogenic belt during Mesozoic times (Birkenmajer 2001; Hervé et al. 2006). The limits and sutures between them are marked by strike-slip zones, further separated by rifts during the Gondwana breakup (Storey et al. 1998; Hervé et al. 2006).

The Antarctic Peninsular configuration can be associated with domains that represent the amalgamation of terrenes of different ages and compositions (Vaughan and Storey 2000), although most of them are parautochthonous. They are associated with turbiditic sequences in an accretionary wedge (the Trinity Peninsula Group and Le May Group) (Birkenmajer 2001; Hervé et al. 2006); low-grade metamorphic complex with turbiditic properties (the Scotia Complex) (Birkenmajer 1994); Jurassic calc-alkaline intrusive rocks, Antarctic Peninsula Batholith (Hervé et al. 2006) or Andean Intrusive Suite (Hawkes 1962; Barton 1961); and Jurassic to Lower Cretaceous basaltic and andesitic lava complex (the Antarctic Peninsula Volcanic Group (Gledhill et al. 1982) (Fig. 5.5b).

The accretionary wedge-related units, intrusive batholitic series, and low-grade metamorphic complex have their counterparts in the Patagonian and Fuegan segments of the Andes (Hervé et al. 2006), representing the continuity of the orogenic process in Mesozoic times, before the Gondwana breakup (Dalziel 1984; Trouw et al. 1997). In this context, the Trinity Peninsula Group was deposited during the Permian-Triassic, in the fore-arc region, as a turbiditic association of pelites and wackes with associated pillows and hyaloclastite, metamorphosed in prehnite-pumpellyite facies (Hyden and Tanner 1981; Hervé et al. 2006).

During the Middle to Upper Jurassic, lacustrine and alluvial beds of Mount Flora Formation were deposited in the back-arc region over the eroded Trinity Peninsula Group. Simultaneously, the crustal units were intruded by the calc-alkaline intermediate to acid magmatism (Andean Intrusive Suite) and its volcanic counterparts (Birkenmajer 2001).

In the Cretaceous to Palaeogene times, the subduction process evolved, and the magmatic arc was shifted to the west, beginning the formation of the South Shetland arc. During the Oligocene, this migration induced an extensional regime into the lithosphere, between the older magmatic arc (Antarctic Peninsula) and the newer arc, opening the Brasfield rift (Birkenmajer 1992, 1994). In general, the magmatism ages in this orogenic zone became newer from south to north, evidencing a migration of the subduction zone (Birkenmajer 1992, 1994). Thus, the geological record of the more southern Snow and Smith Islands to the tip of Robert Island and

Greenwich Island, further to the north, show that magmatism and Jurassic-Cretaceous sedimentary units are more closely related to the Antarctic Peninsula, whereas on King George Island, the Palaeogenic record shows the same (Smellie et al. 1984; Birkenmajer 1994).

The tectonic scenery of the Bransfield retro-arc and the counter clockwise rotation of the continent during the Cenozoic induced subsea and terrestrial volcanism and created a structural framework in the South Shetland Islands that was dominated by tectonostratigraphic compartments limited by directional and transcurrent faults. The chemical signature of the magmatism is predominantly calc-alkaline to tholeiitic (Birkenmajer 1994, 2001). In King George Island, for example, Tectonic structuring is determined from chemical signatures of blocks separated by faults. Here, formal lithostratigraphic units are individualised (Barton 1965; Birkenmajer 1980; Smellie et al. 1984; Birkenmajer 2001), some of which are possibly synchronous but with no clear genetic and temporal relationships.

Thus, the rich geological history of Antarctica ensured the presence of a diverse lithology that includes all types of rocks in the Antarctic continent (Fig. 5.6) and the

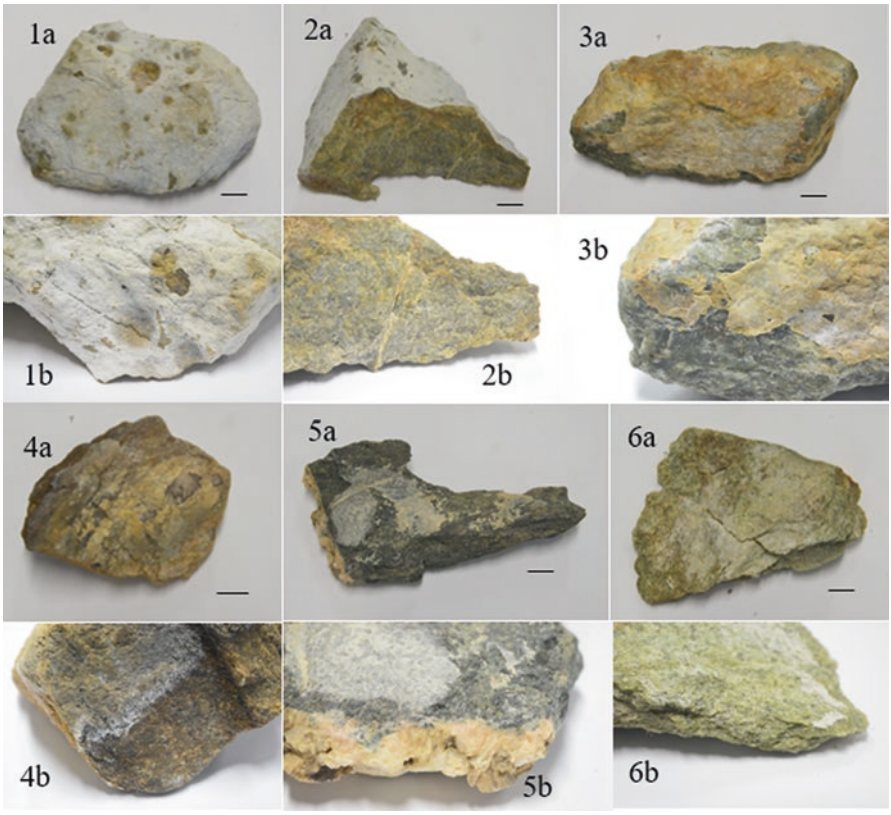

Fig. 5.6 Rocks from the Ellsworth Mountains of Continental Antarctica sources of epilithic and endolithic fungi. (Photos Credits: LH Rosa)

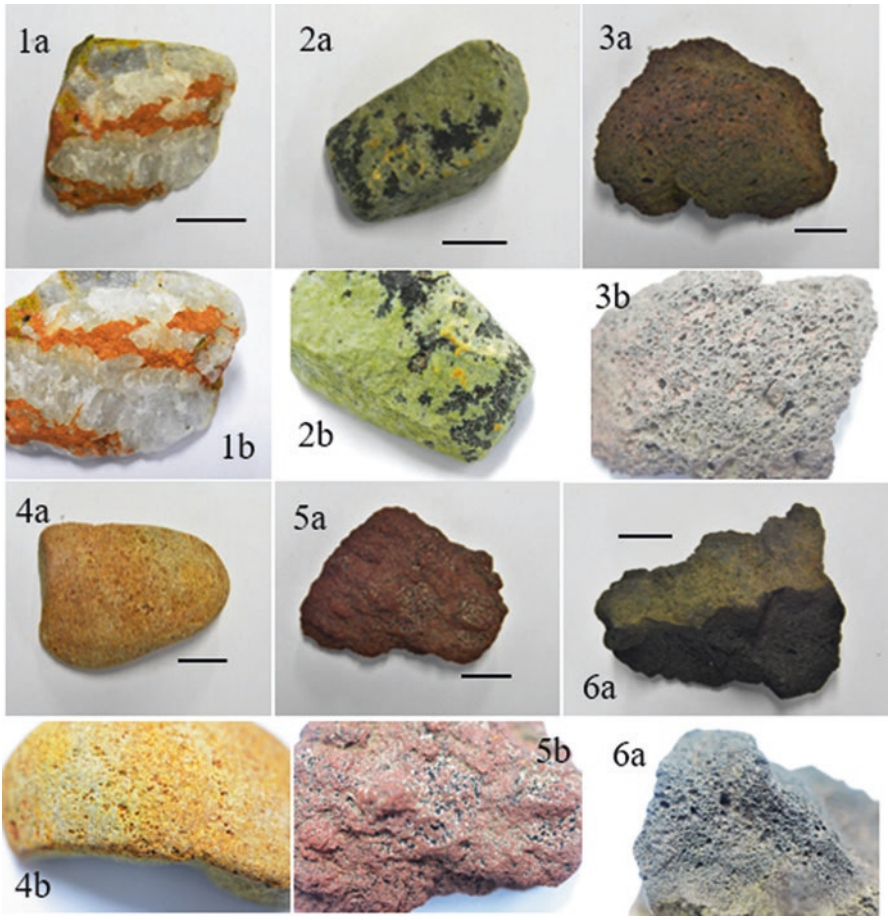

Fig. 5.7 Rocks from the Antarctic Peninsula as sources of epilithic and endolithic fungi. (Photos Credits: LH Rosa)

Antarctic Peninsula (Fig. 5.7). With the development of the polar cap from the Eocene, most of these rocks were covered by thick ice sheets. Nevertheless, in the ice-free areas, where thawing during the summer exposes the surface, it is possible to observe a diversified geology, which includes igneous, metamorphic and sedimentary rocks. These areas, specifically in the South Shetlands Archipelago, the Ellsworth Mountains and the Antarctic Peninsula, rock samples were collected to study fungal diversity (Gonçalves et al. 2017).

5.4 Geological Adaptations Facilitating Hospitable Rock Habitats

The mineral constituents of rocks are weathered by physical (mechanical), chemical and biological processes; each process acts according to the environment and its conditions (Ferris et al. 1987; Banfield et al. 1999; Vaughan et al. 2002). Rocks undergo bio-weathering to form mineral soil through diagenesis or mineral dissolution (Ehrlich 1996; 1998) by the action of microorganisms (May 2003; Gadd et al. 2005, 2006) such as bacteria, fungi and algae (Ehrlich 2002; Burford et al. 2003b; Gleeson et al. 2005, 2006), which excrete chemical agents such as inorganic acids (sulphuric and nitric acids), organic (citric, oxalic and gluconic acids) and binder products such as siderophores (Greek *sideros* = iron, *phores* = transporter) to aid in the process of bio-weathering. Additionally, reactive mineral constituents of rocks (Fe, Mn, S^{2-} or $SO4^{2-}$) undergo oxidation or reduction to assist this phenomenon. Microbial bio-weathering acts on all igneous and sedimentary rocks, including siliceous rocks (silica, silicates and aluminosilicates) and calcareous rocks (Ehrlich 1996, 1998).

The surface of exposed rocks presents environmental constraints such as temperature and humidity fluctuations, intense solar radiation and lack of nutrients that influence the colonisation, growth and development of microorganisms (Gorbushina 2007), making the rocks one of the most inhospitable habitats for microbial life (Staley et al. 1982). The temperature on the surface of the rocks varies greatly (ranging from −45 to +60 °C). Similarly, the availability of water also varies as rock surfaces might go through long periods of almost total desiccation or torrential desert rains may result in the formation of water films on them. Rock surfaces are also exposed to relatively low doses of cosmic radiation at night and extremely high infrared and ultraviolet (UV) radiation on summer days. Sudden rains can cause the availability of nutrients and energy sources to range from zero to abundant on the surface of rocks (Gorbushina 2007).

Black MCF, black yeasts, lichenised fungi, and cyanobacteria are among the most stress-tolerant organisms living on Earth (de Hoog and Grube 2008). They exhibit adaptations to tolerate multiple stresses and are characterised as polytolerant to environments with multiple and variable stress parameters (Gorbushina 2007). Microorganisms ensure their survival and success through community growth (Costerton and Stoodley 2003). Biofilms formed on rocks consist of algae, cyanobacteria, heterotrophic bacteria, lichenised and non-lichenised fungi, as well as free-living organisms (such as MCF), and microscopic animals (such as mites and insects) (Gorbushina and Petersen 2000; Gorbushina 2007).

5.5 Subaerial Biofilms in Rocks

The lithosphere, an area of interaction between a solid substrate and the atmosphere, is an ancient terrestrial and inhospitable niche that first housed microbial associations when biofilms were the only life-forms on Earth (Gorbushina 2007). Microbial

biofilms are represented by heterogeneous matrices of microorganisms concentrated in one interface (usually solid–liquid) and typically surrounded by an extracellular polymer substance (Rosenberg 1989; Hall-Stoodley et al. 2004).

These microbial communities are fossil records, mainly from hydrothermal environments. Supposed filaments in a biofilm, dating back 3.2 billion years, were found in rocks obtained from the bottom of the sea at Pilbara Craton in Australia. Additionally, microcolonies were also identified in a biofilm dating back 3.3–3.4 billion years, found in the formation of Kornberg in South Africa (Westall et al. 2001). These data confirm that the complex organisation, specialisation and functioning of these microbial communities have evolved over the years, and they have been able to establish themselves in various extreme environments such as in ice, hot springs and on rock surfaces (Chertov et al. 2004).

Subaerial biofilms (SABs) are microbial communities that develop on a solid mineral surface exposed to the atmosphere and are mainly composed of algae, bacteria, cyanobacteria, and fungi (Gorbushina 2007). Owing to the considerable environmental adversities that rocks present, colonies of free microorganisms rarely form on this substrate; instead, the formation of SABs in rocks guarantees the survival and success of these organisms. SBA formation follows the topographic profile of the substrate, which can include cracks, pores, mineral grains and cementing material (Gorbushina 2007). Bioreceptivity is the potential that the rock surface presents for colonisation by one or more groups of living organisms without causing bio-weathering (Guillitte 1995) and is directly dependent on petrographic parameters such as mineral composition, porosity and permeability of the rock material, which help to define the microbial community of SABs (Warscheid and Braams 2000). SAB microorganisms are classified according to the shape that they establish on the surface of the rock as epilithic (microorganisms that grow on the rock surface) of the rock and cryptoendolithic (microorganisms that penetrate a few millimetres inside the rock) (Warscheid and Braams 2000).

SABs are initially formed by photoautotrophic microorganisms (algae and cyanobacteria), which use sunlight and CO_2 from the atmosphere as a source of carbon and energy (Gorbushina 2007). Heterotrophic microorganisms (most bacteria and all fungi) require some organic source for their growth, which is provided by the metabolism of phototrophic organisms or the deposition of particles in the atmosphere (Suihko et al. 2007). All these organisms are embedded in arrays of extracellular polymeric substances (EPS) (Characklis and Marshall 1990) that describe SABs (Fig. 5.8a) and its interactions (Fig. 5.8b). The chemical composition of EPS is heterogeneous: more than 99% water, heteropolysaccharides, uronic acids, a few proteins (Konhauser 2007), and nucleic acids (Tolker-Nielsen 2006). EPS function as barriers against toxic metal ions that trap scarce nutrients, prevent bacteriophage and amoeba infections, prevent desiccation (Singleton 2005), provide the microorganisms in the biofilm with carbon and energy sources, and protect the microorganisms from high temperatures and freezing (Konhauser 2007). EPS also aid in the distribution of water in the biofilm during periods of rain and the distribution of the reserve water during periods of drought. Furthermore, it also plays a fundamental role in the maintenance of cell viability and the access to atmospheric water vapour (Gorbushina 2007).

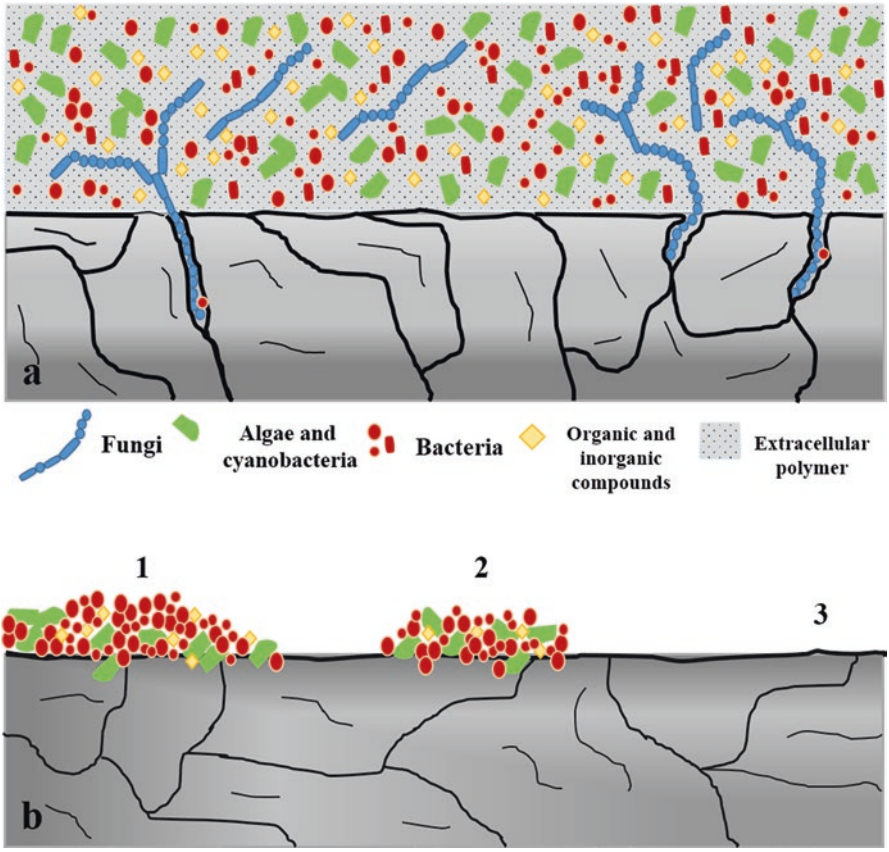

Fig. 5.8 Schematic representation of subaerial biofilms (SABs) and interactions. (**a**) Different microorganisms embedded in an EPS matrix, forming a small microbial ecosystem. (**b**) SABs form between the lithosphere and atmosphere, and the interactions observed at their interface are as follows: (**1**) between the organisms composing the SAB, (**2**) between the biofilm and atmosphere, and (**3**) between the atmosphere and substrate. (Adapted from Gorbushina (2007))

5.6 Fungi-Inhabiting Rocks

Fungi inhabit rocks as free forms or as biofilms by symbiotic associations with photobionts through the process of lichenisation (lichenised fungi, also known as lichens) (Gorbushina 2007). Fungi present on the rock surface contribute to its bioweathering, playing a role in the formation of the soils and, consequently, in the supply of soluble nutrients to the microbial community in this substrate (Burford et al. 2003a).

Fungi-inhabiting rocks can be classified as follows: epilithic fungi, which colonise the rock surface and grow as lichens or in biofilms; endolithic fungi, which grow in pre-existing cracks and crevices of the rocks and are visible on the rock

surface; and cryptoendolithic fungi that grow a free life forms in cavities and pores formed by their mechanical action (Gorbushina 2007). Under extreme conditions restricting life (such as temperature extremes and water and nutrient shortages), fungi actively penetrate the rock and are not visible from the substrate surface (Gorbushina 2007). Fungi-inhabiting rocks can also be classified into two taxonomically and ecologically distinct groups: (i) soil *Hyphomycetes* of epiphytic origin (de Leo et al. 1996) and melanised micro-colonies, with the latter forming compact micro-colonies, and (ii) *Ascomycetes* of the orders *Chaetothyriales*, *Dothideales* and *Capnodiales* (Staley et al. 1982; Gorbushina et al. 1993; Diakumaku et al. 1995; Wollenzien et al. 1995; Sterflinger et al. 1999).

Mycelial growth and the ability of some fungal species to grow dimorphically, i.e. to change their filamentous growth to yeast-like growth, contribute to their predominance in BSA (Gorbushina and Panina 1992; Gorbushina et al. 1993; Wollenzien et al. 1995). The mycelial growth facilitates the penetration and utilization of the nutrients of the rock, while the microcolonial growth or cells similar to the one of a yeast, is adopted in case of nutritional scarcity or environmental stress (Gorbushina 2007).

Black MCF form a polyphyletic group that is found in rocks in the free-living form (Staley et al. 1982; Ruibal et al. 2008). MCF exhibit a meristematic growth pattern, which provides an optimum surface/volume ratio (Wollenzien et al. 1995), decreases water loss and minimises colony contact surface on exposure to sun and other chemical and physical stresses (Sterflinger 2010a). MCF have an in situ and in vitro growth rate of 1–5 mm per month, expend little energy with dispersion and sporulation (Gorbushina 2003; Gorbushina et al. 2003) and can reproduce by isodiametric division (Sterflinger 2006). Owing to their considerable morphological plasticity, they can explore different substrates ranging from saline to acidic (Ruibal et al. 2005, 2008; Plemenitaš and Gunde-Cimerman 2005; Selbmann et al. 2005, 2008, 2014a; Egidi et al. 2014; Isola et al. 2016). MCF are distributed worldwide, and in addition to colonising exposed rock surfaces, they have been isolated from Mediterranean monuments (Urzì et al. 1991; Wollenzien et al. 1995; Ruibal et al. 2005, 2008; Isola et al. 2016), Antarctic rocks (Selbmann et al. 2015a, b), and solar panels (Martin-Sanchez et al. 2018). In addition to morphological adaptations, MCF have distinct cellular characteristics: cells are mainly composed of complex lipids, the presence of pigments and a dense cell wall (Selbmann et al. 2008), as detailed in Table 5.1.

Table 5.1 Special cellular characteristics of micro-colonial fungi (MCF) according to Selbmann et al. (2008)

Cell composition	Cell wall	Pigments
Mono-, di- and triacylglycerols	Chitin	Melanins
Phosphatidylcholine	Melanin	Carotenoids (colourless and brown–red)
Phosphatidylethanolamines	Polysaccharides	
Sterols, sterol ethers		
Phosphatidylethanolamines and free fatty acids		

MCF-inhabiting rocks synthesise the pigment melanin in their cell walls (Gorbushina et al. 1993; Diakumaku et al. 1995), which protects the fungus from UV radiation and gives it mechanical strength to penetrate the cracks of the rocks (Gorbushina 2007). Dadachova and Casadevall (2008) reported that melanised fungi can produce adenosine triphosphate (ATP) by a mechanism called 'radiotropism'. Based on the resistance and adaptability exhibited by melanised fungi in environments with high ionising radiation, the authors concluded that the radiation could alter the electrochemical properties of melanin to enable the pigment to function in energy transduction and, consequently, increase the growth of melanised fungi. Thus, MCF are considered interesting organisms for studies on adaptation to severe conditions (Kogej et al. 2006). Likewise, rocks are interesting targets for research on survival-related characteristics in hostile environments (Gorbushina et al. 2003).

5.7 Fungal Diversity and Distribution in Antarctic Rocks

The fungal communities in rocks of extreme environments such as hot deserts (Gonçalves et al. 2015), historical monuments (Sterflinger 2010b) and rock formations exposed to UV radiation (Ruibal et al. 2008) have been studied for some years. The earliest studies were mainly conducted on cyanobacteria and algae present in rocks in the 1970s and 1980s by Friedmann (1977, 1982) and Friedmann and Ocampo (1976). Friedmann et al. (1987) monitored the climatic conditions to which the cryptoendolithic community was exposed in the Ross Desert in Antarctica. However, considering the territorial expanse of the Antarctic continent, the knowledge about the fungal community-inhabiting rocks in different ecosystems remains limited.

Studies about fungal diversity isolated from Antarctic rocks involve the observation of these microorganisms in the substrate itself, through cultivation techniques or culture-independent techniques. Table 5.2 includes some of the fungal taxa observed in rock samples collected from different sites in the Antarctic continent and peninsula. However, the attempt to cultivate fungi from rock samples collected from northern Victoria Land was not successful (Onofri et al. 1999). Small portions of rocks were inoculated onto plates containing malt extract culture media and incubated at 10 °C and 30 °C; however, the rapid growth of psychrophilic and psychrotolerant fungi prevented the development/growth of pure cultures. For this reason, the description of the new genus *Friedmanniomyces* and the species *Friedmanniomyces endolithicus* was based on the morphological characteristics observed in the natural substrate.

New species of the non-lichenised fungus *Lichenothelia antarctica* was obtained from rocks in the Signy and Lynch Islands, Antarctica (Øvstedal and Lewis Smith 2001). The genus *Lichenothelia* has also been isolated from the stalk of lichenised fungi, and hence, members of this genus have been included in studies of lichenised fungi. Selbmann et al. (2005) isolated 20 meristematic fungi, identified as *Cryomyces*

Table 5.2 Distribution of fungi found in rocks collected from Continental Antarctica and the Antarctic Peninsula

Location	Taxon	References
Continental Antarctica		
Northern Victoria Land	*Friedmanniomyces endolithicus*	Onofri et al. (1999)
Different sites	*Lichenothelia antarctica*	Øvstedal and Lewis Smith (2001)
	Cryomyces antarcticus	Selbmann et al. (2005)
	Cryomyces minteri	
	Friedmanniomyces simplex	
	Friedmanniomyces endolithicus	
	Dothideales spp.	
McMurdo Dry Valleys	*Taphrina antarctica*	Selbmann et al. (2014b)
Ellsworth Mountain	*Acremonium* sp.	Gonçalves et al. (2017)
	Byssochlamys spectabilis	
	Cladosporium halotolerans	
	Cladosporium sp.	
	Debaryomyces hansenii	
	Rhodotorula mucilaginosa	
	Penicillium chrysogenum	
	Penicillium cf. *coffeae*	
	Penicillium citrinum	
	Penicillium tardochrysogenum	
Different sites	*Acarospora* sp.	Coleine et al. (2018)
	Acarosporaceae	
	Alternaria sp.	
	Aspergillus sp.	
	Aureobasidium pullulans	
	Buellia sp.	
	Cryomyces antarcticus	
	Cryptococcus sp.	
	Dothideales	
	Dothideomycetes	
	Eurotiomycetes	
	Friedmanniomyces endolithicus	
	Fusarium proliferatum	
	Lecanorales	
	Lecidea cancriformis	
	Lecidea laboriosa	
	Parmeliaceae	
	Penicillium sp.	
	Pezizales	
	Pleosporales	
	Saitoella coloradoensis	
	Sarcinomyces crustaceus	

(continued)

Table 5.2 (continued)

Location	Taxon	References
	Solicoccozyma aeria	
	Sporormiaceae	
	Verrucaria sp.	
Antarctica Peninsula		
Deception Island	*Eichleriella* sp.	Alves et al. (2019)
	Fusarium sp.	
	Lecanoromycetes sp.	
	Cladophialophora sp.	
	Pseudogymnoascus sp.	
	Rhodotorula mucilaginosa	
	Penicillium sp.	
	Penicillium sp.	
	Phaeosphaeria sp.	
	Protomyces inouyei	
	Pseudogymnoascus destructans	
	Thelebolus globosus	
Robert Island	*Antarctomyces pellizariae*	
	Candida sp.	
	Cladophialophora sp.	
	Cladosporium sp.	
	Cladosporium sp.	
	Cladosporium sp.	
	Curvularia sp.	
	Helotiaceae sp.	
	Protomyces inouyei	
	Vishniacozyma victoriae	
King George Island	*Acremonium* sp.	
	Cladosporium sp.	
Penguin Island	*Cyphellophora* sp.	
	Paracladophialophora sp.	
	Paracladophialophora sp.	
	Penicillium chrysogenum	
Livingston Island	*Paracylindrocarpon* sp.	
Nelson Island	*Glarea* sp.	
	Penicillium tardochrysogenum	

antarcticus, *Cryomyces minteri*, *Friedmanniomyces endolithicus*, *Friedmanniomyces simplex* (species endemic to Antarctica), and six unidentified isolates of the order *Dothideales*, from samples of sandstone, granite and soils from several Antarctic sites.

The isolates were identified by morphological and physiological techniques and sequencing of the internal transcribed spacer (ITS) region. With the exception of

one isolate, all were characterised as psychrophiles capable of growing in a temperature range of 0–20 °C. The fungi had a thick melanised cell wall, produced exopolysaccharide and exhibited meristematic growth and were, therefore, considered well adapted to withstand the extreme conditions in cold deserts such as Antarctica. The cryptoendolithic community demonstrated a life cycle similar to those observed in algae and cyanobacteria, which is considered to be an important strategy for survival in hostile environments and may be further related to possible forms of life that could be found beyond Earth, such as on Mars, where the initial conditions resemble the cold deserts of Antarctica.

Two novel yeast isolates were obtained from Antarctic rock samples collected in northern and southern Victoria Land (from the Dry Valleys region). The yeasts were phylogenetically reclassified within the genus *Taphrina*, and owing to low sequence similarity with other yeasts, and species *Taphrina antarctica* (Selbmann et al. 2014b).

Gonçalves et al. (2017) studied the diversity of cultivable fungi in rocks collected from the Ellsworth Mountains region, located in the ice sheet of western Antarctica in the Heritage Range of the Antarctic continent. Unlike other studies, Gonçalves et al. (2017) obtained 71 isolates that were identified by morphological and molecular techniques as belonging to the genera *Acremonium*, *Byssochlamys*, *Cladosporium*, *Penicillium*, *Debaryomyces,* and *Rhodotorula*. The rock samples were subjected to mineral analysis and classified into quartzites or phyllites with cavities partially filled by secondary materials rich in iron or aluminium. These characteristics suggest that the rocks underwent biogeochemical modification, perhaps caused by fungal bio-weathering.

Using culture-independent techniques such as ITS region sequencing, Coleine et al. (2018) investigated the diversity and structure of the cryptoendolithic fungal community in sandstone samples obtained from along a gradient ranging from 1000 to 3300 m altitude. The predominant organisms were lichenised fungi and *Dothideomycetes*. The operative taxonomical units (OTU) included *Solicoccozyma aeria*, *Lecanorales*, *Acarosporaceae* spp., *Cryomyces antarcticus*, *Friedmanniomyces endolithicus*, *Basidiomycota* spp., *Buellia* sp., *Sporormiaceae* spp., *Fusarium proliferatum,* and *Penicillium* sp. Based on their results, Coleine et al. (2018) suggested that the studied fungal community was highly adapted but had limitations to environmental disturbances and was not influenced by abiotic factors.

Alves et al. (2019) investigated the diversity of cultivable fungi in rocks obtained from different islands (King George, Penguin, Nelson, Robert, Half Moon, Livingston, and Deception Islands) belonging to the South Shetlands Archipelago of in the Antarctic Peninsula. In their study, 386 isolates, identified through molecular biology techniques, were obtained: *Acremonium*, *Antarctomyces*, *Cladophialophora*, *Cladosporium*, *Curvularia*, *Cyphellophora*, *Eichleriella*, *Fusarium*, *Glarea*, *Lecanoromycetes*, *Paracladophialophora*, *Paracylindrocarpon*, *Penicillium*, *Phaeosphaeria*, *Protomyces*, *Pseudogymnoascus*, *Rhodotorula*, *Thelebolus*, and *Vishniacozyma*. In addition, 14 taxa (which included unusual genera or genera identified at high taxonomic hierarchical levels) showed low coverage

and identities when compared to sequences of known species deposited in GenBank, indicating that these taxa may represent new species of Antarctic fungi. Broadly speaking, the diversity, richness and dominance of the fungal community observed in this study were high compared to other Antarctic habitats. The rock samples were also subjected to petrographic analysis and classified into seven types: basalts, andesites, granite, rhyolite, diorite, gaps and tufts. Andesites, basalts, rhyolites and breccias showed the highest fungal diversity and were also the most abundant rocks in the South Shetland Islands, which suggests an important interaction between biological colonisation and regional geology.

5.8 Rock-Inhabiting Fungi and Astrobiology

The theory of panspermia suggests that simple life forms may have collided with Earth after a long period of permanence in space and developed, throughout evolution, the forms of life known today on the planet (Paulino-Lima and Lage 2009). Alternately, lithopanspermia – a theory complementary to panspermia – proposes the transfer and ejection of viable living material off the origin planet, resistance to the conditions of space and intact arrival of the biological material on the target planet (Onofri et al. 2012). Astrobiology addresses the possibility of extra-terrestrial life searching for remnants of processes and structures of biological origin outside Earth (Des Marais et al. 2008; Des Marais and Walter 1999; Gargaud et al. 2011).

The choice of physical, chemical, biological and environmental factors and living organisms as models for astrobiological studies is important because of the following factors: (i) the analogy between geological, biological, or environmental conditions helps to elucidate processes on Earth that can be extrapolated to other planets and validate and interpret information obtained by satellites and other space exploration equipment; (ii) these factors are necessary for testing astrobiological research instruments, field exploration procedures, and, furthermore, preparing astronauts for future space missions; and (iii) to evaluate the possibility of extra-terrestrial life by means of appropriate model microorganisms, it is essential to investigate how life persists and resists these conditions (Fairén et al. 2010; Martins et al. 2017). The motivation for studying astrobiology extends beyond technological development and knowledge about the solar system and galaxies. Astrobiology studies and conclusions are dependent on the development of several interrelated areas that constitute this scenario and are applicable to different sectors such as engineering, biotechnology, food and pharmaceutical industry, philosophy, politics, and economics (Paulino-Lima and Lage 2009).

Choosing a model living organism to elucidate the possibility of existence of extra-terrestrial life is crucial; in the last few decades, extremophilic microorganisms have been garnering interest as suitable model organisms, expanding the concept of limits for microbial growth (Antunes et al. 2011; Ferrer et al. 2012; Stock et al. 2012; Shtarkman et al. 2013). Prokaryotic organisms have already demonstrated an advantage for colonisation and establishment in extreme environments,

but in recent years, studies have shown that besides colonising these environments, eukaryotes may show a greater degree of resistance than that exhibited by prokaryotes (Shtarkman et al. 2013; Horneck et al. 2016; Pacelli et al. 2017). MCF-inhabiting Antarctic rocks exhibit adaptations that enable them to establish in and explore hostile environments, making them potentially suitable eukaryotic models for astrobiology studies. The cold desert in the Dry Valleys of McMurdo, Antarctica, is the largest ice-free area of Antarctica, where the air temperature varies from −20 to −50 °C in winter and −15 to 15 °C in summer, whereas the temperatures tend to be higher on the soil surface. The area is also arid and receives high solar irradiation (Onofri et al. 2008). These conditions have been postulated to resemble the conditions prevailing on Mars (Onofri et al. 2004) and have been simulated to assess the ability of eukaryotic microorganisms to survive at the edge of life.

Cryomyces antarcticus, a cryophilic black MCF with optimal growth below 15 °C and endemic to the region, was isolated from rocks and soil in the deserts of McMurdo Dry Valleys in Antarctica (Selbmann et al. 2005). *Cryomyces antarcticus* is considered as the most extremophilic eukaryote known in the present day and shows resistance to solar radiation, radioactivity, desiccation and oligotrophic conditions, analogous to space and Mars (Onofri et al. 2004, 2008). Therefore, *C. antarcticus* and other species of black rock fungi have been used as eukaryotic models in astrobiological studies and experiments with gamma radiation (Onofri et al. 2004). *Cryomyces antarcticus* is considered as the best model organism for astrobiological studies. Several studies have been conducted to evaluate its phenotypic plasticity and survival in hostile conditions such as space exposures in the European Space Agency-European Technology Exposure Facility (ESA-EUTEF), Lichens and Fungi Experiment (LIFE) (Onofri et al. 2012, 2015) and Biology and Mars Experiment (BIOMEX) (de Vera et al. 2012), proving that its tolerance to extreme conditions extends from high and low temperatures, desiccation, and lack of nutrients to lethal doses of ionising and UV radiation (Onofri et al. 2008, 2012, 2015; Selbmann et al. 2011).

Some pioneering studies have sought to understand the relationship between fungi that inhabit extreme environments limiting life, confined in pristine regions of the planet, and their virulence potential in humans because little is known about the behaviour of these organisms and their response if they come in contact with human beings.

Approximately 5% of fungal species cause some type of disease in mammals (Kwon-Chung and Bennett 1992). Among the disease-causing fungi, some species of melanised fungi are considered opportunistic with significant clinical importance (de Hoog et al. 1994, 1998, 2000). Chromoblastomycosis, a disease which causes lesions on the skin, can be caused by species of the order *Chaetothyriales*, and it exhibits an invasive meristematic phase with muriform cells (Matsumoto et al. 1984). Muriform cells are also known as sclerotic bodies, fumagoid cells (name given to brownish fungal corpuscles), or Medlar cells and are polyhedric fungal elements with septation in two distinct planes, formed to aid adaptation to hostile conditions.

Concomitant to adaptation in extreme environments, virulence factors for humans, plants and animals have been found in unknown species of fungi in unexplored regions, such as Antarctica, promoting attention to the fungal community found in Antarctic rocks. Gonçalves et al. (2017) were the first to describe an opportunistic, in vitro pathogenic behaviour of fungal species isolated from the rocks of the continental Antarctic desert. According to their study, taxa that have one or more physiological characteristics with pathogenic profiles are phylogenetically close to opportunistic and/or mycotoxigenic pathogens and suggesting that these taxa may represent primitive eukaryotic organisms with genomes that can be used to study the evolutionary origins of opportunistic virulence in fungi.

From these results, new questions about the pathogenic potential of fungi isolated from Antarctic rocks were addressed by Alves et al. (2019) in their study, which found 159 taxa capable of growing at 37 °C, belonging to the genera *Penicillium*, *Fusarium*, and *Rhodotorula*. Physiological in vitro tests such as determination of haemolytic activity, protease and phospholipase production, temperature-induced dimorphism, spore diameter, ability to grow at different pH ranges and determination of minimal inhibitory concentration (MIC) of amphotericin B were performed on these isolates to understand and evaluate their pathogenic potential. *Penicillium chrysogenum* obtained from Antarctic rocks exhibited several physiological factors of pathogenicity and has been reported in other studies as an etiological agent of systemic diseases in immunocompromised patients (de Hoog et al. 2000).

5.9 Conclusions and Perspectives

Although extreme conditions on the Antarctic continent limit life, fungi often inhabit the surface and interior of different rocks in the region, either by symbiotic associations or as a free-living form. In these extreme ecosystems, the fungal community may contribute to the bio-weathering of the rocks, playing a role in soil formation and, consequently, in the supply of soluble nutrients to the microbial community in this substrate. Non-lichenised fungi found in the natural environment of the rocks tolerate different stresses through various adaptive strategies and successfully survive in this substrate. The microbial community in these ecosystems is generally composed of endemic taxa with very specific physiological characteristics. Recent studies have reported that cosmopolitan taxa may also be present in Antarctic rocks and form a community comprising epilithic, endolithic, and cryptoendolithic fungi. Understanding the composition of the fungal community in Antarctic rocks, their adaptations to the environmental conditions and the influence of abiotic factors on these microorganisms has considerable importance; currently, however, there are very few studies on the cryptoendolithic community of Antarctica. In this context, studies aiming to investigate the fungal diversity in Antarctic rocks through cultivation techniques and metagenomic approaches and studies related to environmental data such as rock composition and climatic measurements, as well as

the physiological, biochemical, genetic, and evolutionary evaluation, are crucial to understand the dynamics of the fungal community in Antarctica.

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Chapter 6
Fungi in Snow and Glacial Ice of Antarctica

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6.1 The Antarctic Cryosphere

According to glaciologists and palaeoclimatologists, we live in an interglacial environment in the middle of an Ice Age, where the ice mass currently covers 10% of the planet (at the height of the last glacial age, about 18.000–20.000 years ago, 30% of the planet was covered in ice). However, from the environmental viewpoint, 90% of the volume is concentrated only in Antarctica.

Most of this ice is found in the Antarctic ice sheet, covering approximately 12.3 million km^2 with an average thickness of 2.020 m, encompassing 24.7 million km^3 of ice. The maximum thickness of ice found here is 4.776 m, and the oldest existing ice is probably more than 1.5 million years old. The mantle is composed of several domes (the highest reaches 4.093 m in altitude), where the ice drains slowly to its shores, thousands of miles away. The surface of the Antarctic ice sheet is typically semi-parabolic, more than 2.000 km long, with a flat surface at the centre, and a slope that increases towards the coast (Fig. 6.1).

The Antarctic ice sheet can be divided into two major parts, East and West (although the nomenclature, east and west, may sound illogical for a continent centred on the geographical South Pole). The East Antarctic ice sheet is spread over a subglacial topography that is above the mean sea level, covering an area of 10.1 million km^2 (21.7 million km^3 of ice), with a mean thickness of 2.220 m. The region around its main dome (Dome A) is the driest region (it precipitates only 1–2 cm of water, in the form of snow, per year) and the coldest place on Earth (surface tempera-

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Fig. 6.1 Landscape near the West Antarctic ice sheet ice divide. (Photograph of a Brazilian glaciological camp site taken in 2008. Photo Credits: Simões JC)

ture in winter may fall below −90 °C). The West Antarctic ice sheet covers an area of 2.3 million km^2 (3.0 million km^3 of ice), has a mean thickness of 1.300 m and is largely spread on a continental surface positioned below the mean sea level, even covering a trench of depth 2.496 m. The removal of ice from this part of Antarctica would result in the formation of an archipelago.

Ice shelves are the floating parts of the mantle with a thickness varying between 200 and 1.600 m, generally occurring in regions where there is a large embayment of the Antarctic coast and are fixed to the coast. They gain mass by the ice flow from the mantle or by in situ ice accumulation, ending in cliffs that may be 50 m above sea level or 100–350 m below it. They lose mass by the release of icebergs or by melting caused by seawater at their bottom (which plays an important role in forming the bottom water of oceans). Ice shelves cover approximately 44% of the Antarctic coast-the largest ones, Filchner-Ronne and Ross, cover 439.920 km^2 and 510.680 km^2, respectively. In total, it is an additional 1.5 million km^2 of ice, with an average thickness of about 700 m.

The Antarctic Peninsula and adjacent islands have less than 1% of the Antarctic ice volume (mainly comprising small ice masses, including glaciers a few 100 m long, ice caps and the ice covering the Peninsular plateau, which is approximately 120.000 km^2 in area), limited by the topography that controls its shape and flow conditions. However, owing to their small size of these ice masses and because they are closer to the melting point under pressure, they respond more quickly to changes in climatic variables. Figure 6.2 presents a perspective view of the Antarctic cryosphere, where two main components are observed: the ice sheet covering the continent and the sea ice belt. In addition to the mantle, the central plateau of the Antarctic Peninsula can also be observed, covered with ice caps, flowing to the coast in the form of steep outlet glaciers.

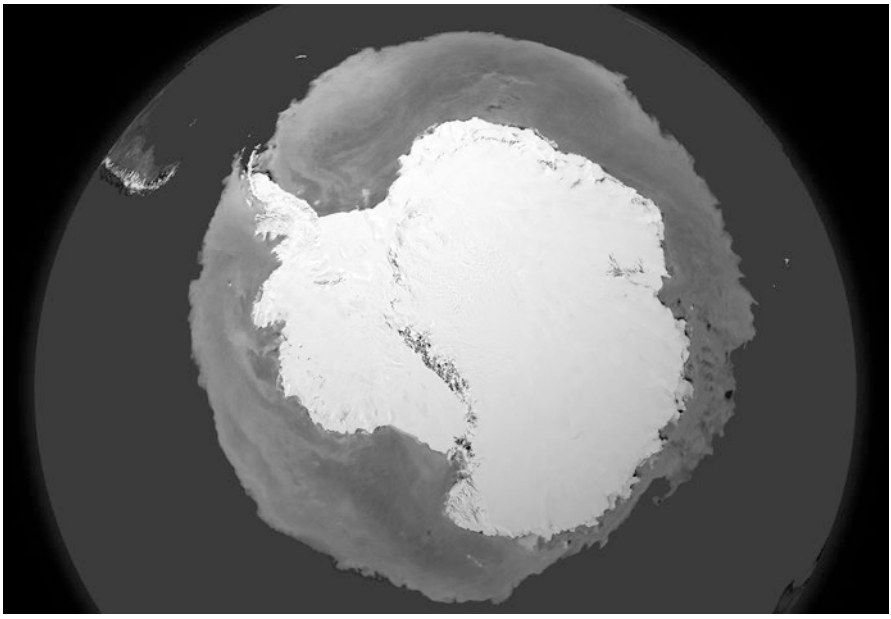

Fig. 6.2 Perspective view of the Antarctic cryosphere. The huge ice sheet (white) covers 99.7% of the continent (across 13.8 million km^2) and the Antarctic Peninsula. The dark spots represent the few mountainous regions with apparent rocks. Around the continent, grey represents sea ice at its maximum annual extent (in September), which can cover up to 20 million km^2 of the Southern Ocean in winters. (Courtesy: NASA/USA)

Around the continent, the sea ice belt can be seen at its maximum extent, typically during the late polar winter (in September), when it covers up to 20 million km^2 of the Southern Ocean. Although it is only a thin layer (averaging not more than 1 m in thickness), it plays an important climatic role in isolating the underlying ocean waters from the atmosphere. Sea ice is formed by the freezing of seawater (−1.83 °C), and its seasonal cycle of formation and melting is known as the environmental phenomenon with the highest annual variation (the area covered by sea ice varies, on average, from 3.0 to 18 million km^2 between summer and winter).

The melting and disintegration of the packed ice is rapid, and the ice coverage may get considerably reduced to only 1.6–3.0 million km^2 from October till the end of February. On average, the Antarctic sea ice does not survive for more than a year, except in some regions such as in the Weddell Sea to the east of the Antarctic Peninsula, the Amundsen Sea and part of the Ross Sea, where it can survive up to 3 years. Importantly, sea ice undergoes drifting by the action of the wind, moving up to 20 km per day.

6.2 Snow and Glacial Ice as Microbial Habitats

Snow is a massive component of the cryosphere that permanently or seasonally spans up to 35% of the Earth's surface (Margesin and Miteva 2011) and represents the source for the formation of glaciers (Pearce et al. 2009). In Antarctica, snow plays an important role in the formation of glacial ice by compression and gradual burial over hundreds and thousands of years (Cowan and Tow 2004). Most of the Antarctic continent is covered by snow (Fig. 6.3), which plays an important role in balancing ice masses and glaciers (Goodison et al. 1999).

As a habitat, snow is directly related to the atmosphere because of constant wind flows of dust, microbial cells and other deposited biological materials present in suspension (Margesin and Miteva 2011). In remote environments, the origin of most of the microbial life seems to be caused by air currents (Abyzov 1993; Pearce et al. 2009), which under the effect of precipitation (in the form of snow) is a capable process of decanting microorganisms suspended in the atmosphere, originating from different parts of the world, towards the terrestrial ecosystems, including Antarctica (Bargagli 2008). Microorganisms inhabiting these snow ecosystems are considered extremophiles and are generally exposed to high incidences of light, UV radiation and seasonal temperature fluctuations (Miteva 2008).

Over the years, the precipitated snow undergoes a process of compaction to become firm ice, which can be considered as a natural means of air sampling from thousands of years ago, where different microorganisms in space and time become trapped in the ice (Abyzov 1993; Ma et al. 1999). Thus, ice can be considered as an excellent matrix for the long-term preservation of microorganisms, allowing the study of contemporary and ancient microbial diversity (Gunde-Cimerman et al. 2003). Ice and snow are important and dominant substrates that are interconnected in the Antarctic environment (Price 2000; Bargagli 2008), with characteristics that restrict the development of life, and are considered as real extreme ecosystems (Maccario et al. 2015).

The glaciers on the planet represent large, thick ice masses that move slowly downwards relative to gravity because of a combination of deformations (Anesio and Laybourn-Parry 2012; Castiella 2014). Glaciers and ice sheets occupy 10% of the Earth's surface (approximately covering 15 million km^2) (Anesio and Laybourn-Parry 2012), and Antarctica holds most of the planet's ice volume (Goodison et al. 1999), where approximately 99.7% of the continent is permanently covered by snow and ice (Convey et al. 2009) with an average thickness of 2 km and a maximum thickness of 4 km (Bargagli 2008) (Fig. 6.4). The active microbial growth in these environments is influenced by ice formation and consequently, the little liquid water available (Gunde-Cimerman et al. 2003). Liquid water is vital in these environments because their distribution reflects the heat balance at certain points inside the ice and the hydraulics forming the drainage system that conveys water through the ice by forming small water channels (Hodson et al. 2008), where microorganisms are able to grow and reproduce.

Fig. 6.3 Summer seasonal snow collection for fungal isolation in Livingston Island and South Shetland Islands in Antarctica. (Photos Credits: LH Rosa)

Fig. 6.4 Glaciers formed at King George Island, South Shetland Islands in Antarctica. (Photos Credits: LH Rosa)

Among physical factors, temperature has considerable influence in determining the type of microorganism that can survive and/or grow in cold ecosystems. Liquid water available in the micro-channels of ice along with the few organic molecules present are the prime conditions for the development of a resident microbial community (Poindexter 2009). Hence, most microorganisms isolated from cold environments are psychrotolerant[1] (also called psychrotrophs) or psychrophilic[2] and are well adapted to cold conditions (Cavicchioli et al. 2002). Therefore, the water activity in snow and glacial ice is an important factor that influences the microbial diversity and activity resident to this extreme habitat. In glacial ecosystems, there are holes called cryoconites,[3] functioning as microhabitats for active microbial communities on the surface of glaciers (Bagshaw et al. 2013). Grzesiak et al. (2015) showed that the gradual exposure of ice as the surface layer of snow melts is an important factor in the spatial variation, chemical composition, abundance of cryoconite holes and the microbial diversity present on the surface of glacial ice. The melting of the superficial snow provides an important supply of inocula, nutrients and water that will cascade into the glacial ecosystems, whereas the accumulation of superficial snow exerts a critical control on the development of the subglacial drainage and determines, to some extent, the degree of interconnectivity in the integrated glacial ecosystem (Fountain et al. 2006; Hodson et al. 2008).

6.3 Antarctic Sea Ice

In the Antarctic cryosphere, beyond snow and ice, the sea ice is also considered a unique habitat that harbours different microorganisms (Fig. 6.5). The sea ice formed in different regions of the Antarctic sea is largely seasonal, with an average range that varies from minimum in February to maximum in September (Zwally et al. 2002; Comiso et al. 2011). A small fraction of the Antarctic sea ice that persists in the austral summer is mainly found in the Weddell Sea; however, in some stricter Antarctic winters, it is possible to find fragments of sea ice in other regions around the coast of the Antarctic continent (Wadhams and Comiso 1992; Vaughan et al. 2013).

Sea ice is a semi-solid heterogeneous matrix containing tiny brine channels and pockets of highly saline water between solid ice crystals (Eicken 2003). Sea ice represents a dynamic ecosystem that changes its physical structure simultaneously with the seasonality of solar radiation at high latitudes and daily fluctuations in air temperature (Hassett and Gradinger 2016). Despite these limiting characteristics,

[1] Psycho-tolerant microorganisms not only grow well at temperatures close to the freezing point of water but also show high growth rates above 20 °C.

[2] Psychrophilic microorganisms show optimal growth at temperatures equal to or less than 15 °C, but cannot grow above 20 °C.

[3] Vertical cylindrical holes that form on the ice surface with a thin layer of particles, debris and microorganisms deposited on the bottom and filled with water.

Fig. 6.5 Sea ice sheet formed at Deception Island, Antarctica. (Photo Credits: LH Rosa)

the sea ice presents itself as a unique habitat for diverse microbial communities in polar environments (Mock and Thomas 2005). The microbial communities in sea ice are critical components of polar marine ecosystems (Robinson et al. 1997) and contribute to primary production in the local food chain (Legendre et al. 1992). It is believed that extreme conditions, together with halotolerance, are a driving force in the evolution of marine microorganisms (Powell et al. 2015), suggesting that the sea ice environment may be an important reservoir of species diversity, including for those of the fungi kingdom (Hassett and Gradinger 2016). However, it is important to note that studies on microbial communities in Antarctic sea ice have concentrated almost exclusively on algae and bacteria, and studies on fungi are inadequate.

6.4 Methods Used to Study Microbial Communities in Snow and Ice

Studies involving microbial diversity in snow, glacial ice and Antarctic sea ice are challenging for a variety of reasons; the remote location of these habitats and the difficult logistics involved in obtaining and transporting these samples are regarded as the main challenges. According to Miteva (2008), the logistic difficulty in obtaining glacial ice is still greater, as the process by which its perforation is performed is slow and requires specialist equipment to obtain the glacial ice core in the best conditions of sterility available for microbiological studies.

To conduct reliable microbiological studies with samples of snow, glacial ice and sea ice, it is important that external contamination of samples during the processing

is avoided to demonstrate that the microbial communities observed in the sample are truly native to that specific habitat, and not any external contamination associated with the methodology used in obtaining the samples. Several processing and decontamination techniques have been proposed, tested and used over the years (Rogers et al. 2004).

To reduce contaminations in snow samples, generally, about 20 cm-thick portions are removed from both the upper layer (to avoid surface contamination from particles carried by the wind) (Antony et al. 2016) and lower layer (to avoid contaminations from the soil layer in contact with the snow) before collecting the target sample. For glacial and sea ice samples, decontamination is usually performed by washing the samples with sodium hypochlorite followed by exposure to UV radiation (Rogers et al. 2004; Miteva et al. 2009), removing the outer layer of the samples (Abyzov 1993; Ma et al. 2000; D'Elia et al. 2009) and using materials previously sterilised by moist heat and/or chemicals such as 70% ethanol for sample collection (Turchetti et al. 2008; de Menezes et al. 2017). The elimination of all external contaminants is fundamental in the authentication of the species found in the samples, and effective decontamination depends on the necessary care taken during the collection, transportation and processing of samples for isolation of the microorganisms or extraction of the genetic material present (Rogers et al. 2004).

The extreme conditions in cryo-environments (minus temperatures, continuous incidence of UV radiation and low availability of water, nutrients and minerals) pose the biggest challenges to isolate and cultivate microorganisms by the direct inoculation of samples obtained from these environments, as these conditions (and the cryo-environment) provide inadequate information regarding the complex nutritional and biomass requirements of the microorganisms present. In addition, the low number of microbial cells and plants in these environments may be damaged, or simply inactive, and may not be able to grow or develop in the different culture media used for their isolation. Thus, physico-chemical studies and previous samples of the target environment that can be analysed can form the basis for preparing specific culture media and growth conditions (Miteva 2008).

Abyzov et al. (1998) utilised melted water from ice core fragments for direct inoculation into different culture media and showed that lower amounts of viable fungi are obtained by direct inoculation of water from deeper and older ice layers. Studies using the same technique of direct inoculation in various culture media were able to obtain strains of viable fungi from ancient glacial ice samples (Ma et al. 1999, 2000; D'Elia et al. 2008, 2009).

In filtration-culture techniques, snow or ice samples are melted and filtered through membranes having pores of specific size, typically 0.45 μm in diameter, for the retention of fungal particles (spores or hyphal fragments), which are subsequently seeded in different culture media compositions (Table 6.1). This technique was effective in getting viable, cultivable microorganisms from Antarctic ice and snow (Turchetti et al. 2008; de Menezes et al. 2017). Elster et al. (2007) used three different pre-concentration methods (filtration, centrifugation or lyophilisation) before inoculating the samples in different solid and liquid culture media. Their study demonstrated that these pre-concentration methods optimised cultivation of

Table 6.1 Culture media used for the isolation of fungi from snow, glacial ice and sea ice

Media	Composition per litre	References
Sabouraud agar	10 g enzymatic digest of casein, 40 g dextrose (glucose), 20 g agar, pH 5.6 ± 0.2	Jacobs et al. (1964), Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013), de Menezes et al. (2017)
Yeast-malt extract agar (YMA)	5 g peptic digest of animal tissue, 3 g yeast extract, 3 g malt extract, 10 g dextrose; 20 g agar, pH 6.2 ± 0.2	Ma et al. (2000)
Nutrient agar (NA)	3 g beef extract, 5 g peptone, 15 g agar, pH 6.8 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013), Sanyal et al. (2018)
Malt extract agar (MEA)	12.8 g maltose, 2.7 g dextrin, 2.4 g glycerol, 0.8 g peptone, 15 g agar, pH 4.7 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Potato dextrose agar (PDA)	40 g potato starch; 20 g dextrose, 15 g agar, pH 5.6 ± 0.2	Ma et al. (2000), D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Mycobiotic agar (MA)	15 g agar, 10 g soybean hydrolysate, 10 g dextrose, 0.5 g cycloheximide, 0.05 g chloramphenicol, pH 6.5 ± 0.2	Ma et al. (2000)
Oatmeal agar (OA)	60 g oat flakes, 12.5 g agar, pH 6.0 ± 0.2	Ma et al. (2000) and Knowlton et al. (2013)
Yeast Extract Peptone agar (YEP)	5 g yeast extract; 5 g bacteriological peptone, 3 g KH_2PO_4, 3 g $(NH_4)_2$ SO_4, 2 g glucose, 15 g agar, pH 7 ± 0.2	Thomas-Hall and Watson (2002)
Supplemented PDA	4 g potato starch, 20 g dextrose, 15 g agar, 0.5 g yeast autolysate, pH 5.6 ± 0.2	Abyzov et al. (2004)
Winogradsky medium	10 g sucrose or glucose, trace $CaCO_3$, 20 g agar, pH 7.2 ± 0.2	Abyzov et al. (2004)
Z medium	0. 467 g $NaNO_3$, 0.059 g Ca $(NO_3).4H_2O$, 0.003 g K_2HPO_4, 0.025 g $MgSO_4.7H_2O$, 0.021 g Na_2CO_3, 10 mL Fe-EDTA, pH 6.6, for solid media add 15 g of agar	Elster et al. (2007)
Bold's Basal Medium (BBM)	10 mL $NaNO_3$ (25 g), 10 mL $CaCl_2.2H_2O$ (2.5 g), 10 mL $MgSO_4.7H_2O$ (7.5 g), 10 mL K_2HPO_4 (7.5 g), 10 mL KH_2PO_4 (17.5 g), 10 mL NaCl (2.5 g), 1 mL EDTA solution, 1 mL Fe solution (0.498 g $FeSO_4.7H_2O$; 0.1 mL H_2SO_4 (96%), 1 mL H_3BO_3 (11.42 g), 1 mL Trace metals solution (8.82 g $ZnSO_4.7H_2O$, 1.44 g $MnCl_2.4H_2O$, 0.71 g MoO_3, 1.57 g $CuSO_4.5H_2O$, 0.49 g $Co(NO_3)_2.6H_2O$), pH 6.6 ± 0.2, for solid media add 15 g of agar	Elster et al. (2007)

(continued)

Table 6.1 (continued)

Media	Composition per litre	References
BG-11	1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 0.006 g citric acid; 0.006 g ferric ammonium citrate, 0.001 g EDTA (disodium salt), 0.02 g Na_2CO_3, 1 mL Trace metal mix A5 (2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.22 g $ZnSO_4 \cdot 7H_2O$, 0.39 g $NaMoO_4 \cdot 2H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 0.49 g $CO(NO_3)_2 \cdot 6H_2O$), pH 7.1 ± 0.2, for solid media add 10 g of agar	Elster et al. (2007)
Rose Bengal agar (RB)	5 g soytone, 10 g dextrose, 1 g KH_2PO_4, 0.05 g rose Bengal, 15 g agar, pH 7.2 ± 0.2	D'Elia et al. (2008, 2009), Turchetti et al. (2008)
Acidic yeast extract agar	30 g yeast extract, 30 g malt extract, 5 g peptone, 10 g dextrose, 20 g agar, pH 4.5 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Meat-liver agar	20 g meat-liver base, 0.75 g D (+)-glucose, 0.75 g starch, 1.2 g sodium sulphite, 0.5 g ammonium ferric citrate, 11 g agar, pH 7.6 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Blood agar	15 g pancreatic digest of casein, 5 g papaic digest of soybean meal, 5 g NaCl, 50 g sheep's blood, 15 g agar, pH 7.3. ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Water agar	Sterile distilled water with 20 g agar	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Yeast extract agar	30 g yeast extract, 30 g malt extract, 5 g peptone, 1 g dextrose, 20 g agar, pH 6.2 ± 0.2	D'Elia et al. (2008, 2009), Knowlton et al. (2013)
Dichloran 18% glycerol agar (DG18)	5 g casein enzymatic digest, 10 g D-glucose, 1 g potassium dihydrogen phosphate, 0.5 g magnesium sulphate, 0.002 g dichloran, 0.1 g chloramphenicol, 15 g agar, pH 5.6 ± 0.2	Turchetti et al. (2008)
Dichloran rose Bengal agar (DRB)	5 g peptic digest of animal tissue, 10 g dextrose, 1 g Monopotassium phosphate 0.5 g magnesium sulphate, 0.025 g rose Bengal 0.1 g chloramphenicol, 0.002 g dichloran, 15 g agar, pH 5.6 ± 0.2	Turchetti et al. (2008)
Tryptone soy broth (TSB)	17 g tryptone, 3 g Soytone—enzymatic digest of soybean meal, 5 g NaCl, 2.5 g glucose, 2.5 g K_2HPO_4, pH 7.3 ± 0.2	Antony et al. (2016)
Zobell marine broth	5 g peptone, 1 g yeast extract, 0.1 g $C_6H_5FeO_7$, 19.45 g NaCl, 8.8 g $MgCl_2$, 3.24 g Na_2SO_4, 1.8 g $CaCl_2$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.8 g KBr, 0.034 g $SrCl_2$, 0.022 g H_3BO_3, 0.004 g Na_2SiO_3, 0.0016 g $(NH_4)(NO_3)$, 0.008 g Na_2HPO_4, 0.0024 g NaF, pH 7.6 ± 0.2	Antony et al. (2016)
Tryptone soy agar (TSA)	15 g tryptone, 5 g soytone - enzymatic digest of soybean meal, 5 g NaCl, 15 g agar, pH 7.3 ± 0.2	Sanyal et al. (2018)

viable microorganisms, and that liquid media provided the best conditions for the isolation of microorganisms in Antarctic ice and snow samples.

Although widely used, some cultivation methods can arbitrarily favour certain taxa in the microbial community to the detriment of others, by either enabling the faster growth rate of some species (based on the type of culture medium and temperature chosen) or owing to the fact that several microorganisms are simply not cultivable. Individually or collectively, these factors can interfere with the accurate interpretation of the microbial diversity in samples, including that for fungi. About 1% of the local microbial community of most ecosystems is able to grow in culture media (Amann et al. 1995). Based on this understanding, independent methods of cultivation have gained interest in recent years, with emphasis on the use of metagenomic techniques.

Metagenomics is based on the direct extraction of DNA from microorganisms in different environmental samples, without requiring their cultivation, which can be used to evaluate microbial diversity (Escobar-Zepeda et al. 2015) by sequencing their amplicons (Antony et al. 2016). In permanently frozen environments, the constant low temperatures provide the ideal conditions for long-term preservation of nucleic acids due to the reduction of the molecular degradation rate (Willerslev et al. 2004). Molecular methods based on polymerase chain reaction (PCR) have the advantage of detecting live, dead, dormant or damaged cell DNA, providing a better representation of the local microbial diversity (Miteva 2008). However, obtaining the low biomass in addition to the difficulty of lysing the obtained cells and obtaining sufficient DNA may hinder the amplification process and the subsequent cloning and analysis of the sequences (Miteva et al. 2009). The popularisation of independent cultivation techniques in terms of cost and analytical tools, as well as the integration of "omics"[4] technologies into all biological approaches will still challenge scientific standards on the ecology of extremophiles (Cowan et al. 2015).

Scanning electron microscopy and fluorescence-based techniques are used in studies involving the characterisation of microbial communities in Antarctic ice and snow to determine cell viability, enumeration and size of cells (Abyzov 1993; Abyzov et al. 2004; D'Elia et al. 2008; Knowlton et al. 2013). Abyzov et al. (2004) have reported the advantage of using epifluorescence microscopy to detect cells and possibly estimate their physiological state in samples selected for study.

Interestingly, Antony et al. (2016) reported notable differences in both the quantity and variety of fungal taxa determined using both culture-dependent and independent techniques in their work focused on the microbial communities in Antarctic snow. Thus, when reviewing studies on microbial diversity, we must bear in mind that only a single methodological approach cannot provide data regarding the real diversity of the microbial communities inhabiting a particular environment. The application of polyphase methodologies (culture-dependent and independent techniques and microscopy) in the evaluation of microbial diversity can increase the accuracy of the results (Elster et al. 2007; Miteva 2008). Although the use of culture-dependent methods may not be able to determine the actual microbial diversity in particular samples/ecosystems, one of the main advantages of these methods is the production of pure microbial cultures (Abyzov et al. 2004). Accordingly, we can

consider the possibility of using these pure microbial cultures in future biotechnology studies for valuable applications.

6.5 Diversity and Ecology of Fungal Community in Antarctic Snow and Ice

Taxonomic studies on fungi in Antarctic snow and ice samples are scarce, and few taxa have been published thus far. Table 6.2 shows the known taxa identified from the samples collected from different substrates in Antarctica. Jacobs et al. (1964) conducted pioneering studies in the central part of the Antarctic continent and isolated the yeast *Candida parapsilosis* and filamentous fungi of the genera *Penicillium* and *Trichoderma* from the snow and glacial ice samples obtained from this region. They concluded that these isolates were probably transported to the region by their own Antarctic expedition or by other previous expeditions and represented a source of human contamination.

According to Abyzov et al. (2004), different methods were used to detect microorganisms in old layers of a glacier in Antarctica at depths of 1.500–2.400 m. Fungi of the genera *Cryptococcus albidus*, *Cystofilobasidium*, *Rhodotorula*, *Pseudozyma*, *Aureobasidium*, *Aspergillus*, and *Penicillium* were isolated from older sections of the Antarctic ice core, dating back 1.000–5.000 years on Lake Vostok in continental Antarctica, and some isolates were found to be heterotrophic psychrotolerant (D'Elia et al. 2009). Furthermore, the number of total and viable yeast cells from the samples of Lake Vostok was higher for the ice accretion obtained from a depth of 3.582 m (D'Elia et al. 2008, 2009). These data indicate that the ecological conditions in these regions of deep ice are sufficient to support heterotrophic metabolism and a high diversity of microorganisms, including fungi (D'Elia et al. 2009). Sanyal et al. (2018) isolated *Leucosporidium* and *Curvibasidium* from glacial cryoconite holes in three distinct regions of East Antarctica. Their study suggested that fungal communities from cryoconite hole environments can provide a rich source of microorganisms, carbon and nutrients to the surrounding glacial ecosystems and participate in the cycling processes of these ecosystems.

Ice core accretions of Vostok 5G evaluated by sequence analysis using metagenomic/metatranscriptomic techniques showed that only 6% of the obtained sequences belonged to the Eukarya domain, especially fungi of the phyla Ascomycota and Basidiomycota (Shtarkman et al. 2013). Another study on ice sections dating back 500.000–175.000 years obtained from two regions on the mainland of Antarctica employed cultivation, microscopy, metagenomics and metatranscriptomic techniques and isolated species belonging to the genera *Alternaria*, *Davidiella* and *Rhodotorula*, and predominantly *Rhodotorula* species (Knowlton et al. 2013). These results indicate that the viability of these microorganisms was "independent of the cold" or "not dependent on cold" conditions at the time of their deposition in the snow, and that low concentrations of atmospheric dust and CO_2 were also related to increased microbial viability.

Table 6.2 Fungi isolated from snow and glacial ice from different regions of Antarctica

Substrate	Location	Taxon	References
Snow	Lichen Valley, Vestvold Hills	*Holtermanniella nyarrowii*	Thomas-Hall and Watson (2002)
	Lichen Valley, South Pole	*Mrakia frigida*	Thomas-Hall et al. (2010)
	Victoria Land	*Vishniacozyma victoriae*	Antony et al. (2016)
	Coppermine Peninsula, Robert Island	*Antarctomyces pellizariae*	de Menezes et al. (2017)
Glacial ice	Ross Island, South Pole	*Trichoderma viride*	Jacobs et al. (1964)
		Penicillium lanosum	
		Penicillium commune	
		Penicillium chrysogenum	
		Candida parapsilosis	
	Lake Vostok, South Pole	*Cryptococcus albidus*	Abyzov et al. (2004)
		Rhodotorula spp.	
	Lake Vostok, South Pole	*Aureobasidium pullulans*	D'Elia et al. (2009)
		Cystofilobasidium informominiatum	
		Rhodotorula mucilaginosa	
		Penicillium chrysogenum	
		Rhodotorula sp.	
		Pseudozyma sp.	
		Cryptococcus sp.	
		Penicillium sp.	
		Aspergillus sp.	
	Lake Vostok 5G region, South Pole	*Alternaria tenuissimum*	Knowlton et al. (2013)
		Davidiella tassiana	
		Rhodotorula mucilaginosa	
	Byrd region, South Pole	*Alternaria alternata*	Knowlton et al. (2013)
		Alternaria sp.	
	Dronning Maud Land, East Antarctica	*Leucosporidium* spp.	Sanyal et al. (2018)
	Larsemann Hills, East Antarctica	*Leucosporidium* spp.	Sanyal et al. (2018)
		Curvibasidium sp.	

Most organisms are capable of supporting only transient periods of cold; microorganisms that inhabit permanently cold environments have cellular processes adapted for growth at low temperatures (Cavicchioli et al. 2002). The formation of ice in the intracellular environment is lethal to living organisms. Fungi have developed various physiological strategies to protect themselves from freezing at low temperatures (Duman and Olsen 1993), which include production of antifreeze proteins

(AFPs) and/or antifreeze glycoproteins (AFGPs) (Ewart et al. 1999; Gilbert et al. 2004; Xiao et al. 2010a, b), exopolysaccharides (EPS) (Tibbett et al. 1998; Polezhaeva et al. 2014), cold-active enzymes (Feller and Gerday 1997; Robinson 2001) and unsaturated fatty acids (Singh et al. 2014).

Furthermore, some fungal species synthesise trehalose in their cells/spores and hyphae, which functions as an important storage substance, protects the cytosol against general stress and stabilises the cell membranes during dehydration (Robinson 2001). Some fungi may increase the concentration and production of glycerol and mannitol to maintain turgor pressure (Robinson 2001). Mannitol appears to play an important role in protecting against water stress and can act as a cryoprotectant (Weinstein et al. 2000).

Different strains of yeast species (*Cryptococcus* and *Rhodotorula*) isolated from Antarctic ice were able to grow and reproduce when subjected to negative temperatures. The incorporation rate of leucine was evaluated to compare macromolecular synthesis under liquid and frozen conditions from −5 to −15 °C in these yeasts. All yeast strains were able to incorporate exogenous leucine in their cellular material. These results demonstrate that these strains of *Cryptococcus* and *Rhodotorula* remain metabolically active under freezing conditions (Amato et al. 2009). Some fungi are adapted to conditions of low water availability, high salt concentrations and low temperatures and can sustain a continuous colonisation on snow and ice (Gunde-Cimerman et al. 2003). Cold-tolerant yeast possibly use a combination of various physiological mechanisms for their survival in the cryosphere (Robinson 2001).

6.6 Biotechnological Applications of Fungi Isolated from Snow and Ice

Microbial communities, including fungi in glacial habitats, appear to be active in various biogeochemical cycles and can perform metabolic functions involved in the cycles of C, N and P (Hodson et al. 2007; Telling et al. 2011; Grzesiak et al. 2015). Yeasts such as *Vishniacozyma victoriae* (synonymous to *Cryptococcus victoriae*) isolated from Antarctic snow can produce different enzymes (amylase, lipase, protease, cellulase, ligninase and β-galactosidase) at low temperatures. These studies suggest that microbial communities growing in the snow could potentially modify the snow chemistry through their metabolic activities (Antony et al. 2016). All isolates of the yeast *Mrakia frigida* obtained from snow samples from different regions of Antarctica produced high amounts of intracellular lipids and exhibited active lipid unsaturation in the fatty acyl chain; these characteristics demonstrate the potential industrial application of *M. frigida* in biofuel synthesis at low temperatures (Thomas-Hall et al. 2010).

Sanyal et al. (2018) evaluated the degradation potential of organic carbon dissolved in Antarctic cryoconite holes and found that *Leucosporidium* sp. and *Curvibasidium* sp. isolates demonstrated high enzymatic activity and extracellular

enzymatic diversity at 4 °C with the production of cellulase, ligninase, amylase, lipase, protease, and β-galactosidase. These results demonstrate that these psychrophilic fungi have significant potential in biotechnological applications because of their diverse and high enzymatic activity at low temperatures and, furthermore, offer economic benefits, such as energy savings in large-scale commercial bioprocesses, which would not require the expensive use of reactors (Feller and Gerday 2003).

6.7 Conclusions and Perspectives

Fungi are broadly distributed in the different substrates and ecosystems of Antarctica. Despite the limited studies on fungal communities in snow and ice published thus far, the detection of cold-tolerant cosmopolitan and endemic taxa suggests that both cold substrates, snow and ice, harbour interesting fungal species living at the edge of life in terms of temperature, low nutrient availability and high exposure to UV radiation. Collecting core ice samples from Antarctic glaciers usually involves expensive and difficult logistics to drill and obtain the old glacial ice fragments. However, with the melting and fragmentation of several glaciers in Antarctica, especially in the Antarctic Peninsula (Fig. 6.6), many small fragments have become available in the sea and beaches. These fragmented ice samples can

Fig. 6.6 Fragments of ice released from glaciers in the Antarctica Peninsula. (Photo Credits: LH Rosa)

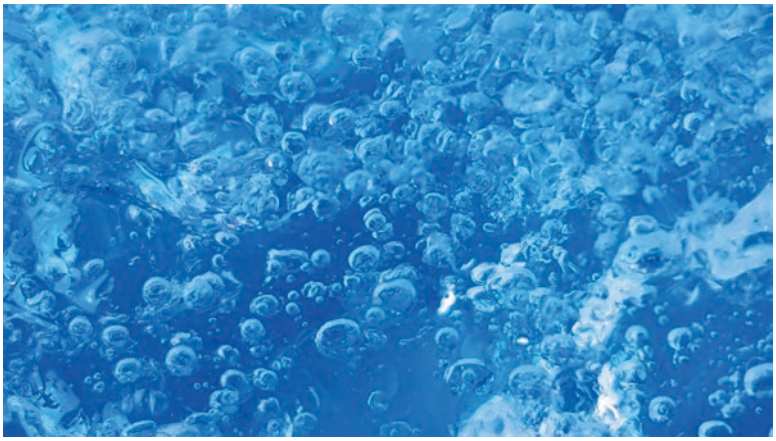

Fig. 6.7 Details of bubbles trapped in fragments of ice collected in Antarctica from glaciers containing atmospheric air of ancient Earth. (Photo Credits: LH Rosa)

be studied as a source of ancient fungi trapped for thousands of years in Antarctica, and after the ice melts, they become available to be dispersed across and out of Antarctica.

In addition, glaciologists use samples of old snow and ice to understand the physico-chemical composition of the atmosphere of the past and associate it to current global climatic changes. Some old ice samples can reveal past atmospheric composition and temperatures of the Earth from the gases trapped in the bubbles of old ice. Consequently, the fungal spores or hyphal fragments trapped in these bubbles may be very old as well (Fig. 6.7).

These fungi inhabiting Antarctic snow and ice may represent taxa that lived in the Earth's atmosphere thousands of years ago, which might provide information regarding the microbial compositions of the planet in the past. In addition, fungi adapted to the extreme conditions of Antarctic snow and ice may exhibit genetic and biochemical pathways to produce antifreeze compounds, tolerance to low nutrient availability and photoprotective activity, which can be of significant value in biotechnological applications.

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Chapter 7
Antarctic Permafrost: An Unexplored Fungal Microhabitat at the Edge of Life

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7.1 Permafrost Environment and Landforms

Permafrost is defined as ground material that has been below 0 °C for 2 or more consecutive years of monitoring and is found primarily in the Arctic and Antarctic regions as well as in high mountainous (alpine) regions, of which the Qinghai-Tibet Plateau, Andes, and Scandinavian Alps are key examples (Bockheim 2015). Perennially frozen permafrost is frequently overlain by a seasonally thawed active layer, whose depth is dependent on air temperatures, moisture content, vegetation coverage, and the snow cover (Michel et al. 2006; Simas et al. 2007; Michel et al. 2012; Moura et al. 2012; Thomazini et al. 2016). The active layer is exposed to freeze-thaw cycles and is, therefore, considered an unstable environment (Bockheim and Hall 2002; Shur and Jorgenson 2007; Jansson and Taş 2014; Czechowski et al. 2016). Permafrost can be hundreds of metres thick (e.g. over 500 m thick in Siberia), while the active layers can range from a few centimetres to several metres in depth (Tarnocai 1980). The Antarctic permafrost may approach 1000 m in thickness (Bockheim and Hall 2002). A transition zone may exist between the permafrost and the active layer, which acts as a temperature buffer and fluctuates between being seasonally frozen and perennially frozen over decadal time scales (Schuur et al. 2015) (Fig. 7.1).

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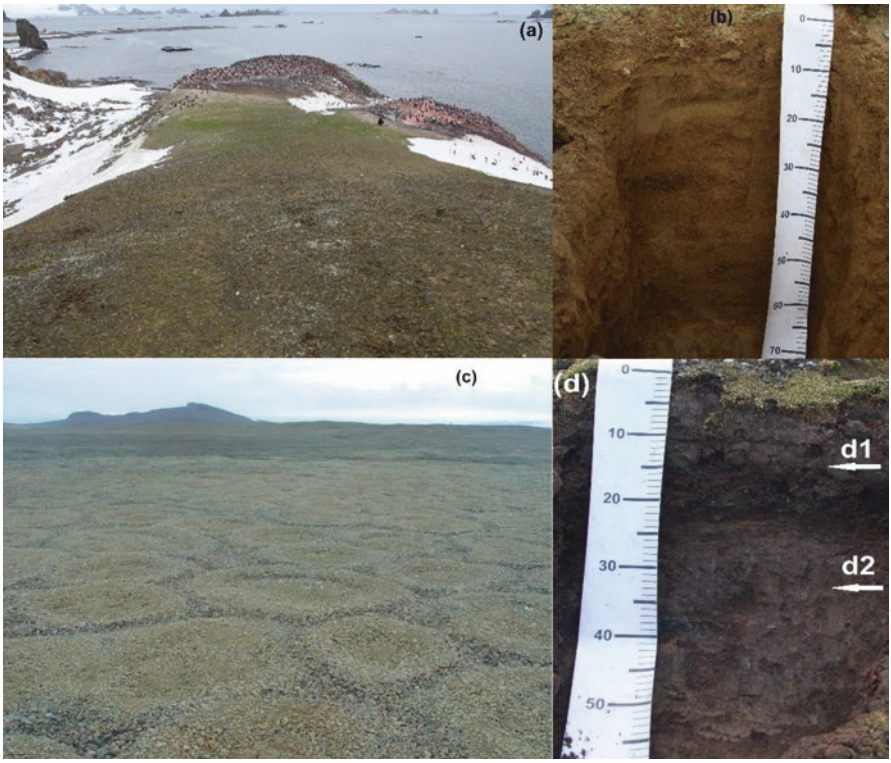

Fig. 7.1 Photographs depicting microbial habitats and permafrost occurrence in Antarctica. (**a**) Former and active penguin rookeries as well as the role of vegetation providing secondary metabolic materials for microbial metabolism at these sites; (**b**) a deep nutrient-rich ornithogenic soil profile formed by intense microbial activity, representing an important hot spot for microorganisms; (**c**) patterned ground indicating permafrost occurrence; and (**d**) a soil profile showing the active layer (**d1**) and the permafrost table starting at approximately 25 cm depth (**d2**). (Photos Credits: A Thomazini)

As a component of the Antarctic landscape, the permafrost ice comprises 92–97% of the total water volume, depending on temperature and sediment texture (e.g. proportion of sand, silt, and clay) (Willerslev et al. 2004). Although it is a strictly thermal phenomena (Steven et al. 2006), permafrost can exhibit differences in its organic matter content, age, soil texture, and ice content throughout its distribution (Shur and Jorgenson 2007; Jansson and Taş 2014). It is estimated that 25% of terrestrial area is covered by permafrost (Jansson and Taş 2014), of which 37% can be found in Antarctica (Goordial et al. 2017), occupying an area of 49,800 km^2 (Fig. 7.2) (Vieira et al. 2010). Underneath the active layer, there is a "permafrost table" (which is permafrost per se) - a physical and biogeochemical barrier to external processes (e.g. ultraviolet radiation) with the underlying horizons firmly cemented by pores, usually filled with ice (Gilichinsky et al. 2007).

Permafrost-affected soils are spatially heterogeneous, especially because of varying frost heave, frost sorting, and mostly frost churning processes that disrupt

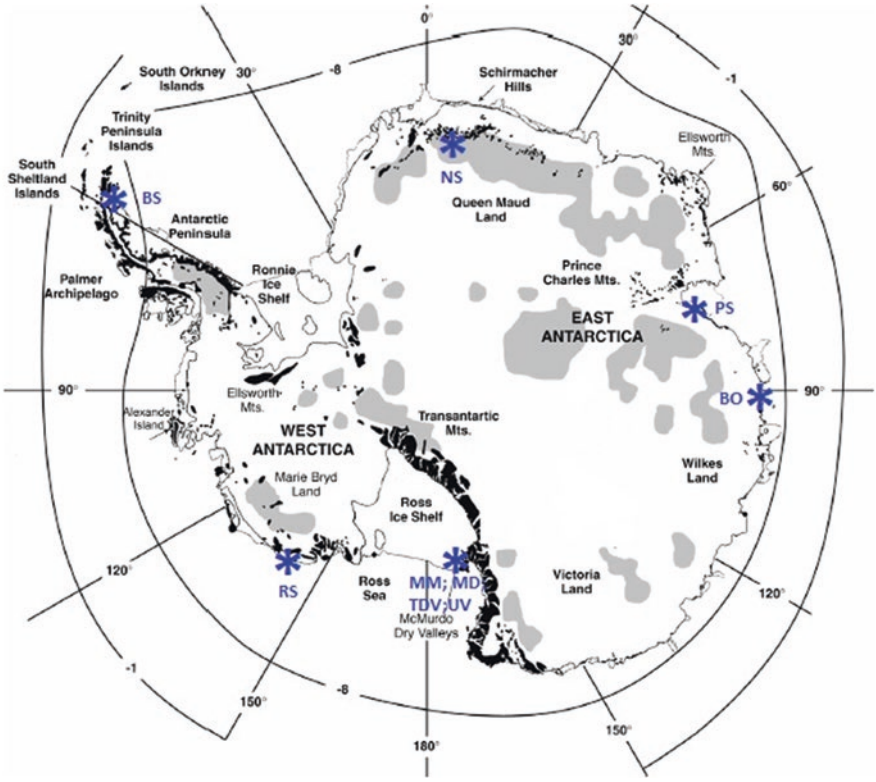

Fig. 7.2 Distribution of permafrost in continental Antarctica. Areas free of ice with probable presence of permafrost are depicted in black. The blue dots represent sampling sites from the published articles reviewed in this chapter. BO Banger Oasis, BS Bellingshausen Station, MD Mayers Dry Valley, MM Mac Murdo Dry Valleys, NS Novolazarevskaya Station, PS Progress Station, RS Russkaya Station, TDV Taylor dry Valleys, UV University Valley. (Map adapted from Bockheim and Hall 2002)

and mix different soil horizons and layers. These processes can facilitate the mixing of organic carbon from the surface into deeper layers (Simas et al. 2007). Arctic permafrost-affected soils contain large stores of frozen organic carbon; in contrast, lower organic matter accumulation is the prevalent feature of Antarctic soils and permafrost. This is attributed to several advances and retreats of vegetation and organic matter deposition during glacial/interglacial periods. Organic matter accumulated during warmer periods subsequently becomes frozen during the glacial age (Zimov et al. 2006; Vonk et al. 2013).

Despite the lower organic carbon content, some Antarctic soils have well-preserved organic carbon readily available for biological degradation (Simas et al. 2007; Michel et al. 2012). Therefore, ornithogenic soils may be highly susceptible to permafrost degradation under climate change. Although Antarctic permafrost is much less studied than its Arctic counterpart, Antarctic soils and permafrost represent a more extreme environment because of the combination of freezing

temperatures, aridity, and low organic matter content. However, little is known about the microbial communities in Antarctic permafrost.

The landforms associated with permafrost soils have typical periglacial or paraglacial features (Francelino et al. 2011), such as ice-wedges, taliks, cryopegs, massive ground ice, frost boils, thermokarst depressions, uplift marine terraces, biogenic landforms, organic accumulations, and broken soil horizons, each providing a unique habitat for microbial growth.

Ice-wedge polygons are formed when the frozen ground contracts and cracks during the winter months, splitting it into polygonal blocks (Kerfoot 1972). During the spring, these cracks are filled with melt water, which may freeze again and forms ice wedges. As the temperature rises, the inner ground between the cracks expands and elevates, forming the polygon centre (Kerfoot 1972). The ice wedges are overlain with active layer soil, creating the polygon trough (Schuur et al. 2015). The ice wedges grow over subsequent years of freeze and thaw cycles, resulting in a high-centred polygon surrounded by lower troughs.

Taliks are unfrozen masses of ground soil found within the permafrost, most commonly under wetlands because of the ability of water to transfer heat. Cryopegs are supercooled groundwater brine lenses that remain liquid even under negative temperatures because of their high salinity (Gilichinsky et al. 2003). Permafrost-affected soils may have a marked seasonal variation of soluble nutrients (nitrogen, phosphorus, calcium, magnesium, and potassium), based on the variations of these nutrients in the active layer, forming gradients. As meltwater is produced at the surface, nutrients are solubilized and can leach and move along the thermal gradient, enriching the top frozen permafrost layer (Tarnocai et al. 2009). This results in the upper layer of permafrost becoming a sink of soluble materials and nutrients, especially organic carbon, during permafrost formation, and a source of nutrients and organic carbon (especially by CO_2 losses) during permafrost degradation (Thomazini et al. 2016).

7.2 Microbial Communities in the Antarctic Permafrost

Although the Antarctic environment is considered to have little diversity, Antarctica has complex ecosystems with unique properties and different substrates that can harbour microhabitats and several microbial groups. Among these unexplored microhabitats, permafrost represents a unique habitat to study microorganisms that are on the edge of life.

The oldest known permafrost sample dates back three million years (Jansson and Taş 2014). In Antarctica, permafrost samples dating back to 170,000 years have been obtained (Kochkina et al. 2001). The atmospheric and ecological context of the period in which the permafrost was formed (e.g. atmosphere rich in CO_2, presence of vegetation and water, and others) could be preserved through freezing during the glaciation periods. In this manner, living beings inhabiting a particular location were possibly preserved in ice, and although the plants and animals trapped during

this glacial transition no longer exist, evidence suggests that many microorganisms were able to adapt and even remain active (Jansson and Taş 2014).

Antarctic permafrost temperatures are rarely measured and vary from −18 to −27 °C (Vorobyova et al. 1997). Owing to the constant low temperatures of permafrost, this substrate might contain cells, dormant propagules, spores and/or genetic material that are possibly the oldest on Earth, which may reveal important information about our ecosystems from the past (Willerslev et al. 2004). Thus, permafrost represents a unique ecological niche for microorganisms adapted to cold conditions (Jansson and Taş 2014).

Permafrost is, therefore, an extreme environment for microorganisms, especially those endemic to the region. Microorganisms inhabiting permafrost must be able to survive long exposures to temperatures below zero degrees on geological time scales, low liquid water levels and extremely low nutrient and metabolite transfer rates (Steven et al. 2006). Owing to its extreme characteristics as an environment, the microbial communities present in permafrost tend to present high rates of endemism (Czechowski et al. 2016).

Despite its extreme conditions, permafrost is considered as a microbial habitat, and even though it imposes several limitations to known organisms, the biodiversity in permafrost is relatively high, showing that it is not merely a frozen habitat with surviving microorganisms (Zucconi et al. 2012; Kochkina et al. 2014). Bacteria and fungi are the most commonly studied microorganisms in permafrost. Some bacteria have been reported to sustain biological activity in permafrost (Jansson and Taş 2014). In contrast, fungi are present in the form of spores (Vorobyova et al. 2001), hyphal fragments and resistant propagules in permafrost, without any apparent metabolic activity. The ratio of abundance between fungi and bacteria in permafrost appears to be low, probably because of low water activity, low carbon and nitrogen content, and conditions that restrict the growth and dispersion of fungi (Dreesens et al. 2014; Goordial et al. 2017). The depth and age of permafrost do not appear to have an influence on the fungal concentration in the sample, since fungal populations are microfocals and can be detected in any portion of the sample, regardless of depth or age of the sediments (Ozerskaya et al. 2009).

Permafrost is indeed an extreme environment. Therefore, the living communities present in this environment should be able to overcome two main limiting factors to life as we know it on Earth: extremely low temperatures and low liquid water content. Studies on the diversity of fungi inhabiting the deep permafrost layer have been conducted at both poles; however, only the list of species found in the Arctic permafrost has been documented thus far (Ozerskaya et al. 2009).

7.3 Fungi in Antarctic Permafrost

Few studies have aimed to identify fungal communities inhabiting the Antarctic permafrost (Kochkina et al. 2001, 2012; Gilichinsky et al. 2007; Zucconi et al. 2012; Goordial et al. 2016, 2017). Most of our current knowledge regarding

permafrost fungi is based on the research on Arctic permafrost. In a review article, Ozerskaya et al. (2009) reported that 33 families and 77 species of fungi are present in the Arctic permafrost, compiled from those listed in nine published articles.

Taking into consideration the data compiled in these earlier studies, the current work presents the list of fungi reported in the Antarctic permafrost and also describes the spatial distribution of this information throughout the continent (Table 7.1). The nomenclature found in the MycoBank database (http://www.mycobank.org/) was followed. In total, 70 species of fungi, distributed among 32 families and 4 phyla, were found in the Antarctic permafrost.

The 32 families listed here have been reported in only five published articles regarding Antarctic permafrost, indicating that the fungal diversity in the Antarctic permafrost is possibly even higher, despite insufficient sampling. The most diverse fungi found in the Antarctic permafrost are fungi that form conidia (e.g. *Aspergillus* spp., *Chrysosporium* spp., *Cladosporium* spp. and *Penicillium* spp.), possibly representing a dormant state in these taxa, which allows them the longest period of survival in this microhabitat (Ozerskaya et al. 2009). Among the 22 fungal species reported, the number of species belonging to the genus *Penicillium* was the highest, reported in four out of nine study locations; in contrast, only four species of *Cladosporium* were reported, but they were found in eight out of the nine study locations. The genera *Cordyceps* and *Akanthomyces*, isolated from Antarctic permafrost, can also be added to the list of Antarctic fungi proposed by Bridge and Spooner (2012).

Most of the reported species belonged to the *Aspergillaceae* family, represented by species of the genera *Penicillium* and *Aspergillus*/*Eurotium.* Since the discovery of penicillin by Alexander Fleming in 1928, the genus *Penicillium* has been extensively studied. This genus is ubiquitous and is present in the different substrates and environments of Antarctica (Gomes et al. 2018). In general, the genus *Penicillium* is considered highly important in the medical field because of its ability to produce several biologically active substances and even synthesize mycotoxins and other allergens (Zhelifonova et al. 2009). However, very few *Penicillium* spp. found in Antarctica have been chemically investigated with regard to this potential (Gomes et al. 2018).

The genus *Cladosporium* was also widely distributed among permafrost samples. These fungi cause several diseases in plants and animals, resulting in serious environmental damages (Bensch et al. 2012). The *Cladosporium* spp. isolated from permafrost samples included *Cladosporium sphaerospermum*, a potent human pathogen (de Hoog et al. 2000; Yano et al. 2003); *Cladosporium antarcticum*, derived from the Antarctic lichen *Caloplaca regalis* (Schubert et al. 2007), showing that the Antarctic ecosystems can reveal new and undiscovered endemic species; *Cladosporium herbarum* (reported in four of the nine study locations), a common fungi found worldwide (Schubert et al. 2007), was considered as the most widely distributed fungal species in the Antarctic permafrost. *Cladosporium herbarum* causes leaf spots on plants with commercial interest (Barbosa et al. 2001) and can also cause opportunistic mycosis in immunosuppressed patients (Dreborg et al. 1986; Malling et al. 1986).

Table 7.1 List of fungal species reported from samples of Antarctic permafrost

Taxa	Region	Deep range (cm)	Sediment age (years)	Method	Reference
Ascomycota					
Aspergillaceae					
Aspergillus sp.	TDV, MM	*	170.000	Cultivable	Kochkina et al. (2001), Gilichinsky et al. (2007)
Aspergillus sydowii	MM, BS	320–330	7486 ± 40	Cultivable	Gilichinsky et al. (2007), Kochkina et al. (2012)
A. versicolor	MM	*	*	Cultivable	Kochkina et al. (2012)
Eurotium amstelodami	MM	340	*	Cultivable	Zucconi et al. (2012)
Penicillium aurantiogriseu	MM	*	*	Cultivable	Gilichinsky et al. (2007)
P. brevicompactum	BS	500–520	7490 ± 40	Cultivable	Kochkina et al. (2012)
P. chrysogenum	BS, OS, BO, MM	100–625	705–7495 ± 40	Cultivable	Kochkina et al. (2012), Zucconi et al. (2012)
P. citrinum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. commune	MM	*	*	Cultivable	Kochkina et al. (2012)
P. expansum	OS, MM	60–316	> 50.000	Cultivable	Kochkina et al. (2012), Zucconi et al. (2012)
P. funiculosum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. glabrum	MM	342	*	Cultivable	Zucconi et al. (2012), Kochkina et al. (2012)
P. glandicola	MM	*	*	Cultivable	Kochkina et al. (2012)
P. implicatum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. jensenii	MM	*	*	Cultivable	Kochkina et al. (2012)
P. miczynskii	MM	*	*	Cultivable	Kochkina et al. (2012)
P. minioluteum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. palitans	MM	233–343	*	Cultivable	Zucconi et al. (2012)
P. purpurogenum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. rugulosum	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Penicillium sp.	MD, MM	234–344	30.000	Cultivable	Kochkina et al. (2001), Zucconi et al. (2012), Gilichinsky et al. (2007)

(continued)

Table 7.1 (continued)

Taxa	Region	Deep range (cm)	Sediment age (years)	Method	Reference
P. variabile	BS	500–625	7492 ± 40	Cultivable	Kochkina et al. (2012)
P. verrucosum	MM	*	*	Cultivable	Gilichinsky et al. (2007)
P. viridicatum	MM	*	*	Cultivable	Kochkina et al. (2012)
P. waksmanii	BS	615–625	7497 ± 40	Cultivable	Kochkina et al. (2012)
Apiosporaceae					
Arthrinium sp.	MD	*	30.000	Cultivable	Kochkina et al. (2001)
Arthrinium arundinis	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Chaetomiaceae					
Chaetomium	MD	*	30.000	Cultivable	Kochkina et al. (2001)
Cladosporiaceae					
Cladosporium sp.	MD, TDV	*	30.000–170.000	Cultivable	Kochkina et al. (2001)
Cladosporium antarcticum	BO	115–125	705 ± 110	Cultivable	Kochkina et al. (2012)
C. cladosporioides	MM, RS	20–337	*	Cultivable	Gilichinsky et al. (2007), Kochkina et al. (2012), Zucconi et al. (2012)
C. herbarum	MM, BO, RS, NS,	15,338	705–> 50.000	Cultivable	Gilichinsky et al. (2007), Kochkina et al. (2012), Zucconi et al. (2012)
C. sphaerospermum	BS	615–625	7494 ± 40	Cultivable	Kochkina et al. (2012)
Clavicipitaceae					
Cordyceps bassiana	MM	316	*	Cultivable	Zucconi et al. (2012)
Cordycipitaceae					
Akanthomyces lecanii	MM	*	*	Cultivable	Kochkina et al. (2012)
A. muscarius	BS	500–520	7489 ± 40	Cultivable	Kochkina et al. (2012)
Engyodontium album	MM	233	*	Cultivable	Zucconi et al. (2012)
Dematiaceae					
Dematiaceae sp.	MD, TDV	*	30.000–170.000	Cultivable	Kochkina et al. (2001)
Diaporthaceae					

Diaporthe helianthi	UI	*	*	Uncultivable	Kochkina et al. (2012)
Didymellaceae					
Juxtiphoma eupyrena	BO	115–125	705 ± 110	Cultivable	Kochkina et al. (2012)
Phoma leveillei	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Dipodascaceae					
Dipodascus sp.	TDV	*	170.000	Cultivable	Kochkina et al. (2001)
Geotrichum candidum	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Dothioraceae					
Aureobasidium pullulans var. *pullulans*	RS, MM, PS	115–336	*	Cultivable	Kochkina et al. (2012), Zucconi et al. (2012)
Herpotrichiellaceae					
Exophiala sp.	RS	115–120	*	Cultivable	Kochkina et al. (2012)
Hypocreaceae					
Gliocladium sp.	RS	115–120	*	Cultivable	Kochkina et al. (2012)
Trichoderma harzianum	BS	500–520	7493 ± 40	Cultivable	Kochkina et al. (2012)
Incertae sedis					
Chaetophoma sp.	BO	115–125	705 ± 110	Cultivable	Kochkina et al. (2012)
Microascaceae					
Wardomyces anomalus	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Morchellaceae					
Imaia gigantea	UI	*	*	Uncultivable	Kochkina et al. (2012)
Myxotrichaceae					
Oidiodendron sp.	MM, TDV	*	170.000	Cultivable	Gilichinsky et al. (2007)
Nectriaceae					
Fusarium oxysporum	MM	*	*	Cultivable	Kochkina et al. (2012)
Fusarium sp.	TDV	*	170.000	Cultivable	Kochkina et al. (2001)
Onygenaceae					
Chrysosporium europae	BS	320–330	7487 ± 40	Cultivable	Kochkina et al. (2012)

(continued)

Table 7.1 (continued)

Taxa	Region	Deep range (cm)	Sediment age (years)	Method	Reference
Xerochrysium xerophilum	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Orbiliaceae					
Dwayaangam colodena	UI	*	*	Uncultivable	Kochkina et al. (2012)
Plectosphaerellaceae					
Verticillium sp.	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Metapochonia suchlasporia	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Pleosporaceae					
Alternaria alternata	MM	316–335	*	Cultivable	Zucconi et al. (2012)
Ulocladium botrytis	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Ramalinaceae					
Lecania brialmontii	UI	*	*	Uncultivable	Kochkina et al. (2012)
Roccellaceae					
Roccella gracilis	UI	*	*	Uncultivable	Kochkina et al. (2012)
Saccharomycetaceae					
Candida albicans	UI	*	*	Uncultivable	Kochkina et al. (2012)
Pichia kluyveri	UI	*	*	Uncultivable	Kochkina et al. (2012)
Undetermined					
Ascochyta sp.	RS	115–120	*	Cultivable	Kochkina et al. (2012)
Ascomycota incertae sedis	RS	115–140	*	Cultivable	Kochkina et al. (2012)
Chaetothyriales	UV	*	*	Cultivable	Goordial et al. (2016)
Coelomycetes incertae sedis	BO, OS	115–240	705 ± 110	Cultivable	Kochkina et al. (2012)
Cryomyces sp.	PS	230–240	*	Cultivable	Kochkina et al. (2012)
Dothideomycetes	UV, MM	5–55	*	Uncultivable	Goordial et al. (2016, 2017)
Eurotiomycete	UV, MM	12–30	*	Uncultivable	Goordial et al. (2016, 2017)
Leotiomycetes	UV, MM	0–22	*	Uncultivable	Goordial et al. (2016, 2017)
Saccharomycetes	UV	5–55	*	Uncultivable	Goordial et al. (2016)

Sordariomycetes	MM	22	*	Uncultivable	Goordial et al. (2017)
Basidiomycota					
Cortinariaceae					
Cortinarius scaurus f. *phaeophyllus*	UI	*	*	Uncultivable	Kochkina et al. (2012)
Sporidiobolaceae					
Rhodotorula mucilaginosa	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Rhodotorula sp.	UV	37–42	*	Cultivable	Goordial et al. (2016)
Sporidiobolus metaroseus	MM	233	*	Cultivable	Zucconi et al. (2012)
Tremellaceae					
Cryptococcus pseudolongus	UI	*	*	Uncultivable	Kochkina et al. (2012)
Filobasidium stepposum	MM	339	*	Cultivable	Zucconi et al. (2012)
Undetermined					
Basidiomycetes sp.	MD, TDV, MM	*	30.000–170.000	Cultivable	Kochkina et al. (2001), Gilichinsky et al. (2007)
Basidiomycota incertae sedis	UV	0–5	*	Uncultivable	Goordial et al. (2016)
Tremellomycetes	UV, MM	0–22	*	Uncultivable	Goordial et al. (2016, 2017)
Streptophyta	MM	22	*	Uncultivable	Goordial et al. (2017)
Zygomycota					
Mortierellaceae					
Mortierella sp.	MM	*	*	Cultivable	Gilichinsky et al. (2007)
Mucoraceae					
Rhizopus oryzae	MM	316	*	Cultivable	Zucconi et al. (2012)
Undetermined phyla					
Mucedinaceae					
Mucedinaceae sp.	MD, TDV	*	30.000–170.000	Cultivable	Kochkina et al. 2001

Abbreviations: *BO* Banger Oasis, *BS* Bellingshausen Station, *MD* Mayers Dry Valley, *MM* Mac Murdo Dry Valleys, *NS* Novolazarevskaya Station, *PS* Progress Station, *RS* Russkaya Station, *TDV* Taylor dry Valleys, *UV* University Valley, * uninformed.

Species belonging to the genus *Oidiodendron* were also isolated from different Antarctic substrates, such as marine macroalgae (Loque et al. 2010) and soils (Ding et al. 2016; Wentzel et al. 2018). The genus *Oidiodendron* is considered medically important as it exhibits cytotoxic activity against lymphocytic leukaemia (Li et al. 2012) and synthesizes novel antibiotics for medicinal use (Andersen and Rasmussen 1984).

These studies demonstrate that data collection regarding fungal biodiversity in permafrost is important to discover fungal metabolites of potential value in the fields of medicine, environmental research and biotechnology, in general. Concomitantly, it is also important to consider fungal species with pathogenic potential against plants, animals and humans that have, thus far, remained "imprisoned" in the Antarctic permafrost. Owing to climate change and global warming, these pathogenic fungal species could get introduced into the temperate and tropical regions of the planet; therefore, researchers conducting studies in Antarctica must take precautionary measures to minimize the risk of transferring and introducing these species into other regions of the planet.

The preservation of microorganisms in permafrost depends on the original composition and density of the ancient microbial communities, the level of microbial adaptations to low temperatures and the protective properties of the environment (Kochkina et al. 2001). All these factors are related to the evolution of microbial communities since ancient times, the geological history of the sediments and the conditions for their transition to the state of being permanently frozen, instead of the age and depth of said sediments (Kochkina et al. 2001).

The biomass levels, diversity and structure of the fungal community in low-temperature environments are significantly affected by temperature and water availability (Jansson and Taş 2014). Thus, fungal adaptation to low temperatures might have led to the evolution of fungal enzymes with unique properties (Kochkina et al. 2001). Most of the terrestrial biosphere is characterized by low temperatures (< 5 °C), and therefore, it is important to understand and consider microbial activities and adaptations as well as the ecological interactions of microbial communities that are active in this habitat (Elster et al. 2017).

The preservation of viable communities that are trapped in the Antarctic permafrost leads to key questions regarding the biological surviving mechanisms, biogeochemical processes, life forms, metabolic state of microorganisms, and ability to repair in situ DNA damage (Gilichinsky et al. 2007). In studies employing different "omics" approaches to determine the metagenome present in the Antarctic permafrost, it was possible to observe that some RNAs and proteins were likely preserved for long periods of time in this frozen environment or, yet, that they were sustained in tissues that are surviving in a dormant state. In contrast, a few groups of microorganisms are able to actively express those genes, translating them into proteins, even in freezing temperature conditions (Hultman et al. 2015). Furthermore, studies have reported relatively high expressions of cold-shock proteins in these microorganisms, presumably for survival under freezing conditions (Hultman et al. 2015). In contrast, the concentration of transport proteins in these samples was low, presumably, in response to the low transcript transportation required in permafrost.

To survive under low-temperature conditions, fungi have developed different strategies, such as formation of multicellular spores, increased melanin concentration in the fungal cell wall, production of cryoprotectants (e.g. antifreeze proteins that reduce ice crystals present in the cytoplasm), increased intracellular trehalose concentration (to protect the cytosol from stress), production of polyols (glycerol and mannitol), production of unsaturated fatty acids (to provide increased membrane fluidity) and the synthesis of low temperature-functioning enzymes (lipases, polygalacturonase, amylase and phosphatase) (Robinson 2001; Ozerskaya et al. 2009; Buzzini et al. 2012). Isolation of novel or rare microorganisms, discovery of new metabolites and obtaining information regarding the microbial diversity in Antarctica have collectively generated interest in Antarctic fungi (Kochkina et al. 2014). In this context, it is essential to achieve better organization and dispersion of published data on Antarctic microorganisms. While some studies have published comprehensive data regarding the depth and age of the sediments, the species identified and the sampling sites have not been clearly correlated in these studies, making it difficult for readers to understand or even analyse the microbial diversity.

Despite insufficient sampling, Antarctic permafrost shows considerable richness in its fungal community, possessing an incredibly wide biotechnological potential as well as presenting concerns regarding its potential to harbour pathogenic agents that could cause diseases in plants and animals. When compared to other terrestrial ecosystems, even the ones found in Antarctica, the permafrost continues to be an extreme environment that remains unexplored with regard to its fungal communities, necessitating an increase in future scientific efforts to conduct further research in such a unique environment.

7.4 Role of Permafrost in Global Climatic Changes

Man-made environmental changes have been causing increasingly noticeable losses, making them the focus of greater discussions. Antarctica is considered a key area in relation to the global climate and, apparently, the least disturbed portion of the planet in the context of direct anthropological impacts (Guglielmin and Cannone 2012; Vieira et al. 2010), but at the same time, it is particularly important to global climate change (Maxwell and Barrie 1989; Ramos et al. 2017). However, several environmental changes can directly or indirectly affect Antarctica, such as the increase in global temperature caused by high rates of greenhouse gas emissions (Jansson and Taş 2014; Zhu et al. 2014; Thomazini et al. 2014, 2015, 2016). One of the most direct consequences of global warming on the polar ice caps is the substantial melting of glaciers and permafrost.

According to Guglielmin and Cannone (2012), the surface temperature on continental Antarctica has been showing a strong warming tendency since 1997 (the beginning of their temperature records), with an average increase of 0.31 °C annually. A direct consequence of this increase in temperature was the melting of permafrost (which has been showing a yearly increase in average temperature by 0.1 °C)

at the rate of 1 cm every year, which is inevitably converted into the active layer of the soil. Besides that, moreover, these environmental responses have a strong correlation with the increase in total solar radiation induced by the expansion of the holes in the ozone layer (Solomon 1999). Indeed, the current influx of UVA and UVB radiation is higher in the Antarctic continent than it is in any other part of the planet (Solomon 1999).

The potential effects of climate change in Antarctica could include altered thickness and moisture content in permafrost, increase in ice-free areas, changes in vegetation, soil alterations (Bockheim 1993) and even changes in the microbial population. Specifically, the physical variables influencing frozen soils include stages of defrosting, decrease in snow coverage, increase in nutrient availability and alterations in liquid water availability (Nikrad et al. 2016).

Several gases can be found inside the pores present in permafrost (such as oxygen, carbon dioxide, nitrogen and methane). The oxygen and nitrogen concentrations inside these pores are similar to those found in the atmosphere, while the concentrations of methane and carbon dioxide can be higher, which is directly linked to climate change (Steven et al. 2006). The defrosting of permafrost would result in the release of considerable amounts of carbon and nutrients, which can be used to speed microbial development (Jansson and Taş 2014) and promote microbial degradation of the cryo-acquired carbon, leading to the biogenic production of methane (Mondav et al. 2014). These processes, in turn, would create a positive feedback loop for climate change, speeding the process of defrosting even further (Mondav et al. 2014; Nikrad et al. 2016).

Based on laboratory data simulating the permafrost defrosting process, it was possible to observe the occurrence of rapid changes in microbial gene abundancy and in the groups of metabolically active organisms (Mackelprang et al. 2011). For instance, with only 1 week of defrosting at 5 °C, the permafrost metagenome became more similar to the ones from the active layer samples compared to the ones from other permafrost samples and was even able to show the release of CO_2 and methane (Mackelprang et al. 2011). Moreover, increase in temperature and humidity increased microbial activity in other non-psychrophilic microorganisms (Nikrad et al. 2016), besides providing a better opportunity for exogenous microorganisms to develop. The possibility of community succession by non-indigenous microorganisms in this environment could lead to the extinction of highly specialized endemic fungi that are not very competitive (Selbmann et al. 2012).

The end of the dormant state in many fungi, mediated by global warming, may reveal some isolates that are potentially harmful to human, animal and plant health. Although there is a lack of empirical evidence regarding such pathogenic fungi, a similar *phenomenon* was reported when the eukaryotic pathogen virus *Pithovirus sibericum* was isolated (Legendre et al. 2014) from the Siberian permafrost, dating back to 30,000 years. The global impact of rapid climate change makes the polar and alpine environments places of concern for Science and society, because of the ecological impacts resulting from these changes that have already been observed at the ecosystem level (Elster et al. 2017). The landscape of frozen ecosystems is rapidly changing, and unfortunately, our knowledge of fungal activity in frozen soils is advancing slower than the pace at which climate change is occurring (Nikrad et al. 2016).

7.5 Conclusion and Perspectives

Despite the limited published data on fungi inhabiting the Antarctic permafrost, it is possible to infer that the Antarctic permafrost is moderately rich in viable fungi. The fungal species found in the ancient permafrost samples highlight the certainty of the evolution of specific adaptive strategies for these fungi to survive under a permanent state of freezing. Concomitantly, it is possible to isolate fungal species with known pathogenic potential (such as *C. sphaerospermum*) as well as encounter species of considerable biotechnological interest (such as *Oidiodendron* spp.). Further research is necessary to monitor permafrost melting in Antarctica and to broaden our knowledge regarding the diversity of fungi present in such environments, with the intent to advance our understanding of its ecology, community structure and biotechnological potential and the role of agents of opportunistic diseases in plants and animals.

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Chapter 8
Fungi Associated with Plants and Lichens of Antarctica

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8.1 Introduction

Microorganisms seem to occupy virtually every niche on earth (Strobel 2003), including the polar region. In Antarctica, the microbial communities are an important part of its ecosystem, controlling most of the biological flux of carbon, nutrients, and energy (Wynn-Williams 1990). Despite the limited extent of terrestrial biota and suitable habitats, many fungal species have been reported from Antarctica, which have been isolated from soil, rock, permafrost, freshwater lakes, seawater, marine sediments, glacial ice, snow, terrestrial and marine invertebrates, wood, lichen thalli, macroalgae, bryophytes, and vascular plants (Bridge and Worland 2008). Nevertheless, the knowledge of microbial diversity in Antarctica is still low compared with temperate and tropical ecosystems, mainly due to the harsh environmental conditions of this continent (Yergeau et al. 2007). Studies on fungi associated with Antarctic plants and lichens were initiated with the works of Pugh and Allosopp (1982), Fletcher et al. (1985), Gamundi and Spinedi (1988), Onofri and Tosi (1989), Del Frante and Caretta (1990), Baublis et al. (1991), and Möller and

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Dreyfuss (1996), who detected a rich fungal community on the surface of or within host tissues.

Most of the Antarctic fungi are cold-adapted cosmopolitan taxa, of which propagules are transported to Antarctica by birds, mammals, air and ocean currents and anthropogenic means such as tourism vessels. Fungi are usually dispersed, can colonise many substrates and survive at diverse environmental conditions (Ruisi et al. 2007). Most of the propagules arriving in Antarctica, however, are unable to grow under the harsh conditions of this continent, or when they grow, require plenty of time to complete their life cycles (Ruisi et al. 2007). According to Furbino et al. (2017), they can grow at least under the Antarctic summer conditions, showing a mesophilic or psychrophilic behaviour. Until now, only a tiny part of the fungi found in Antarctica is considered endemic, but the percentage may increase with further studies on the yet incompletely known Antarctic fungal diversity.

All plants in a natural ecosystem seem to have some relation with endophytic or some other associated fungi (Rodriguez et al. 2008). According to Bacon and White (2000), microbial endophytes are those microorganisms that live inside the plant, for at least part of their life cycle, without causing any visible damage to the plant's tissue. As a complement, Schulz and Boyle (2005) pointed out that the term 'endophyte' was used to define organisms that use various strategies of symbiosis, ranging from facultatively saprobiotic, to parasitic, to exploitive, to mutualistic strategies. For such asymptomatic interactions to occur, there are probably multiple, balanced, antagonistic relations between the endophytic fungi, the pathogens and the plant defence mechanism. The plant defence mechanism provides defence against the harmful effects of pathogens and the endophytes, whereas the endophytes furnish toxins that prevent a competition between them and the pathogens besides modulating the immunological response of the plant (Schulz et al. 2015). According to a report by Higgins et al. (2007), the endophytic assemblages of plants that survive in an extreme environment, such as that in Antarctica, may represent a promising source of novel species of fungi, which can help in understanding the ecological role and evolution of plant-fungal symbioses. Most studies of fungal endophytes have focused on species living in vascular plants, such as the only two native vascular plants of Antarctica, *Colobanthus quitensis* (Kunth) Bartl. (*Caryophyllaceae*) and *Deschampsia antarctica* Desv. (*Poaceae*). However, this fungal group can also live inside non-vascular plants such as mosses and liverworts, the two lineages of bryophytes that are important in the polar regions in terms of species diversity, biomass, and ecosystem functioning (Longton 1988, Zhang et al. 2013b). In general, a taxonomically diverse suite of fungi has been reported to interact with bryophytes as pathogens, parasites, saprobes and commensals (Davey and Currah 2006).

This chapter provides an overview of the current knowledge of the fungal diversity associated with bryophytes, vascular plants, and lichens in Antarctica.

8.2 Bryophytes of Antarctica: A Promising Hotspot of Extremophile Fungi

Antarctica's plant vegetation is dominated by mosses (Bryophyta, 111 species) (Ochyra et al. 2008) and liverworts (Marchantiophyta, 27 species) (Bednarek-Ochyra et al. 2000), in contrast to only two native and one invasive species of flowering plants. In certain areas of the continent, the presence of extensive moss carpets is the only bit of green that can be seen for miles. The relevance of bryophytes as a major constituent of local plant diversity has been acknowledged by naming a plateau in Signy Island after a moss species: the *Andreaea* plateau.

Bryophytes constitute the second largest group of land plants after angiosperms; they are widely distributed in all terrestrial ecosystems and conspicuous vegetational elements in, e.g., tropical cloud forests, temperate rain forests, bogs, moss tundras, and desert crusts (Buck and Goffinet 2000; Frey and Stech 2009). The use of bryophytes as bioindicators and as a source of biochemical compounds is well known (Welch 1948; Ando and Matsuo 1984; Fernández and Serrano 2009). Bryophyte species are often widely distributed, for example, about a half of the Antarctic moss species are of bipolar distribution (Ochyra et al. 2008; Câmara et al. 2018), with or without intermediary occurrences in (tropical) high mountain areas.

Observations on the Antarctic vegetation started as early as Antarctica's discovery in the early nineteenth century. The earliest record of Antarctic vegetation probably is a note in Adam Young's diary written while sailing aboard the ship *Williams* in 1819, in which he registered the presence of 'mosses' in King George Island. There are few other records registering the presence of mosses from about the same period in different ship logs, but since these expeditions focused on whale and seal hunting, no plant specimens were collected (or no collections survived).

The first time a plant was collected and properly cited by its name was by James Eights (1798–1882): *Polytrichastrum alpinum* (Hedw.) G.L.Sm. (Fig. 8.1) with the vouchers are preserved in herbaria (US and NY). However, the first professional botanist to land and collect samples from Antarctica was J.D. Hooker (1817–1911), who joined the James Clark Ross expedition 1839–1843 on the *Erebus* and *Terror*. Hooker performed the first professional collection of Antarctica's vegetation and

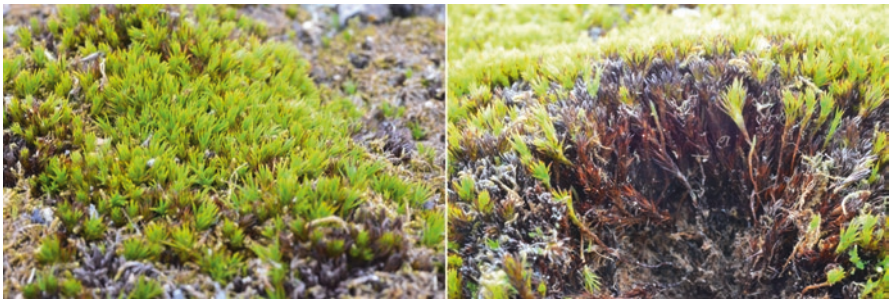

Fig. 8.1 *Polytrichastrum alpinum* (Hedw.) G.L.Sm. (Photos Credits: LH Rosa)

described two new species. His findings were published in the Flora Antarctica or formally *The Botany of the Antarctic Voyage of H.M. Discovery Ships Erebus and Terror in the years 1839–1843, under the Command of Captain Sir James Clark Ross* (Hooker 1844) and the vouchers are preserved in herbarium of The Natural History Museum (BM).

After this period, Antarctic's vegetation raised little interest until the end of the eighteenth century and the beginning of the nineteenth century, when Jules Cardot (1860–1934) investigated the moss collections during the *Belgica* expedition (1897–1899), the largest collection of Antarctic mosses by then. Some other expeditions followed, slowly expanding our understanding of the Antarctic flora. A detailed history of early botanical explorations in Antarctica is outlined in Ochyra et al. (2008).

Currently, the largest bryophyte collection from Antarctica is deposited in the herbaria of the British Antarctic Survey (BAS) and the Australian Antarctic Division (AAD). In South America, the largest collection of bryophytes from Antarctica is located at the University of Brasilia herbarium (UB), comprising specimens recently collected during projects in the frame of the Brazilian Antarctic program (PROANTAR). The most extensive and comprehensive work on Antarctic vegetation is the 'Illustrated moss flora of Antarctica' by Ochyra et al. (2008), a masterpiece with descriptions, illustrations, keys and comments; the collections made by the author are deposited at herbarium KRAM (Institute of Botany, Polish Academy of Sciences, Kraków). Smaller collections of the Antarctic flora are present in other herbaria, for example, bryophytes collected during the Zaneveld expedition in 1964 at herbarium L of Naturalis Biodiversity Center, which were only recently identified and made accessible.

Due to the climate peculiarities of Antarctica, most of the plant diversity is present in the maritime Antarctica region, especially the South Shetland archipelago. The only two native flowering plants are limited to maritime Antarctica as well and reach south to Alexander Island. The species diversity and abundance decreases drastically with increasing latitude, and only very few mosses occur in the Continental Antarctica, which can be explained by the extreme local environmental conditions.

Even though a comprehensive moss flora for Antarctic is available (Ochyra et al. 2008), recent new collections resulted in new records and improved our knowledge about the geographical ranges of many taxa (Câmara et al. 2017, 2018; Henriques et al. 2018). Moreover, recent reports from Deception Island have revealed drastic changes in its flora composition over the last 15 years. Molecular species identification (DNA barcoding) as well as phylogenetic and phylogeographic analyses based on DNA fingerprinting and sequence data resulted in new insights into the delimitation, relationships and intraspecific genetic diversity of Antarctic mosses (Skotnicki et al. 2005; Hedenäs 2012; Biersma et al. 2017, 2018a, b; Câmara et al. 2018, 2019). Among the results are a molecular confirmation of the existence of bipolar species, taxonomic changes such as the description of a new genus and a new species of *Bartramia* in Livingstone Island (South Shetlands), previously only reported from

sub-Antarctic islands. These results indicate the continuous need of collecting and research on Antarctic bryophytes, employing an integrative taxonomic approach.

Even with limited human impact, several Antarctic Specially Protected Areas (ASPAs) were established to safeguard the vegetation (e.g. ASPAs 106, 108, 109, 112, 140), however, no critical assessment of threat level for the local flora has been done specifically for Antarctica, this is particularly important as species are still poorly known and human impact has been increasing fast, especially due to tourism. A comprehensive study on threatened species is still missing from the region, and studies like these rely largely on correct identification of species, also the levels of diversity can only be understood after phylogenetic studies, and still very few exist. In addition, maritime Antarctica, where most of the plant species occur, is among the areas in the planet that has been facing the highest increase in temperature on the planet, with unpredicted consequences for the local flora. It is expected, in the near future, that more species will reach Antarctica, so it is fundamental to fully describe and understand the current flora composition in detail, in order to better access future changes that will occur. To accomplish that, a comprehensive and reliable list of species, their status of conservation, species distribution and phylogenetic data are needed.

Bryophytes comprise one of the richest fungal microhabitats in the Antarctic environment (Yu et al. 2014), but further studies are needed to characterise the diversity, distribution and ecological roles of associated fungi in different regions (Zhang et al. 2013a, b). Lindo and Gonzalez (2010) proposed the term 'bryosphere' for the combined complex of living and dead moss tissue and associated organisms, which represent a tightly coupled system of both above- and belowground processes. The bryosphere can be thought of as similar to an inquiline system whereby mosses host a numerous and diverse community of microorganisms and invertebrates including fungi, bacteria, cyanobacteria, tardigrades, nematodes, mites and springtails (Lindo and Gonzalez 2010).

Almost all bryophyte-associated fungi were isolated from acrocarpous moss species. Of these, *Bryum argenteum* Hedw., *B. pseudotriquetrum* (Hedw.) G. Gaertn., B. Mey. & Scherb., *Ceratodon purpureus* (Hedw.) Brid., *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth., *Hennediella heimii* (Hedw.) R.H. Zander, *Polytrichastrum alpinum* (Hedw.) G.L. Sm., *Polytrichum strictum* Brid., *Schistidium antarctici* (Card.) L.I. Savicz & Smirnova, *Syntrichia magellanica* (Mont.) R.H. Zander, and *S. sarconeurum* Ochyra & R.H. Zander are common and widespread in Antarctica, whereas *Bryoerythrophyllum recurvirostrum* (Hedw.) P.C. Chen, *Campylopus pyriformis* (F. Schulz) Brid. and *Coscinodon lowianum* (J.H. Willis) Ochyra are more narrowly distributed (Ochyra et al. 2008). Studies on pleurocarpous mosses and (foliose) liverworts are much rarer, having analysed only two species each, *Sanionia uncinata* (Hedw.) Loeske and *Warnstorfia sarmentosa* (Wahlenb.) Hedenäs as well as *Cephaloziella varians* (Gottsche) Steph. and *Barbilophozia hatcheri* (A. Evans) Loeske, respectively. All studies analysing bryophyte-associated fungi are shortly discussed below, with all respective taxa listed in Table 8.1.

Table 8.1 Fungi associated with Antarctic bryophytes belonging to different habitats

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
MacRobertson lLand	*Bryum* sp.	*Cladosporium herbarum*	Fletcher et al. (1985)
Davis Mossel Lake area	*Bryum pseudotriquetrum*	*Pseudogymnoascus pannorum*	Kerry (1990)
	Bryoerythrophyllum recurvirostrum	*Phoma herbarum*	
		Phialophora fastigiata	
		Penicillium spp.	
		Mortierella spp.	
Mawson Station, between buildings	*Bryum pseudotriquetrum*	*Pseudogymnoascus pannorum*	
		Phoma herbarum	
		Epicoccum nigrum	
		Penicillium spp.	
		Mucor spp.	
		Mortierella spp.	
		Cladosporium spp.	
Mawson outskirts, icecliffs site	*Coscinodon lawianus* (as *Grimmia lawiana*)	*Thelebolus microsporus*	
		Phoma herbarum	
		Epicoccum nigrum	
		Cladosporium spp.	
Valley W of Station	*Bryum pseudotriquetrum*	*Pseudogymnoascus pannorum*	
		Phoma herbarum	
		Epicoccum nigrum	
King George Island	Unidentified moss taxa	*Phoma* sp.	Möller and Dreyfuss (1996)
		Pseudogymnoascus pannorum var. *pannorum*	
		Sterile mycelium	
		Chalara sp.	
		Chaunopycnis alba	
		Phaeoseptoria sp.	
		Phomopsis sp.	
		Mortierella gamsii	
		Thelebolus microsporus	
		Monascella sp.	
		Acremonium rutilum	
		Phialophora malorum	
		Pycnostysanus sp.	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Pseudogymnoascus pannorum var. *vinaceus*	
		Myrioconium sp.	
		Paecilomyces variotii	
		Penicillium janthinellum	
		Phialophora cf. *alba*	
		Phaeosphaeria eustoma	
		Arthroderma cf. *cuniculi*	
		Monocillium sp.	
		Penicillium brevicompactum	
		Penicillium glabrum	
		Phialophora hyaline	
		Phialophora melinii	
		Phoma sp. 1	
		Tolypocladium rubicola	
Crater Cirque	Unidentified moss taxa	*Acremonium charticola*	Zucconi et al. (1996)
		Cladosporium herbarum	
		Pseudogymnoascus pannorum var. *pannorum* no. 5	
		Phoma herbarum	
		Pink yeast no. 3	
		White yeast no. 2	
Kay Island	Unidentified moss taxa	*Acremonium strictum*	
		Arthrobotrys ferox no. 2	
		Arthrobotrys ferox no. 3	
	Unidentified moss taxa and soil under moss	*Phoma sorghina*	
		Pink yeast no. 1	
'Carezza Lake'	Unidentified moss taxa and soil under moss	*Dendryphiella salina*	
	Unidentified moss taxa	*Pseudogymnoascus pannorum* var. *pannorum* no. 3	
	Unidentified moss taxa and soil under moss	*Pseudogymnoascus pannorum* var. *pannorum* no. 6	
		Phoma sp. no. 1	
Edmonson Point	Unidentified moss taxa	*Arthrobotrys ferox*	
Baker Rocks		*Dendryphiella salina*	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
'Giardino'	Unidentified moss taxa and soil under moss	*Pseudogymnoascus pannorum* var. *pannorum* no. 2	
'Campo Icaro'		*Pseudogymnoascus pannorum* var. *pannorum* no. 4	
		Pink yeast no. 2	
		White yeast no. 1	
Gondwana Station	Unidentified moss taxa	*Phoma* sp. no. 2	
Edmonson Point		*Arthrobotrys ferox*	Fenice et al. (1997)
Kay Island		*Arthrobotrys ferox* no. 2	
		Arthrobotrys ferox no. 3	
	Unidentified moss taxa and soil under moss	*Phoma sorghina*	
	Unidentified moss taxa	*Verticillium* cf. *lecanii* no. 3	
	Unidentified moss taxa and under moss	Pink yeast no. 1	
Mt Melbourne	Unidentified moss taxa	*Chaetomium* sp. no. 2	
Crater Cirque		*Cladosporium herbarum*	
		Phoma sp. no. 4	
		Verticillium cf. *lecanii* no. 1	
Lake 'Carezza'		*Dendryphiella salina*	
		Pseudogymnoascus pannorum var. *pannorum* no. 3	
	Unidentified moss taxa and soil under moss	*Pseudogymnoascus pannorum* var. *pannorum* no. 6	
		Phoma sp. no. 1	
Baker Rocks	Unidentified moss taxa	*Dendryphiella salina*	
'Giardino'	Unidentified moss taxa and soil under moss	*Pseudogymnoascus pannorum* var. *pannorum* no. 2	
Gondwana Station	Unidentified moss taxa	*Phoma* sp. no. 2	
Vegetation Island		*Thelebolus microsporus*	
'Campo Icaro'	Unidentified moss taxa and soil under moss	*Pseudogymnoascus pannorum* var. *pannorum* no. 4	
		Pink yeast no. 2	
		White yeast no. 1	
Windmill Islands	*Bryum pseudotriquetrum*	*Arthrobotrys* sp.	Azmi and Seppelt (1998)

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Chrysosporium sp.	
		Chrysosporium pannorum	
		Pseudogymnoascus sp.	
		Mortierella sp.	
		Mortierella gamsii	
		Mycelia sterilia	
		Nectria peziza	
		Penicillium sp.	
		Penicillium chrysogenum	
		Penicillium expansum	
		Penicillium palitans	
		Phialophora malorum	
		Phoma herbarum	
		Phoma sp. no. 1	
		Thelebolus microsporus	
	Ceratodon purpureus	*Arthrobotrys* sp.	
		Chrysosporium sp.	
		Chrysosporium pannorum	
		Pseudogymnoascus sp.	
		Mycelia sterilia	
		Penicillium sp.	
		Penicillium expansum	
		Penicillium palitans	
		Phialophora malorum	
		Phoma herbarum	
		Phoma sp. no. 1	
		Phoma sp. no. 2	
		Thelebolus microsporus	
	Grimmia antarctici	*Acremonium* sp.	
		Chrysosporium sp.	
		Chrysosporium pannorum	
		Mortierella sp.	
		Mortierella gamsii	
		Mucor piriformis	
		Mycelia sterilia	
		Nectria peziza	
		Penicillium sp.	
		Penicillium chrysogenum	
		Penicillium palitans	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Phialophora malorum	
		Phoma herbarum	
		Phoma sp. no. 1	
		Phoma sp. no. 2	
		Thelebolus microsporus	
Windmill Islands/ Wilkes Land	*Schistidium antarctici* (as *Grimmia antarctici*)	*Phoma* sp.	McRe and Seppelt (1999)
		Yellow yeast	
		Trichoderma viride	
		Phoma herbarum	
		Penicillium solitum	
		Clear yeast	
		Phoma spp.	
		Mortierella minutissina	
	Bryum pseudotriquetrum	*Phoma* spp.	
		Thelebolus microsporus	
Robinson Ridge	*Schistidium antarctici* (as *Grimmia antarctici*)	*Penicillium solitum*	McRae et al. (1999)
		Penicillium palitans	
Ardery Island		*Penicillium corylophilum*	
Bailey Peninsula		*Penicillium antarcticum*	
		Penicillium palitans	
		Penicillium corylophilum	
Marble Point	*Bryum argenteum*	*Embellisia* sp. no. 2	Bradner et al. (2000)
		Penicillium sp.	
		Trichoderma sp.	
Livingston Island	Unidentified moss taxa and soil samples	*Rhodotorula minuta*	Pavlova et al. (2001)
		Rhodotorula mucilaginosa	
		Naganishia albida	
		Papiliotrema laurentii	
		Candida oleophila	
Gondwana Lake	*Bryum pseudotriquetrum*	*Cladosporium cladosporioides*	Tosi et al. (2002a, b)
		Cladosporium sphaerospermum	
		Naganishia albida	
		Papiliotrema laurentii	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Pseudogymnoascus pannorum var. *pannorum*	
		Pseudogymnoascus pannorum var. *vinaceus*	
		Mortierella antarctica	
		Penicillium minioluteum	
		Phoma herbarum	
		Rhodotorula minuta	
		Verticillium lecanii	
		White yeast	
		Mycelia sterilia	
Prior Island	*Bryum pseudotriquetrum*	*Cladosporium cladosporioides*	
		Cladosporium sphaerospermum	
		Phoma herbarum	
		Verticillium lecanii	
		White yeast	
Bruce Point	*Bryum pseudotriquetrum*	*Cladosporium cladosporioides*	
		Papiliotrema laurentii	
		Pseudogymnoascus pannorum var. *vinaceus*	
		Phoma herbarum	
		Rhodotorula minuta	
		White yeast	
Adelie Cove	*Bryum pseudotriquetrum*	*Cadophora malorum*	
		Pseudogymnoascus pannorum var. *vinaceus*	
		Mortierella antarctica	
		Phoma herbarum	
		Verticillium lecanii	
		Verticillium psalliotae	
		White yeast	
		Pink yeast	
Tinker Glacier	*Bryum pseudotriquetrum*	*Pseudogymnoascus pannorum* var. *vinaceus*	
		Mortierella antarctica	
		Phoma herbarum	
		Scolecobasidium salinum	
		White yeast	
Snowy Point	*Bryum pseudotriquetrum*	*Pseudogymnoascus pannorum* var. *vinaceus*	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Phoma herbarum	
		Mycelia sterilia	
Edmonson Point	*Ceratodon purpureus*	*Pseudogymnoascus pannorum* var. *vinaceus*	
		Phoma herbarum	
		Rhodotorula minuta	
		Thelebolus microsporus	
		White yeast	
Cape Irizar	*Syntrichia magellanica* (as *S. princeps*)	*Cladosporium cladosporioides*	
		Pseudogymnoascus pannorum var. *pannorum*	
		Phoma herbarum	
		White yeast	
		Pink yeast	
		Mycelia sterilia	
173 Camp Icare	*Syntrichia magellanica* (as *S. princeps*)	*Pseudogymnoascus pannorum* var. *vinaceus*	
		Phoma herbarum	
		White yeast	
Starr Nunatak	*Syntrichia magellanica* (as *S. princeps*)	*Cadophora malorum*	
		Cladosporium cladosporioides	
		Candida albidus	
		Pseudogymnoascus pannorum var. *pannorum*	
		Phoma herbarum	
		Pink yeast	
		Mycelia sterilia	
Cape Reynolds	*Bryum argenteum*	*Pseudogymnoascus pannorum* var. *pannorum*	
		Phoma herbarum	
		Mycelia sterilia	
Inexpressible Island	*Bryum argenteum*	*Naganishia albida*	
		Phoma herbarum	
		Zygosaccharomyces sp.	
		Mycelia sterilia	
Cape Satsrugi	*Schistidium antarctici*	*Papiliotrema laurentii*	
		Cryptococcus sp.	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Pseudogymnoascus pannorum var. *pannorum*	
		Mortierella antarctica	
		Phoma herbarum	
		Rhodotorula minuta	
		Mycelia sterilia	
Kay Island	*Syntrichia sarconeurum* (as *Sarconeurum glaciale*)	*Cadophora malorum*	
		Cladosporium cladosporioides	
		Pseudogymnoascus pannorum var. *pannorum*	
		Mortierella antarctica	
		Phoma herbarum	
		Verticillium lamellicola	
		Verticillium lecanii	
		White yeast	
Kohler Head	*Schistidium antarctici* + *Hennediella heimii*	*Cladosporium cladosporioides*	
		Cladosporium herbarum	
		Conidiobolus sp.	
		Vanrija humicola	
		Pseudogymnoascus pannorum var. *pannorum*	
		Paecilomyces farinosus	
		Penicillium sp.	
		Phoma herbarum	
		Rhodotorula minuta	
		Saccharomyces cerevisiae	
		Verticillium lecanii	
		Verticillium psalliotae	
		Zygosaccharomyces sp.	
		White yeast	
		Pink yeast	
		Mycelia sterilia	
Harrow Peaks	*Bryum pseudotriquetrum* + *Syntrichia princeps*	*Arthrobotrys superba*	
		Cadophora malorum	
		Cladosporium cladosporioides	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Cladosporium herbarum	
		Naganishia albida	
		Papiliotrema laurentii	
		Pseudogymnoascus pannorum var. *pannorum*	
		Pseudogymnoascus pannorum var. *vinaceus*	
		Mortierella antarctica	
		Phoma herbarum	
		Scolecobasidium salinum	
		Pink yeast	
		Mycelia sterilia	
Mount Melbourne	*Campylopus pyriformis*	*Aureobasidium pullulans*	
		Chaetomium gracile	
		Paecilomyces farinosus	
		Penicillium brevicompactum	
		Penicillium minioluteum	
		Saccharomyces cerevisiae	
Rothera Point, Adelaide Island	*Cephaloziella varians*	*Pseudogymnoascus pannorum*	Hughes et al. (2003)
		Mortierella parvispora	
		Phoma herbarum	
		Pythium sp.	
Fildes Region, King George Island	*Barbilophozia hatcheri*	*Hyaloscyphaceae* sp.	Zhang et al. (2013b)
		Helotiales sp. no. 1	
		Rhizoscyphus sp.	
		Annulohypoxylon sp.	
		Xylariaceae sp.	
		Hyphodiscus sp.	
		Helotiales sp. no. 2	
		Dermateaceae sp.	
		Helotiales sp. no. 3	
	Chorisodontium aciphyllum	*Hyphodiscus* sp.	
		Rhizoscyphus sp.	
		Hyaloscyphaceae sp.	
		Sporidiobolales sp.	
		Unidentified fungus	
		Helotiales sp. no. 5	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
	Sanionia uncinata	Unidentified fungus	
		Hyphodiscus sp.	
		Mrakia sp.	
		Eocronartium sp.	
		Xenopolyscytalum sp.	
		Rhodotorula sp. no. 1	
		Rhodotorula sp. no. 2	
		Helotiales sp. no. 4	
		Rhizoscyphus sp.	
		Thelebolus sp.	
		Chaetomium sp.	
		Scopulariopsis sp.	
Fildes Region, King George Island	*Chorisodontium aciphyllum*	*Cryptococcus fildesensis*	Zhang et al. (2014)
Admiralty Bay, King George Island	*Schistidium antarctici*	*Mortierella alpina*	Melo et al. (2014)
Ongul islands	Unidentified moss taxa	*Phoma herbarum*	Hirose et al. 2016
		Phaeosphaeria sp. no. 2	
		Cladophialophora minutissima	
Langhovde	Unidentified moss taxa	*Phoma herbarum*	
		Alternaria sp.	
		Phaeosphaeria sp. no. 2	
		Tetracladium sp.	
		Leotiomycetes sp. no. 1	
		Cladosporium sp.	
		Dothideomycetes sp.	
		Ascomycota sp.	
		Cladophialophora minutissima	
Breidvågnipa	Unidentified moss taxa	*Cladophialophora minutissima*	
Skarvsnes	Unidentified moss taxa	*Phoma herbarum*	
		Alternaria sp.	
		Phaeosphaeria sp. no. 2	
		Tetracladium sp.	
		Leotiomycetes sp. no. 1	
		Dothideomycetes sp.	
		Ascomycota sp.	
		Cladophialophora minutissima	
Skallen	Unidentified moss taxa	*Phoma herbarum*	

(continued)

Table 8.1 (continued)

Island/region	Bryophyte host taxon	Proposed fungal taxa	Reference
		Alternaria sp.	
		Phaeosphaeria sp. no. 2	
		Tetracladium sp.	
		Ascomycota sp.	
Riiser-Larsen	Unidentified moss taxa	*Phoma herbarum* *Cladosporium* sp. *Dothideomycetes* sp. *Cladophialophora minutissima*	
		Cladosporium sp.	
		Dothideomycetes sp.	
		Cladophialophora minutissima	
Different island of the South Shetland Islands	Unidentified moss taxa	*Candida davisiana*	Ferreira et al. (2018)
		Debaryomyces hansenii	
		Protomyces inouyei	
		Antarctomyces sp.	
		Microbotryozyma collariae	
		Bannozyma arctica	
		Cystobasidium laryngis	
		Dioszegia antarctica	
		Dioszegia hungarica	
		Filobasidium sp.	
		Filobasidium magnum	
		Holtermanniella wattica	
		Leucosporidium sp.	
		Leucosporidium creatinivorum	
		Leucosporidium fragarium	
		Mrakia frigida	
		Mrakia gelida	
		Piskurozyma fildesensis	
		Phenoliferia glacialis	
		Phenoliferia psychrophenolica	
		Rhodotorula mucilaginosa	

The first study by Fletcher et al. (1985) isolated *Cladosporium herbarum* from *Bryum* sp. from MacRobertson Land. Kerry (1990) compared the microflora of different samples of mosses (*Bryoerythrophyllum recurvirostrum*, *Bryum pseudotriquetrum* and *Coscinodon lawianus* [as *Grimmia lawiana* J.H. Willis]) from uncontaminated and contaminated sites, viz. sites within Mawson Station and from remote sites like Mossell Lake area in the Vestfold Hills and Rum Doodle in Mac Robertson Land. *Phoma herbarum* was isolated from all moss samples from Mossell Lake (Vestfold Hills) and from most moss samples from the Mawson region (station, ice cliffs, and valley sites). *Epicoccum nigrum* was isolated from all moss and algal sites at Mawson, but less frequently than *P. herbarum*.

Fenice et al. (1997) isolated 33 fungal strains recuperated from different samples, including moss in different sites located in Victoria Land (continental Antarctica). Both Azmi and Seppelt (1998) and McRe and Seppelt (1999) focused on the fungi associated with moss samples collected at Windmill Islands, Wilkes Land. In the former study, fungi were isolated from *Bryum pseudotriquetrum*, *Ceratodon purpureus,* and *Schistidium antarctici* (as *Grimmia antarctici* Cardot). Among the most frequently obtained fungal taxa were *Phoma herbarum*, *Chrysosporium* sp., *Penicillium* sp., and *Thelebolus microsporus*. The latter study evaluated the fungal diversity of *Bryum pseudotriquetrum* and *Schistidium antarctici* as well and observed that the number of fungal species recovered from the mosses was very small and *Phoma* was the most common taxon found in every moss sample. *Phoma herbarum*, *Trichoderma viride*, *Mortierella minutissima*, *Penicillium solitum,* and *T. microsporus* were the other taxa recovered. McRe et al. (1999) isolated and identified an undescribed species *Penicillium antarcticum*. Among the isolated fungi, there was one recovered from the moss *G. antarctica* collected at Bailey Peninsula; additionally, other *Penicillium* species were obtained. Bradner et al. (2000) identified a probably new fungus, *Embellisia* sp. 2, associated with leafy stems of *Bryum argenteum* collected at Marble Point in Southern Victoria Land, and two other associated fungi identified as *Penicillium* sp. and *Trichoderma* sp. were obtained.

Five yeast isolates were isolated from moss and soil samples in Livingston Island by Pavlova et al. (2001) and identified as *Rhodotorula minuta*, *Rhodotorula mucilaginosa*, *Naganishia albida*, *Papiliotrema laurentii* and *Candida oleophila*, according to their morphological, cultural and physiological characteristics. Tosi et al. (2002a, b) obtained 120 fungal isolates belonging to 18 genera recovered from the moss species *B. pseudotriquetrum*, *C. purpureus*, *Syntrichia princeps* (De Not.) Mitt., *B. argenteum*, *Schistidium antarctici* (Card.) L.I. Savicz & Smirnova, *Sarconeurum glaciale* (C. Muell) Card. & Bryhn, *Schistidium antarctici* + *Hennediella heimii* (Hedw.) R.H. Zander, *Bryum pseudotriquetrum* + *Syntrichia princeps*, and *Campylopus pyriformis* (F. Schulz) Brid. collected in Victoria Land on the West Coast of the Ross Sea. Among the isolates identified, the filamentous fungi most frequently observed were *Cladosporium cladosporioides*, *P. pannorum* var. *pannorum*, *P. pannorum* var. *vinaceus*, *Mortierella antarctica*, *P. herbarum*, *R. minuta,* and *Verticillium lecanii*; among the yeasts, *Cryptococcus* was the most frequently isolated genus.

Hughes et al. (2003) isolated the first fungi from colonies of a foliose liverwort, *Cephaloziella varians*, collected from Rothera Point, Adelaide Island, Antarctic Peninsula, which were identified as *P. pannorum*, *Mortierella parvispora*, *P. herbarum,* and *Pythium* sp. The response of fungal isolates to solar radiation and temperature was examined, and after 3 h of exposure to solar radiation at >287 nm, the hyphal extension rates of all species were reduced; however, exposure to solar radiation of >400 nm displayed no effect on hyphal growth. According to the authors, a comparison of growth response to solar radiation and temperature showed that the species that were most resistant to UV radiation grew the fastest at higher temperatures. These data suggest that solar UV-B radiation reduces the growth of fungi on the soil surface in the Antarctic terrestrial environment.

Zhang et al. (2013b) obtained 128 endophytic fungal isolates associated with 3 bryophytes species representing foliose liverworts [*Barbilophozia hatcheri* (A. Evans) Loeske], acrocarpous mosses [*Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth.] and pleurocarpous mosses [*Sanionia uncinata* (Hedw.) Loeske], collected in the Fildes Region (Fildes Peninsula, Ardley Island and adjacent islands), located in the Southwestern part of King George Island, South Shetland Islands. The isolates were identified to 21 taxa, among which were 15 *Ascomycota*, 5 *Basidiomycota* and 1 unidentified fungus. The dominant fungal endophytes were *Hyaloscyphaceae* sp. in *B. hatcheri*, *Rhizoscyphus* sp. in *C. aciphyllum* and the unidentified fungus in *S. uncinata*; their relative frequencies were 33.3, 32.1 and 80.0%, respectively. The fungal taxa isolated from *B. hatcheri*, *C. aciphyllum* and *S. uncinata* were 9, 6 and 12, respectively; only *Hyphodiscus* sp. and *Rhizoscyphus* sp. coexisted in all 3 bryophyte species. Zhang et al. (2013a) studied the endophytic fungal communities associated with three bryophyte species, the liverwort *B. hatcheri*, the mosses *C. aciphyllum* and *S. uncinata* in the Fildes Region, King George Island, using clone library analysis. A total of 680 clones belonging to 93 Operational Taxonomic Units (OTUs) were found, and according to sequence similarity and phylogenetic analysis, 78 belonged to the phylum *Ascomycota*, 13 to the phylum *Basidiomycota*, 1 to the phylum *Zygomycota* and 1 to an unknown phylum. Among the 93 OTUs, the most common orders in the Ascomycota were *Helotiales* (42 OTUs) and *Chaetothyriales* (14 OTUs). The most common orders in the *Basidiomycota* were found to be *Sebacinales* (3 OTUs) and *Platygloeales* (3 OTUs). The only order in the *Zygomycota* was *Mortierellales*, represented by one OTU.

Yu et al. (2014) recovered endophytic fungi of 31 morphotypes from bryophyte tissues of a wide range of bryophyte species, including *Andreaea* sp., *B. hatcheri*, *C. aciphyllum*, *Polytrichastrum alpinum*, *Polytrichum strictum*, *S. uncinata* and *Warnstorfia sarmentosa* collected in Barton Peninsula, King George Island. The fungal isolates were categorised into 16 groups through ITS sequence analysis (Internal transcribed spacer) and belonged to the phyla *Ascomycota* (12), *Basidiomycota* (1), *Oomycota* (1), and *Zygomycota* (2). Among these endophytes, the genomic sequences of isolates EFOMIA 02, 04 and 15 were 100% homologous with those of *Lecythophora hoffmannii* (GenBank code AB231012), Fungal sp. (HM123665) and *Antarctomyces psychrotophicus* (AM489755), respectively, retrieved from the GenBank database. The genomic sequence of isolate EFOMIA

05 was 96% homologous with that of *Massarina rubi* (AF383963), the genomic sequence of EFOMIA 09 was 97% homologous with that of *M. parvispora* (EU484297), the genomic sequence of EFOMIA 10 was 98% homologous with Fungal sp. (EU240043) and the genomic sequences of EFOMIA 08, 12 and 13 were 99% homologous with those of *Pseudogymnoascus* sp. (JX171173), *Coniochaeta ligniaria* (AY198390) and *Pythium* sp. (AB299389), respectively. Among the remaining isolates, the genomic sequences of EFOMIA 01, 03, 06, 07, 11, 14 and 16 exhibited similarity to those of uncultured fungi retrieved from the GenBank database. The temperature preferences of 15 fungal endophytes were also evaluated after the isolates were incubated at 4, 10, 15, 25, and 30 °C for 1 month. The authors concluded that growth rate measurements in a wide range of temperatures confirmed that most of the fungal strains were both mesophilic and psychrotolerant (Yu et al. 2014).

Zhang et al. (2014) investigated the fungal diversity associated with the liverwort *B. hatcheri* and the mosses *C. aciphyllum* and *S. uncinata* collected in the Fildes Region, King George Island. Twenty-nine yeasts were isolated with the majority belonging to known species including *Rhodotorula psychrophenolica*, *Mrakia psychrophilia* and *Mrakia robertii*. However, two of them, each isolated from a different sample of moss, belonged to a novel species identified as *Cryptococcus fildesensis*, a psychrophilic basidiomycetous yeast recovered from the moss *Chorisodontium aciphyllum*.

Melo et al. (2014) observed the growth of the fungus (strain ITA1-CCMA 952, identified by ITS sequence comparison as *Mortierella alpina*) from the tissues of the moss *S. antarctici* found in Admiralty Bay, King George Island. Tests showed that the isolate exhibited a broad optimal growth temperature range of 15–25 °C and produced essential fatty acids including high amounts of polyunsaturated fatty acids (PUFAs), such as y-linolenic, arachidonic and oleic acids. The crude extract from the isolate demonstrated a strong antioxidant activity with an EC_{50} value of 48.7 μg mL^{-1} and antibacterial activities against *Escherichia coli* with an MIC of 26.9 μg mL^{-1}; against *Pseudomonas aeruginosa* and *Enterococcus faecalis* with an MIC of 107 μg mL^{-1}; and against *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Salmonella typhi* with an MIC of 215.3 μg mL^{-1}. Chemical investigations of the extract indicated the presence of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), demonstrating that the isolate is a promising candidate for the biotechnological production of antibiotics, antioxidant substances and PUFAs.

Hirose et al. (2016) collected 205 unidentified moss samples from 41 locations in 6 ice-free regions: 5 regions in the Lützow-Holm Bay area (East Ongul Island, Langhovde, Skallen, Skarvsnes and Breidvågnipa) and 1 in the Mt. Riiser-Larsen region of the Amundsen Bay area, approximately 500 km from the Lützow-Holm Bay area, in East Antarctica. The authors recovered 289 fungal isolates, which were classified into 23 molecular taxonomic units (MOTUs). The most frequent MOTUs were *P. herbarum* (70%), *Alternaria* sp. (20%), *Phaeosphaeria* sp. 2 (13%) and *Tetracladium* sp. (5%), and the less frequent ones were *Leotiomycetes* sp., *Pleosporales* sp., *Phaeosphaeria* sp., *Cosmospora* sp., and *Helotiales* sp. The major

fungal MOTUs were six ice-free coastal outcrops. *P. herbarum* was found to be significantly more strongly associated with the *Bryum pseudotriquetrum*/*Bryum archangelicum* Bruch & Schimp. complex and *C. purpureus*. *Alternaria* sp. was more strongly associated with the *B. pseudotriquetrum*/*B. archangelicum* complex than with the other moss species. *Tetracladium* sp. was significantly more strongly associated with *H. heimii*. *Cladophialophora minutissima* was less frequent at higher altitudes and had a significantly strong association with *C. purpureus* (Hirose et al. 2016).

Ferreira et al. (2018) determined the diversity of epiphytic yeasts in 75 samples of unidentified bryophytes collected in 18 different sampling points of the Antarctic Peninsula. Ninety-two epiphytic yeasts were isolated that belonged to 22 distinct yeast species isolated, of which the majority belonged to the *Basidiomycota*, and *Vishniacozyma victoriae* and *Debaryomyces hansenii* were the most frequent.

8.3 Rings on Antarctic Mosses

According to Fenton et al. (1983), patches of some Antarctic moss species can show patterns of concentric arcs or rings on their surfaces, which can be up to 5 m in diameter. They are supposedly caused by fungal infection that spreads outwards from an initial infection point, advances as continuous concentric lines and infects the moss. These radial fungal advances can result in a circular 'fairy ring', although usually the ring is broken and the infection spreads in the shape of an arc (Fig. 8.2). Fenton et al. (1983) studied the rate of fungal advance on the moss *Chorisodontium aciphyllum* (Hook. f. & Wilson) Broth. between 1974 and 1976 at Signy Island in the South Orkney Islands, where they established photo-quadrats in the selected sites in which the rate of advancement was monitored, and the authors observed that more than one infection band was produced per year. Microscopic examination of the infected moss samples revealed that the ascomycetes species *Coleroa turfosorum*, *Bryosphaeria megaspora* and *Epibryon chorisodontii* were associated with the rings.

Fig. 8.2 Concentric fungal rings on *Sanionia uncinata*: (**a**) complete rings; (**b**) detailed part of the ring. (Photos Credits: LH Rosa)

Additionally, Tojo et al. (2012) identified a new species *Pythium polare* (*Oomycota*) isolated from brown-discoloured stem leaves of *Sanionia uncinata* collected at King George Island, South Shetland Islands. Pawłowska et al. (2017) re-examined, re-described and compared the holotype of *Thyronectria hyperantarctica* with new collections of specimens of *Bryum* sp. with visible moribund rings collected in between 2014 and 2015 from three localities on King George Island. Phylogenetic analysis of the epitype revealed that its closest phylogenetic affinity was with the fungus family *Tilachlidiaceae* and it formed a monophyletic group in this lineage with other species. Therefore, the new monotypic fungal genus *Psychronectria* was described to accommodate *T. hyperantarctica*. *Psychronectria hyperantarctica* was also described for the first time, which is responsible for moribund ring formation in Antarctic moss mats. However, as these studies reported different fungi as supposed phytopathogenic agents of the 'fairy ring', further detailed studies will be necessary to clarify which fungal species are responsible for the disease and affect which mosses across the Antarctic Peninsula.

8.4 Fungi Associated with the Vascular Plants *Deschampsia antarctica* and *Colobanthus quitensis*

Once it was discovered that the two angiosperms [*Colobanthus quitensis* (Fig. 8.3a) and *Deschampsia antarctica* (Fig. 8.3b)] found in Antarctica shelter communities of endophytic, saprobic and parasitic fungi were used as models to study the ecology and evolution of this community (Rosa et al. 2009; Rosa et al. 2010) and to study the dispersal of these communities (Santiago et al. 2017). Such studies can also help reveal the method of speciation in fungal webs found in Antarctica and the effect of climate change on the Antarctic biota (Santiago et al. 2015). These plants can also be used to find interesting, extremophilic endophytic fungi (Rosa et al. 2009).

Fig. 8.3 (**a**) *Colobanthus quitensis*; (**b**) *Deschampsia antarctica*. (Photos Credits: LH Rosa)

The distribution of vascular plants in Antarctica is relatively low (Peat et al. 2007; Lopez-Bueno et al. 2009), and the flora is dominated by basal plants (Convey et al. 2000). These plants can survive under extreme conditions of low temperature and water availability, elevated UV-B radiation levels, destructively strong winds, poorly developed soils with low organic matter and nutrient contents as well as irregular nutrient distribution and freeze-thaw events (Convey et al. 2014). As the major part of the continent is permanently covered with ice or snow, about 0.3% of the Antarctic land area is available for plant colonisation; such areas are mainly located along the Antarctic coast and on the islands associated with Antarctica, such as the South Shetland Islands (Convey et al. 2009).

Both plants display physiological, biochemical and anatomical adaptations which allow them to survive there (Alberdi et al. 2002; Bravo and Griffith 2005; Ruhland and Krna 2010; Kellmann-Sopyła and Giełwanowska 2015) providing protection against prolonged periods of freezing (Snider et al. 2000; Alberdi et al. 2002) and desiccation (Malinowski and Beleski 2000). Temperature and water availability are the most important factors of the biology of Antarctic terrestrial organisms (Chwedorzewska 2009). The plants employ different strategies to resist cold temperatures (Bravo et al. 2001). *Deschampsia antarctica* is considered a highly frost-tolerant plant, able to tolerate freezing, resisting temperatures as low as −26.6 °C, while *C. quitensis* is considered a supercooling plant, not able to tolerate freezing, avoiding it, resisting until −9.4 °C (Bravo et al. 2001; Alberdi et al. 2002).

There are a few studies that have reported the association of the endophytic and related fungi community to both angiosperms of Antarctica. Concerning the fungal assemblages of *C. quitensis*, there are three published works by Moller and Dreyfuss (1996), Rosa et al. (2010) and Santiago et al. (2017), while for *D. antarctica* there are five published works: Moller and Dreyfuss (1996), Rosa et al. (2009), Upson et al. (2009), Santiago et al. (2012) and Santiago et al. (2017). We have tried to produce a thorough inventory of endophytic and associated fungi which are linked with the two angiosperms from Antarctica (Table 8.2).

Moller and Dreyfuss (1996) recovered and identified 20 fungal species from *D. antarctica* and *C. quitensis* identified as *Phoma* sp., *Phaeoseptoria* sp., *Phaeosphaeria microscopica*, *Volucrispora graminea*, *Phialophora malorum*, *Chromelosporium ollare,* and *Fusarium* sp. All of them were considered cosmopolitan species, of which *Phaeoseptoria* sp., *P. malorum* and *Fusarium* sp. were already described as phytopathogens, and *P. microscopica* was described as an endophyte. The most frequently isolated species (8%) were *Phoma* sp. II and *Phaeoseptoria* sp.

Most *D. antarctica* endophytic fungi were recovered from its leaves (Fig. 8.4). Rosa et al. (2009) obtained 26 fungal isolates from the leaves of 91 plant specimens. Only the genera *Alternaria* and *Phaeosphaeria* were identified and both were described as phytopathogens. *Alternaria* made up 57.7% of the total fungi and was, thus, the most abundant.

Upson et al. (2009) obtained 243 fungal isolates from roots or rhizosphere of *D. antarctica* and *C. quitensis*. *Oculimacula* sp. and *Hydrocina chaetocladia* were recovered from roots; while *Sarcosomataceae* sp., *Hymenoscyphus ericae* aggre-

Table 8.2 Fungi associated with *Colobanthus quitensis* and *Deschampsia antarctica* from different sites of Antarctica

Island/region	Host	Proposed fungal taxa	Reference
King George Island	Leaves of *Deschampsia antarctica* and *Colobanthus quitensis*	*Phoma* sp. II	Moller and Dreyfuss (1996)
		Phaeoseptoria sp.	
		Phaeosphaeria microscopica	
		Volucrispora graminea	
		Phialophora malorum	
		Chromelosporium ollare	
		Fusarium sp.	
	Leaves of *D. antarctica*	*Alternaria* sp. 1	Rosa et al. (2009)
		Alternaria sp. 2	
		Alternaria sp. 3	
		Alternaria sp. 4	
		Alternaria sp. 5	
		Alternaria sp. 6	
		Alternaria sp. 7	
		Phaeosphaeria sp. 1	
		Phaeosphaeria sp. 2	
		Phaeosphaeria sp. 3	
		Phaeosphaeria sp. 4	
		Phaeosphaeria sp. 5	
King George Island	Rhizosphere of *D. antarctica*	*Sarcosomataceae* sp.	Upson et al. (2009)
		Hymenoscyphus ericae aggregate	
South George	Rhizosphere of *D. antarctica*	*Gyoerffyella* sp.	
		Leptodontidium orchidicola	
Léonie Island	Rhizosphere of *D. antarctica*	*Polyscytalum pustulans*	
Signy Island	Rhizosphere of *D. antarctica*	Ericoid endophyte	
		Mollisia minutella	
		Oculimacula yallundae	
Livingston Island	Roots of *D. antarctica*	Uncultured fungus clone	
Coronation Island	Roots of *D. antarctica*	*Hydrocina chaetocladia*	
King George Island	Leaves of *C. quitensis*	*Aspergillus reptans*	Rosa et al. (2010)
		Cadophora luteo-olivacea	
		Davidiella tassiana	
		Entrophospora sp.	

(continued)

Table 8.2 (continued)

Island/region	Host	Proposed fungal taxa	Reference
		Fusarium proliferatum	
		Pseudogymnoascus pannorum	
		Microdochium phragmitis	
		Mycocentrospora sp.	
		Phaeosphaeria sp.	
		Entrophospora sp.	
		Vishniacozyma victoriae	
		Rhodotorula mucilaginosa	
		Sporidiobolus ruineniae	
King George Island	Leaves of *D. antarctica*	*Alternaria* sp. 1	Santiago et al. (2012)
		Alternaria sp. 2	
		Alternaria sp. 3	
		Alternaria sp. 4	
		Antarctomyces psychrotrophicus	
		Cadophora luteo-olivacea	
		Helgardia sp.	
		Herpotrichia sp.	
		Oculimacula sp.	
		Phaeosphaeria herpotrichoides	
		Phaeosphaeria sp. 1	
		Phaeosphaeria sp. 2	
		Phaeosphaeria sp. 3	
		Phaeosphaeria sp. 4	
		Phaeosphaeria sp. 5	
		Phaeosphaeria sp. 6	
		Phaeosphaeria sp. 7	
King George Island	Leaves of *D. antarctica*	*Vishniacozyma victoriae*	Santiago et al. (2017)
		Cystobasidium. laryngis	
	Leaves of *C. quitensis*	*Leucosporidium* aff. *golubevii*	

gate, *Gyoerffyella* sp., *Leptodontidium orchidicola*, *Polyscytalum*, Ericoid endophyte, and *Mollisia minutella* were obtained from rhizosphere.

One hundred and eighty-eight fungal isolates were obtained from leaves of *C. quitensis* at King George Island (Rosa et al. 2010), represented by species such as *Aspergillus reptans*, *Davidiella tassiana*, *Entrophospora* sp., *Fusarium proliferatum*, *Microdochium phragmitis*, *Mycocentrospora* sp., *Phaeosphaeria microscopica*, *Cadophora luteo-olivacea,* and *Pseudogymnoascus pannorum*. *Davidiella tassiana*, *Entrophospora* sp., *P. microscopica* and *C. luteo-olivacea* have already

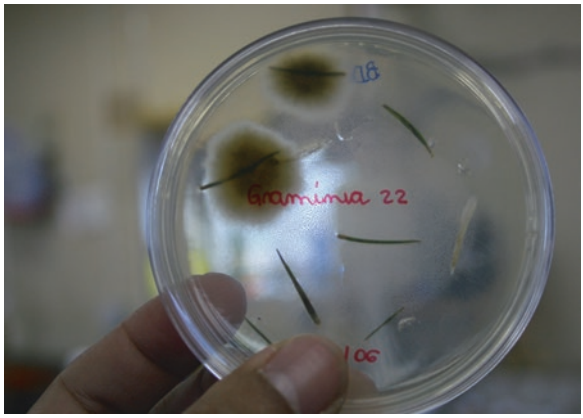

Fig. 8.4 Endophytic fungus in leaves of *Deschampsia antarctica*. (Photo Credits: LH Rosa)

been identified as endophytic fungi, and *F. proliferatum*, *M. phragmitis* and *Mycocentrospora* sp. were described as phytopathogens. The most prevalent species found in this study was *D. tassiana* and it had an abundance of 20.2%.

Santiago et al. (2012) obtained 313 fungal isolates from *D. antarctica*, at the King George Island, and identified the cosmopolitan taxa *Alternaria* sp. 1, *Helgardia* sp., *Herpotrichia* sp., *Oculimacula* sp. and *Phaeosphaeria herpotrichoides*. Only two endemic species were isolated, *Antarctomyces psychrotrophicus* and *C. luteo-olivacea*. Santiago et al. (2017) reported that 80 endophytic yeasts were obtained from leaves of 60 specimens of *C. quitensis* identified as *Vishniacozyma victoriae* (most abundant), *Rhodotorula mucilaginosa*, *Sporidiobolus ruineniae* and *Leucosporidium* aff. *golubevii*, all representing cosmopolitan taxa. Twenty-eight isolates were recovered and identified from leaves of 56 specimens of *D. antarctica*, identified as *Vishniacozyma victoriae* (most abundant) and *Cystobasidium larynges.*

8.5 Non-lichenised Fungi Associated with Thalli of Antarctic Lichens

Lichens represent fungi in a lichenised state, or technically called lichenised fungi (Pereira et al. 2007; Victoria et al. 2013; Cao et al. 2017). Lichens cover large areas of Antarctica and are the pioneer organisms growing in harsh environments (Poelking et al. 2014) and probably are the major contributors to biomass and diversity (Domaschke et al. 2012). Lichens can be considered as organisms with distinctive characteristics obtained by the combination of fungal adaptation abilities and high algae productivity. The characteristics unique to these organisms are their ability to develop certain protective mechanisms, adaptation to temperature and radiation and desiccation survival (Fernandes et al. 2014). Additionally, González et al. (2018) suggested that the lichen species *Stereocaulon alpinum*, *Sphaerophorus*

globosus, *Leptogium puberulum,* and *Cladonia* sp. from Livingston Island, Antarctica, were associated with the microbial abundance and soil functioning in the region, which may be responsible for the changes in distribution of key species linked to climate change.

Few studies have demonstrated the association of non-lichenised fungi with the thalli of lichens in Antarctica (Table 8.3). Among them, Möller and Dreyfuss (1996) reported the presence of non-lichenised fungi from 14 Antarctic lichens, represented

Table 8.3 Non-lichenised fungi associated with lichen thalli from Antarctica

Island/Region	Host	Proposed fungal taxa	Reference
King George Island	*Usnea antarctica*, *Usnea aurantiaco-atra*, *Caloplaca regalis*, *Xanthoria elegans,* and *Ramalina terebrata*	*Phoma* sp. II	Möller and Dreyfuss (1996)
		Pseudogymnoascus pannorum	
		Chalara sp.	
		Chaunopycnis alba	
		Cylindrocarpon sp.	
		Phomopsis sp.	
		Mortierella gamsii	
		Phaeosphaeria microscopica	
		Thelebolus microspores	
		Cladosporium herbarum	
		Monascella sp.	
		Acremonium rutilum	
		Chalara constricta	
		Volucrispora graminea	
		Phialophora malorum	
		Pycnostysanus sp.	
		Camarosporium sp. I	
		Libertella sp.	
		Myrioconium sp.	
		Phialophora cf. *alba*	
		Scytalidium sp.	
		Chaetomium globosum	
		Phaeosphaeria eustoma	
		Acremonium butyri	
		Acremonium cerealis	
		Acremonium psychrophilum	
		Alternaria alternata	
		Alternaria cf. *chlamydospora*	

(continued)

Table 8.3 (continued)

Island/Region	Host	Proposed fungal taxa	Reference
		Ascochyta sp.	
		Camarosporium sp. II	
		Camarosporium sp. III	
		Chaunopycnis ovalispora	
		Cladosporium sp.	
		Epicoccum nigrum	
		Exophiala sp.	
		Lichenoconium cf. *xanthoriae*	
		Myceliophthora sp.	
		Ovadendron sulfureo-ochraceum	
		Tricellula cf. *aquatica*	
		Trichocladium opacum	
		Trichurus spiralis	
		Thelebolus sp.	
Southern and Northern Victoria Island	*Lecanora fuscobrunnea*	Unknown black fungus	Selbmann et al. (2013)
	Usnea antarctica	*Elasticomyces elasticus*	
	Lecanora sp.	*Elasticomyces elasticus*	
	Lecanora fuscobrunnea	*Elasticomyces elasticus*	
	Lecidea cancriformis	*Friedmanniomyces endolithicus*	
	Xanthoria elegans	Unknown black fungus	
	Xanthoria elegans	Unknown black fungus	
	Umbilicaria decussata	Unknown black fungus	
	Rhizocarpon sp.	Unknown black fungus	
	Lecidea sp.	Unknown black fungus	
	Acarospora flavocordia	Unknown black fungus	
Elephant Island	*Usnea antarctica*	*Antarctomyces psychrotrophicus*	Santiago et al. (2015)
		Bensingtonia yamatoana	
		Candida parapsilosis	
		Cryptococcus aquaticus	
		Naganishia friedmannii	
		Goffeauzyma gilvescens	
		Tremella sp.	
		Solicoccozyma terricola	
		Vishniacozyma victoriae	
		Debaryomyces hansenii	
		Fusarium sp. 1	
		Fusarium cf. *globosum*	

(continued)

Table 8.3 (continued)

Island/Region	Host	Proposed fungal taxa	Reference
		Holtermanniella watticus	
		Leucosporidiella creatinivora	
		Mortierella sp.	
		Penicillium sp. 1	
		Penicillium chrysogenum	
		Penicillium sp. 2	
		Pseudogymnoascus sp. 7	
		Pseudogymnoascus sp. 1	
		Pseudogymnoascus sp. 10	
		Pseudogymnoascus sp. 9	
		Pseudogymnoascus sp. 5	
		Rhodotorula laryngis	
		Rhodotorula mucilaginosa	
		Thelebolus sp.	
		Yarrowia lipolytica	
King George Island	*Usnea aurantiaco-atra*	*Leptosphaeriaceae* sp.	
		Bensingtonia yamatoana	
		Candida davisiana	
		Cladosporium sp. 1	
		Cladosporium sp. 2	
		Vishniacozyma victoriae	
		Debaryomyces hansenii	
		Fusarium sp. 3	
		Pseudogymnoascus sp. 3	
		Pseudogymnoascus sp. 4	
		Pseudogymnoascus sp. 8	
		Pseudogymnoascus sp. 12	
		Mrakia frigida	
		Purpureocillium lilacinum	
		Penicillium sp. 3	
		Penicillium crustosum	
		Penicillium sp. 4	
		Penicillium sp. 5	
		Thelebolus sp. 1	
		Thelebolus sp. 2	
Deception Island	*Usnea antarctica*	*Antarctomyces psychrotrophicus*	
		Bensingtonia yamatoana	
		Capnobotryella sp.	
		Cladosporium sp. 1	

(continued)

Table 8.3 (continued)

Island/Region	Host	Proposed fungal taxa	Reference
		Cryptococcus foliicola	
		Vishniacozyma victoriae	
		Catenulostroma sp.	
		Debaryomyces hansenii	
		Fusarium sp. 4	
		Fusarium cf. *globosum*	
		Pseudogymnoascus sp. 1	
		Pseudogymnoascus sp. 3	
		Pseudogymnoascus sp. 4	
		Pseudogymnoascus sp. 5	
		Pseudogymnoascus sp. 6	
		Pseudogymnoascus sp. 11	
		Pseudogymnoascus sp. 12	
		Mrakia frigida	
		Penicillium sp. 6	
		Capnodiales sp.	
		Pseudogymnoascus sp. 2	
		Rhodotorula arctica	
		Rhodotorula creatinivora	
		Rhodotorula laryngis	
		Rhodotorula mucilaginosa	
		Thelebolus sp.	
		Thelebolus cf. *globosus*	
Deception Island	*Usnea antarctica*	*Cryptococcus victoriae*	Duarte et al. (2016)
		Rhodotorula laryngis	
Livingston Island	*Ramalina terebrata*	*Cryptococcus victoriae*	
		Mrakia sp.	
		Leucosporidiella fragaria	
		Rhodotorula larynges	
		Rhodotorula glacialis	
		Rhodotorula sp. 1	
	Usnea aurantiaco-atra	*Cryptococcus victoriae*	
		Rhodotorula laryngis	
Half Moon Island	*Ramalina terebrata*	*Cryptococcus victoriae*	
		Holtermanniella watticus	
		Rhodotorula larynges	
	Usnea aurantiaco-atra	*Cryptococcus victoriae*	
		Mrakia sp.	

(continued)

Table 8.3 (continued)

Island/Region	Host	Proposed fungal taxa	Reference
		Rhodotorula arctica	
		Rhodotorula glacialis	
King George Island	*Usnea aurantiaco-atra*	*Cryptococcus victoriae*	
		Holtermanniella nyarrowii	
		Mrakia sp.	
		Rhodotorula larynges	
	Usnea antarctica	*Cryptococcus fildesensis*	
		Rhodotorula larynges	
	Ramalina terebrata	*Cryptococcus victoriae*	
		Cryptococcus sp. 2	
		Mrakiella aquatica	
		Rhodotorula larynges	
		Rhodotorula arctica	
Penguin Island	*Usnea aurantiaco-atra*	*Leotiaceae* sp. 1	
		Sarcinomyces sp. 1	
		Cryptococcus antarcticus	
		Cryptococcus victoriae	
		Cryptococcus fildesensis	
		Mrakia sp.	
		Rhodotorula larynges	
		Rhodotorula glacialis	
	Usnea antarctica	*Sarcinomyces* sp. 1	
		Cryptococcus victoriae	
		Cryptococcus sp. 3	
		Mrakia sp.	
		Rhodotorula larynges	
		Rhodotorula arctica	
		Rhodotorula sp. 2	
Nelson Island	*Usnea aurantiaco-atra*	*Cryptococcus victoriae*	
		Tremella indecorata	
		Rhodotorula arctica	

by 89 non-lichenised fungal taxa of different genera. The most abundant taxa identified were *Phoma* sp., *Pseudogymnoascus* sp., *Chalara* sp., and *Phaeoseptonia* sp. However, the authors identified the fungi recovered using only morphological taxonomy techniques, which may have underestimated the diversity of the species obtained. A report by Selbmann et al. (2013) was the first study which reported non-lichenised fungi associated with lichen thalli and isolated 13 fungal isolates from 16 lichen samples, collected from seven sites in North and South Victoria in Antarctica. The isolates were identified in nine unknown black fungi, three *Elasticomyces elasticus* and one *Friedmanniomyces endolithicus*.

Fig. 8.5 Thalli of the lichen *Usnea antarctica* that was studied for its non-lichenised fungal assemblages and proposed to be a lichensphere, a protected natural microhabitat for the non-lichenised fungal assemblages living in extreme environments of Antarctica (Santiago et al. 2015). (Photo Credits: LH Rosa)

The study of the association of non-lichenised fungi with *Usnea antarctica* (Fig. 8.5) and *Usnea aurantiaco-atra*, in Antarctica, was performed by Santiago et al. (2015), who obtained rich and diverse fungal assemblages. The most abundant non-lichenised fungi they found were the taxa *Pseudogymnoascus* sp., *Thelebolus* sp., *Antarctomyces psychrotrophicus,* and *Vishniacozyma victoriae*, which were considered endemic and/or highly adapted to environments of Antarctica. Additionally, with these results, Santiago et al. (2015) suggested that lichen thalli represent a hotspot of non-lichenised fungal diversity, called lichensphere, that may provide a natural microhabitat protected with conditions capable of helping non-lichenised fungi and other forms of Antarctic life to survive and disperse in the extreme environments of Antarctica.

The yeasts associated with Antarctic lichens were also described by Duarte et al. (2016). In their study, 200 yeasts were recovered from lichens (69 isolates from *Ramalina terebrata*, 87 from *Usnea aurantiaco-atra* and 44 from *Usnea antarctica*) in 6 South Shetland Islands, and among these isolates, 18 taxa were identified. The most abundant yeast isolated in this study was *Vishniacozyma victoriae*, followed by *Rhodotorula laryngis*.

8.6 Conclusion and Perspectives

Plants and lichens present in different regions of Antarctica probably shelter the most rich and diverse Antarctic fungal assemblages and comprise an important component of the Antarctic ecosystems. Antarctic bryophytes, vascular plants and lichens are reservoirs of cosmopolitan, cold-adapted and endemic fungi. They represent microhabitats that are hotspots for fungal diversity. These fungi mainly include mosses and lichens, which aid in the survival of Antarctic plants and lichens. Additionally, the fungi associated with plants and lichens are essential to the Antarctic ecosystem as they control most of the biological flux of carbon, nutrients and energy under the extreme conditions prevalent in the region.

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Chapter 9
Fungus-Invertebrate Interactions in Antarctica

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9.1 Introduction

Antarctica (Fig. 9.1) is characterised by its extreme isolation from other continents and lack of ice-free areas, which have a clear influence on its biodiversity. The intense glaciation and glacial cycling from the Miocene to Pleistocene time periods, combined with the isolated nature of this continent, reduce the possibility of refugia, contributing to the reduced diversity and distribution of terrestrial and freshwater organisms (microbial, plant and animal life alike) (Convey 2017; Convey et al. 2018). This is a major contrast with the Arctic, which had easier routes of terrestrial recolonisation due to its continuous contact with the landmasses of North America and Eurasia. Antarctic marine habitats, while much more extensive and biodiverse, have been isolated from lower latitudes over multimillion-year timescales, since the formation of the Antarctic Circumpolar Current (Clarke et al. 2005; Barnes et al. 2006; Fraser et al. 2017).

The marine diversity of the Southern Ocean that surrounds the continent of Antarctica and its outlying sub-Antarctic island groups has received considerable research attention (CAML 2005–2010; De Broyer et al. 2014). However, even though often perceived simply as 'white rocks' or frozen lakes/rivers devoid of life, the terrestrial habitats of the Antarctic also hide a complex network of biogeographic domains and unique and often endemic biodiversity, spread out across three main areas, the sub-, maritime, and continental (or frigid) Antarctic, and currently 16 'Antarctic Conservation Biogeographic Regions' (ACBRs) (Pugh and Convey

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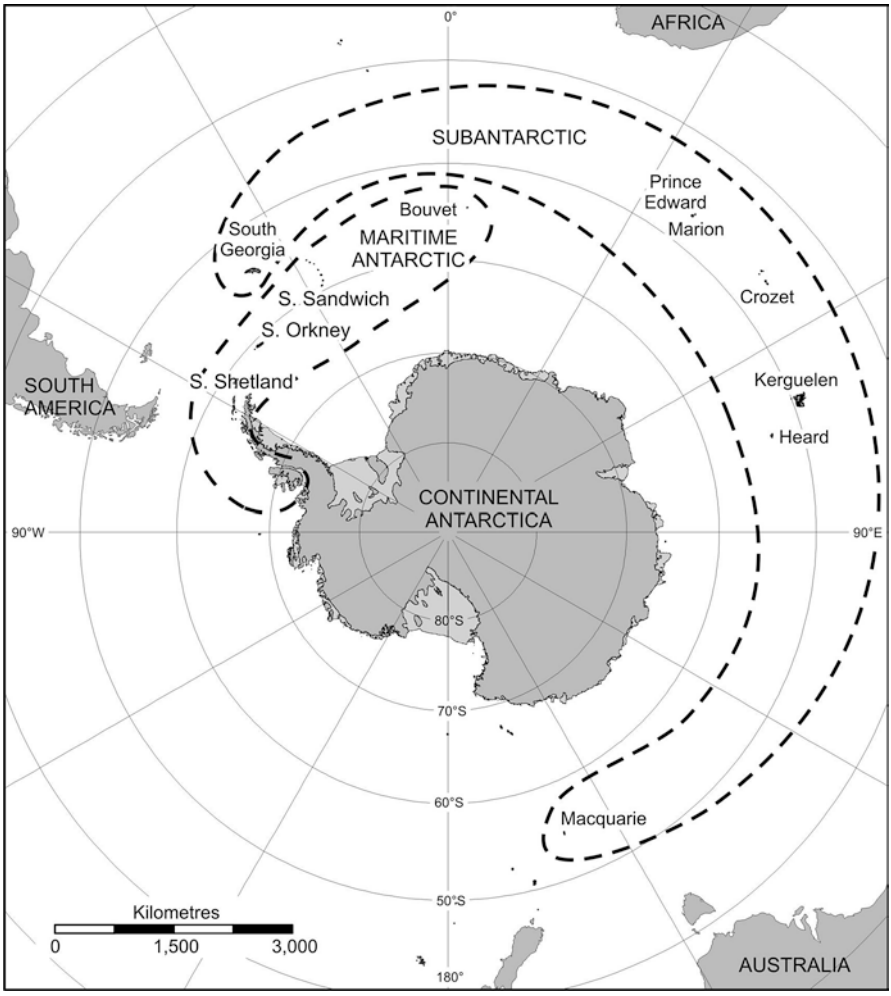

Fig. 9.1 The biogeographic regions of Antarctica (Convey 2017)

2008; Convey et al. 2008; Terauds et al. 2012; Chown and Convey 2016; Terauds and Lee 2016; Convey 2017). Most of the known Antarctic terrestrial biodiversity is found in fragmented and island-like areas of seasonally ice- and snow-free habitats in coastal regions, especially along the Antarctic Peninsula and continental coastline, and in the major mountain ranges inland (although the individual largest ice-free areas are formed by the McMurdo Dry Valleys of southern Victoria Land). Convey (2017) provided an overview of the terrestrial biogeography and biodiversity of the Antarctic and the natural and human-induced processes affecting its ecosystems.

Although it mostly reflects a history of long-term isolation, the invertebrate fauna of the Antarctic is also limited by the continent's extreme environmental conditions

(Convey 2017), and contemporary diversity is limited to extremophiles with the appropriate ecophysiological adaptations. The continent's current terrestrial fauna consists of nematodes, insects (only two species of dipterans on the continent), springtails, tardigrades, mites, and rotifers; other taxa such as molluscs, spiders and earthworms and a wider diversity of insect groups can be found in the less extreme sub-Antarctic regions (see Convey and Lebouvier 2009, Chown and Convey 2016 and Convey 2017, for reviews). Antarctic marine organisms are far more diverse (De Broyer et al. 2014); however, far less is known about marine fungi and their interactions with other taxa.

A wide variety of invertebrates has been studied in detail (terrestrial microarthropods, the krill *Euphausia superba* (Fig. 9.2c), the flightless midge *Belgica antarctica* (Fig. 9.3d) and various marine invertebrates being amongst the best-known examples (see, e.g. Block et al. 2009; Chown and Convey 2016; Cui et al. 2016; Everatt et al. 2014; Peck 2018) in terms of their physiology, adaptation, evolution and ecology. However, very few studies have addressed the interactions between fungi and Antarctic invertebrates. Indeed, Cui et al. (2016) highlighted the complete lack of previous studies regarding the diversity and ecological function of microorganisms associated with *E. superba*; this has resulted in a striking gap of knowledge given that krill are widely known as a key species in the Antarctic marine trophic web.

In this chapter, we review the limited available literature on the associations of invertebrates and fungi across the different environments of Antarctica and the

Fig. 9.2 Examples of Antarctic marine invertebrates associated with fungi. (**a**) *Ophiuroidea* sp., (**b**) Echinodermata, (**c**) *Euphausia superba*, (**d**) *Glyptonotus antarcticus*, and (**e**) *Nacella concinna*. (Photos credits: FM Pellizari)

Fig. 9.3 (**a**) Example of one of the few habitats of terrestrial invertebrates in Antarctica, with mosses and Acari (black spots) (King George Island, South Shetland Islands), (**b**) two Acari species: on the right (brown) *Gamasellus racovitzai* and on the left (black) *Alaskozetes antarcticus*, (**c**) *Cryptopygus antarcticus*, and (**d**) *Belgica antarctica*. (Photos credits: EM Biersma)

diversity involved in these associations, and discuss how interactions may be affected by impacts such as climate change, invasive species and increasing human presence and activity in the region. Research to date has largely focused on terrestrial ecosystems, and hence most examples described in this chapter are drawn from terrestrial studies, although we provide links to marine studies where possible.

9.1.1 Antarctic Terrestrial Biota

In the maritime and continental Antarctic, with the exceptions of steep cliffs and exposed mountain ridges, most terrestrial habitats are covered seasonally by snow and/or ice, providing organisms protection from extreme temperature variations and wind abrasion (Convey et al. 2018). While habitats in the maritime and continental zones may be free of seasonal snow cover for periods ranging from only days or weeks to even 5 months, many sub-Antarctic islands experience only intermittent snow cover, which is often restricted to higher altitudes. Even on the coldest sub-Antarctic islands, subnivean microhabitat temperatures are often sufficient to allow year-round biological activity (Convey 1996a). This is not the case in the maritime and continental zones, where biological processes are arrested by low winter temperatures (Walton 1984).

Antarctic soils are typically poorly developed, with low organic content (Beyer and Bölter, 2002). Formation, development and stability of soils are heavily influenced by cryoturbation (Thomas et al. 2008). There is a clear dichotomy between the sub-Antarctic and the other two zones, with only the latter possessing a widespread permafrost layer. Brown soils are associated only with the larger stands of flowering plants in the maritime Antarctic but are more widespread in the sub-Antarctic. Deep peat deposits have developed since the last glaciation under extensive valley bog communities in the sub-Antarctic. Significant deep moss banks are much more restricted (Fig. 9.3a) in the maritime Antarctic and not found in the continental Antarctic (Fenton 1980, 1982; Fenton and Smith 1981; Royles and Griffiths 2014) and differ from those of the sub-Antarctic in being well preserved by inclusion in permafrost, with little or no decay, and even retaining viability over at least 1500 years of preservation (Roads et al. 2014).

Terrestrial vertebrates are mostly absent in the Antarctic and are limited to a single endemic insectivorous passerine and two species of freshwater ducks in South Georgia and Îles Kerguelen, and two scavenging sheathbills, one each present along the Antarctic Peninsula, Scotia Arc islands, South Georgia and on Marion Island. There are no native mammals, reptiles, amphibians or freshwater fish. Nutrient availability in terrestrial habitats is strongly dependent on the Antarctic marine vertebrate fauna (Bokhorst and Convey 2016). Most such habitats are coastal, but some birds also breed on nunataks several hundred kilometres inland. Antarctic terrestrial fauna, therefore, consist almost entirely of invertebrates.

The milder sub-Antarctic islands host a range of 'higher' insects and other arthropod groups. The most diverse groups are Diptera and Coleoptera although these, and all other groups (e.g. Araneae, Isopoda, Lepidoptera, Hymenoptera, Hemiptera) are represented by very few species in terms of absolute numbers. Terrestrial diversity also includes molluscs and annelid worms, as well as diverse communities of micro-arthropods [Acari (Fig. 9.3b), Collembola (Fig. 9.3c)] and micro-invertebrates (Nematoda, Tardigrada, Rotifera). Overall diversity is lower in the maritime Antarctic, with only two chironomid midges (Diptera) present. Micro-arthropods and other micro-invertebrates are well represented. Although, again, species diversity is low, population densities are often very high, and comparable with or greater than many temperate and even tropical ecosystems. The continental Antarctic fauna includes no insects, and is restricted to micro-arthropods and micro-invertebrates. Though they are of similar diversity overall to the maritime Antarctic, they are much more limited in their spatial distribution. This zone includes the simplest faunal ecosystems on the planet, where even nematodes are apparently absent (Convey and McInnes, 2005; Hodgson et al. 2010). In all three regions, the fauna present includes taxa with characteristic trophic preferences (e.g. algivory, bacterivory, fungivory, predation); however, little detailed autecological work has been attempted, and the specific diets of Antarctic taxa are virtually unknown (Hogg et al. 2006).

9.2 Fungi-Invertebrate Interactions

Interactions between fungi and invertebrates in the Antarctic (both on land and in water) have seldom been studied. This is partly because many taxa have only been found recently and also is a consequence of the known hardships of working with extremophiles. However, there is clearly an assortment of species that actively interact with each other (Bridge and Worland 2008; Bridge and Spooner 2012). Fungal species have been found in the carcasses of dead animals (e.g. Bridge et al. 2005, 2008), predating on micro-invertebrates (e.g. Onofri and Tosi 1992; McInnes 2003) or being utilised as a source of food (e.g. Bokhorst et al. 2007). In the following sections, we present an overview of the interactions known to date across some major invertebrate groups.

9.2.1 Fungal Isolation from Invertebrates

Various methods to recover fungi from invertebrates have been described. Small invertebrates can be surface sterilised by washing in 70% ethanol for 30–60 s and after that transferring to Petri dishes containing culture media (Bridge and Worland 2004; Bridge et al. 2005, 2008; Bridge and Denton 2007). From the marine environment, Henríquez et al. (2014) obtained approximately 1 cm^3 pieces from the inner tissues of invertebrates, which were excised under sterile conditions using a scalpel and forceps, and directly spread them onto Petri dishes containing different culture media. Godinho et al. (2019) collected invertebrates, which were washed twice in a sterile solution 0.9% NaCl (for those sampled in land ecosystems) or seawater (for those from marine ecosystems) for 2–4 min. After that, the invertebrates were ground and placed in Petri dishes containing media for fungal growth. Different media were used for fungal development, such as Sabouraud agar, potato dextrose agar, malt extract agar, marine agar and others, which were supplemented with antibacterial antibiotics (usually chloramphenicol) for the inhibition of bacterial contamination. Table 9.1 summarises the fungi isolated from invertebrates in Antarctica.

9.2.2 Marine Invertebrates

Relative to their abundance, the mycological associations of marine invertebrates are very poorly studied, and the literature is largely restricted to the studies reported by Duarte et al. (2013), who first reported the occurrence of species of yeasts isolated from Antarctic marine invertebrates, including gastropods, tunicates, and isopods (Table 9.1). Cui et al. (2016) recently reported 42 taxa of fungi from a single species of crustacean (the very abundant krill, *Euphasia superba*). Godinho et al. (2019) studied the distribution and diversity of fungi associated with 10 species of

Table 9.1 Fungi isolated from terrestrial and marine invertebrates of Antarctica

Island/ Region	Invertebrate	Proposed taxa	References
Signy Island	*Eretmoptera murphyi* (larvae)	*Alternaria* sp.	Bridge and Denton (2007)
		Arthroderma sp.	
		Acremonium strictum	
		Antarctomyces psychrotrophicus	
		Mortierella gamsii	
		Pythium sp.	
Unknown	*Tetranychus urticae*	*Acremonium implicatum*	Bridge and Worland (2008)
		Cladosporium sp.	
		Lecanicillium lecanii	
	Polyphagotarsonemus latus	*Beauveria bassiana*	
	Dinothrombium giganteum	*Aspergillus flavus*	
	Eotranchyus sp.	*Cladosporium cladosporoides*	
	Thrombidium gigas	*Aspergillus flavus*	
	Oribatid sp.	*Lecanicillium lecanii*	
		Simplicillium lamellicola	
	Abacarus hystrix	*Lecanicillium lecanii*	
Bird Island	*Hydromedion sparsutum*	*Pirella circinans*	Bridge et al. (2008)
Nelson Island	*Alaskozetes antarcticus*	*Neozygites acaridis*	Bridge and Worland (2004)
Adelaide Island	*Cryptopygus antarcticus*	*Paecilomyces antarcticus*	Bridge et al. (2005)
King George Island	Unidentified sea squirt	*Candida sake*	Duarte et al. (2013)
		Wickerhamomyces anomalus	
		Rhodotorula mucilaginosa	
	Unidentified sea sponge	*Debaryomyces hansenii*	
		Bullera pseudoalba	
		Cryptococcus laurentii	
		Rhodotorula mucilaginosa	
	Salpa sp.	*Metschnikowia australis*	
		Cryptococcus victoriae	
		Cystofilobasidium capitatum	
		Cystofilobasidium infirmominiatum	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Rhodotorula mucilaginosa	
	Unidentified sea star	*Meyerozyma guilliermondii*	
		Cryptococcus adeliensis	
		Cryptococcus albidosimilis	
		Cystofilobasidium infirmominiatum	
		Guehomyces pullulans	
	Unidentified *s*ea isopod	*Meyerozyma guilliermondii*	
	Unidentified sea snail	*Meyerozyma guilliermondii*	
	Nacella concinna (Fig. 9.2e)	*Wickerhamomyces anomalus*	
		Cryptococcus laurentii	
		Rhodotorula mucilaginosa	
	Unidentified sea urchin	*Cryptococcus laurentii*	
		Rhodotorula laryngis	
		Rhodotorula mucilaginosa	
	Marine sponge	*Acremonium* sp.	Henríquez et al. (2014)
		Aspergillus versicolor	
		Aureobasidium pullulans	
		Cladosporium cladosporioides	
		Cladosporium sp.	
		Pseudogymnoascus pannorum	
		Penicillium commune	
		Cladosporium sp.	
		Pseudogymnoascus sp.	
		Penicillium polonicum	
		Penicillium solitum	
		Phoma herbarum	
		Phoma sp.	
		Pseudeurotium sp.	
		Pseudogymnoascus pannorum	
		Pseudogymnoascus sp.	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Thelebolus sp.	
		Pseudogymnoascus sp.	
		Thelebolus sp.	
	Tedania sp.	*Cystofilobasidium infirmominiatum*	Vaca et al. (2013)
		Metschnikowia australis	
	Leucosporidiella sp.	*Cystofilobasidium infirmominiatum*	
	Dendrilla sp., *Hymeniacidon* sp., *Poecilosclerida* sp.	*Metschnikowia australis*	
Antarctic Peninsula	*Laevilacunaria antarctica*	*Antarctomyces psychrotrophicus*	Godinho et al. (2019)
		Metschnikowia australis	
		Pseudogymnoascus destructans	
		Pseudogymnoascus verrucosus	
		Vishniacozyma victoriae	
		Pseudogymnoascus cf. *destructans*	
	Antarctonemertes valida	*Cladosporium* sp. 1	
		Didymella longicolla	
		Glaciozyma martinii	
		Metschnikowia sp. 1	
		Mollisia sp.	
		Mortierella sp. 1	
		Mrakia sp.	
		Penicillium brevicompactum	
		Penicillium sp. 1	
		Penicillium sp. 2	
		Penicillium sp. 3	
		Pestalotiopsis kenyana	
	Ascidia sp.	*Antarctomyces psychrotrophicus*	
	Halyclystus antarticus	*Metschnikowia* sp. 1	
		Metschnikowia sp. 2	
	Lumbricillus sp.	*Aspergillus* sp. 1	
		Aspergillus sp. 2	
		Didymella coffeae-arabicae	
		Letendraea sp.	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Metschnikowia australis	
		Nothophoma macrospora	
		Penicillium sp. 1	
		Penicillium swiecickii	
		Penicillium sp. 2	
		Pestalotiopsis kenyana	
		Phoma sp.	
		Pseudogymnoascus cf. *destructans*	
		Thelebolus cf. *globosus*	
	Magelonidae sp.	*Metschnikowia* sp. 2	
		Mrakia frigida	
	Nacella concinna	*Antarctomyces psychrotrophicus*	
		Aspergillus sp. 1	
		Aspergillus sp. 2	
		Candida sp.	
		Candida spencermartinsiae	
		Candida zeylanoides	
		Cladosporium halotolerans	
		Cladosporium sp. 1	
		Clavispora lusitaniae	
		Debaryomyces hansenii	
		Pseudogymnoascus destructans	
		Geotrichum sp.	
		Glaciozyma martinii	
		Holtermanniella festucosa	
		Metschnikowia sp. 2	
		Meyerozyma guilliermondii	
		Mortierella sp. 1	
		Mortierella sp. 2	
		Mortierella sp. 3	
		Mrakia frigida	
		Penicillium sp. 1	
		Penicillium sp. 2	
		Penicillium sp. 3	
		Penicillium sp. 4	

(continued)

Table 9.1 (continued)

Island/ Region	Invertebrate	Proposed taxa	References
		Polypaecilum botryoides	
		Pseudogymnoascus cf. *destructans*	
		Pseudogymnoascus pannorum	
		Pseudogymnoascus verrucosus	
		Rhodotorula mucilaginosa	
		Thelebolus balaustiformis	
		Thelebolus cf. *globosus*	
		Tolypocladium tundrense	
	Ophiuroidea (Fig. 9.2a)	*Pseudogymnoascus* cf. *destructans*	
		Penicillium sp. 5	
		Metschnikowia sp. 2	
	Tigriopus kingsejongensis	*Debaryomyces hansenii*	
		Pseudogymnoascus pannorum	
		Penicillium sp. 3	
		Penicillium sp. 5	
		Pseudogymnoascus appendiculatus	
		Pseudogymnoascus cf. *destructans*	
		Pseudogymnoascus verrucosus	
		Septoria chromolaenae	
	Trepaxonemata sp.	*Cladosporium* sp. 1	
		Debaryomyces hansenii	
		Penicillium sp. 3	

invertebrates from Antarctica and recovered a rich and diverse community with 83 taxa from 27 distinct genera. The most abundant fungi associated with the Antarctic invertebrates were *Cladosporium* sp., *Debaryomyces hansenii*, *Glaciozyma martinii*, *Metschnikowia australis*, *Pseudogymnoascus destructans*, *Thelebolus* cf. *globosus*, *Pseudogymnoascus pannorum*, *Tolypocladium tundrense*, and different *Penicillium* and *Metschnikowia* species. Godinho et al. (2019) showed that the cryptic fungi recovered from Antarctic invertebrates displayed phylogenetic relationships with species that occurred in other cold, temperate and tropical regions of the world, including endemic and cosmopolitan cold-adapted taxa.

9.2.3 Nematodes

Nematodes are among the most abundant and widespread invertebrates occurring on land in Antarctica, only being absent from some of the most isolated inland regions of the continent (Convey and McInnes 2005; Hodgson et al. 2010). Nematophagous (or 'nematode-trapping') fungi, which are globally widespread and occur naturally in habitats with organic detritus (Gray et al. 1982), are also present in Antarctica, with several taxa reported from locations in the maritime Antarctic (Duddington et al. 1973; Spaull 1973; Gray et al. 1982; Gray and Smith 1984; Gray 1985; Velázquez et al. 2017). As noted by Gray et al. (1982), these predacious fungi have a role in the transfer of energy through the food chain, while also potentially controlling the population levels of their prey. More generally, however, Nielsen et al. (2011) suggested that nematodes, which are mostly bacterial grazers, could help the growth of fungi by reducing the ecological dominance of bacteria in the habitat.

9.2.4 Tardigrada and Rotifera

To date, the only study specifically mentioning fungal interactions with tardigrades (water bears) and rotifers is that of McInnes (2003), who reported a new fungal species (*Lecophagus antarcticus*) which was found attacking both invertebrates by means of trapping with adhesive pegs arising from vegetative hyphae (a behaviour previously reported in other members of the genus *Lecophagus*; Vechhi et al. 2016).

9.2.5 Collembola, Arachnida and Insecta

The first published record of interaction between fungi and terrestrial Arthropoda in Antarctica is that of Onofri and Tosi (1992), who reported *Arthrobotrys ferox* predating on the springtail *Gressittacantha terranova* in Kay Island, Edmonson Point and Baker Rocks (Wood Bay, Victoria Land, Antarctica), by means of what they described as 'organs consisting of ovoidal cells surrounded by an adhesive secretion (sic) and supported by a 2-celled stalk'. According to these authors, this species was the first sample of predaceous hyphomycetes collected in continental Antarctica and also the first recorded to predate on springtails in the Antarctic continent. The 2000s saw an increased number of studies, mostly from Bridge and collaborators (Bridge and Worland 2004, 2008; Bridge et al. 2005, 2008; Bridge and Denton 2007; Bridge and Spooner 2012).

Bridge and Worland (2004) discovered *Neozygites*, an entomophthoralean fungus, on living mites at Nelson Island, South Shetland Islands, off the north-west coast of the Antarctic Peninsula. Subsequently, Bridge et al. (2005) isolated a new

species *Paecilomyces antarcticus* from the carapace of dead springtails collected near Rothera Research Station, Adelaide Island, although they could not identify a precise role of the fungus. *Paecilomyces* spp. have been previously reported to grow on wintering insect larvae (ARSEF 2018); however, in the study by Bridge et al. (2005), there was no visible fungal growth before the fungus was cultured in the laboratory. Bokhorst et al. (2007) suggested that the springtail *Cryptopygus antarcticus* feeds facultatively on fungi (among other organic material, such as algae and dead matter) (see also Tilbrook 1970; Broady 1979; Burn 1984; Block 1985; Cannon 1986), suggesting a closer relationship between the findings reported separately in these studies. However, *C. antarcticus* is thought to primarily be an algivore, preferentially grazing on certain microalgal species, rather than being a generalist microbivore (Worland and Lukešová, 2000). Members of the springtail genus *Friesea* are often considered to be fungivorous, but no autoecological studies have been carried out on any of the several Antarctic species to confirm this (Greenslade 2018a, b). Likewise, the oribatid mite *Halozetes belgicae* has been observed grazing on both microalgae and fungal hyphae within the thalli of supralittoral lichens at locations in the South Shetland Islands and along the Antarctic Peninsula (P. Convey, pers. obs.). The sub-Antarctic snail, *Notodiscus hookeri*, obtains specific micronutrients by grazing on lichens (Gadea et al. 2017, 2018).

Bridge and Denton (2007) isolated viable propagules of *Ascomycetes*, *Zygomycetes* and *Oomycetes* from the intestinal tract of *Eretmoptera murphyi*, a chironomid midge native to sub-Antarctic South Georgia, which was introduced accidentally by humans to Signy Island in the maritime Antarctic through plant transplant experiments (Block et al. 1984; Convey and Block 1996). This is a rare example of a study dealing directly with fungi found inside a living host in Antarctica. Normally, fungal growth only becomes apparent in dead animals, as reported by Bridge et al. (2008), who found an association between *Pirella circinans*, a coprophilous fungus, and the South Georgian endemic beetle *Hydromedion sparsutum*. This type of fungus is normally reported from the dung of small mammals, which are absent in South Georgia, and was the only fungus recovered in the insect cadavers, indicating a probable close association between the species.

The presence of entomopathogenic fungi gives potential for examination of their applicability as biological controls (see below). The studies of Bridge and Spooner (2012) and Velázquez et al. (2017) highlighted the importance of identifying and quantifying trophic interactions within the habitats where fungi and invertebrates are found. Bridge and Spooner (2012) noted that it is harder to determine the roles of fungi associated with invertebrates in Antarctica, mainly because the very specific life cycle and adaptive features required by these animals to survive in the extreme environmental conditions of the Antarctic influence the formation of fungal epizootics (see also Bridge and Worland 2008).

9.2.6 *Entomopathogenic Fungi*

No studies have yet formally documented the entomopathogenic potential of fungi in the Antarctic. As noted above, Bridge and Denton (2007) documented the microfungal composition of the intestinal tract of the South Georgian flightless midge *E. murphyi*, using materials collected from the invasive population on Signy Island, and suggested the possibility of the insect larvae working as vectors for fungal introductions. However, it was not possible to ascertain whether the fungal species identified were present on Signy Island before the fly's introduction, since all of them had previously been reported from Antarctica. Bridge and Worland (2008) found *Neozygites*, another pathogenic fungus, associated with the mite *Alaskozetes antarcticus*, and reviewed knowledge of fungal pathogens across Antarctica, highlighting the increasing number of studies on nematodes, flowering plants and mosses (Pegler et al. 1980; Gray and Smith 1984; Bridge et al. 2008) in comparison to those involving arthropods.

Bridge and Denton (2007) also considered the possibility of microfungi being found in hosts elsewhere in the world, such as *Lecanicillium lecanii*, and being able to adapt to the Antarctic if they were introduced, since they generally tolerate wide temperature ranges (Brasier et al. 1999; Nikoh and Fukatsu 2000; Hughes and Lawley 2003). Bridge et al. (2014) highlighted the potential of entomopathogenic fungi to make temperature-resistant mycoinsecticides. Edgington et al. (2014) studied the insecticidal potential of two fungi (*Pseudogymnoascus* and *Mortierella*) found around Rothera Research Station, Adelaide Island, and on Signy Island, finding two species of the latter (*M. alpina* and *M. sygniensis*) to cause significant mortality in larvae and adults of the tested insect species, suggesting a potential to be used elsewhere as pest control. Such pesticides may eventually play a role in combating biological invasions.

9.3 Invasive Species

The introduction of alien species is a matter of concern in any environment in the world, as it can have direct (e.g. predation of bird eggs and terrestrial invertebrates, trampling and grazing of plants) and indirect (e.g. alteration of habitat structure leading to changes in species dominance or behaviour) repercussions on native species and the stability of the local ecology. This is particularly the case in extreme environments where life has adapted very specifically to the driving physical environmental stressors and generally has very little ability to compete effectively with new arriving species (Convey 1996b). One of the main sources of biological invasions in the Antarctic, as globally, is human activity (e.g. Smith 1996; Azmi and Seppelt 1998; Frenot et al. 2005; Chwedorzewska 2009; Lee and Chown 2009; Convey 2010; Lityńska-Zając et al. 2012; Chwedorzewska et al. 2013; Galera et al. 2018). Accidental introductions and deliberate transplant experiments have shown

that a wide range of flora, fauna and microbes are capable of surviving and establishing viable populations, while an even greater number have been recorded on a transient or synanthropic basis (Frenot et al. 2005; Greenslade 2006; Convey 2017).

Whilst survey data documenting the introduction of invertebrates, and their ecological impacts, remain limited in Antarctica, the subject has received increasing attention in recent years (see, e.g. Lee and Chown 2009; Lityńska-Zając et al. 2012; Bartlett 2018a, b; Hughes et al. 2015, 2018; Gonçalves et al. 2017a, b). It is not uncommon for invasive species to be restricted to areas where human occupation is continuous, disappearing as soon as humans leave (Convey 2017; Potocka and Krzemińska 2018). However, some non-native species do become established in the natural environment, where they can cause competitive displacement and local extinction of native species and add new trophic links in terrestrial ecosystems.

Introduction of flowering plants, bryophytes and microbes has accompanied human activity. Given the possible evolutionary isolation of Antarctic microbes, the introduction of fungal strains from outside Antarctica (or even between regions within Antarctica) and the consequential potential for damage to this unique biological resource should not be underestimated (Smith 1996; Wynn-Williams 1996a, b; Frenot et al. 2005; Chown and Convey, 2007; Bridge and Hughes 2010; Cowan et al. 2011; Augustyniuk-Kram et al. 2013; Hughes et al. 2015, 2018).

Finally, Gonçalves et al. (2017a) suggested that some continental Antarctic fungi may be pathogenic to humans and, through humans who come in contact with them, they could possibly spread to other parts of the world. The same can be said about the possibility of the major migrating vertebrates of the Antarctic (de Sousa et al. 2017; Gonçalves et al. 2017b), which can carry pathogens to other parts of the world.

9.4 Conclusion and Perspectives

As a demonstration of the untapped potential for discovery of fungi associated with invertebrates of Antarctica, Cui et al. (2016) reported 42 types of fungi isolated from a single crustacean species (*E. superba*). Some of these produce cytotoxic compounds that may help protect the crustacean against mammalian predators and pathogenic bacteria. The potential, for example, for the discovery of cytotoxic and entomopathogenic compounds from Antarctic fungi is likely to lead to an upsurge in bioprospecting studies. Clearly, much future effort is required to isolate fungi from marine invertebrate taxa, a virtually unexplored field at present. There is also an urgent need for the improved survey and monitoring of microbial – including fungal – diversity across Antarctica, including the assessment of the native or invasive status of isolates. Studies on the impacts of future climate changes must be extended to include microbial groups, and in the context of the current chapter, focus in particular on potential changes in the interactions between fungi and invertebrates (e.g. see Bridge and Spooner 2012).

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Chapter 10
Sub-Antarctic and Antarctic Marine Ecosystems: An Unexplored Ecosystem of Fungal Diversity

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10.1 Introduction

There are various marine ecosystems in the world which are practically unexplored in terms of their microbial diversity, ecological role, and biotechnological potential. There are few published studies on the Antarctic marine microbiology, as compared with the rest of the world. The biological dynamics of marine ecosystems seem to be related to the presence of the microbiota living there, which dominate the living biomass of the oceans.

Various microorganisms, including bacteria, archaea, viruses, fungi, and protists, can be found in different ocean substrates (Glöckner et al. 2012). Microorganisms play an important role in the formation of the marine trophic pyramid by acting as photosynthetic primary producers (Richmond 2004), herbivores (Ramanan et al.

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2016), consumers (Rivkin et al. 1996), parasites and decomposers responsible for cycling organic matter and oxygen to sustain life in the oceans (Glöckner et al. 2012), and by restoring the biogeochemical cycling of carbon, nitrogen, silica, iron, and other trace elements (Nelson et al. 1996; Moore et al. 2002; Morel and Price 2003; Voss et al. 2013).

Marine fungi are categorized into two major ecological groups by Kohlmeyer and Kohlmeyer (1979): (i) obligate marine fungi, which are described as 'those that grow and sporulate exclusively in a marine or estuarine habitat', and (ii) facultative marine fungi which are described as 'those from freshwater and terrestrial milieus able to grow and possibly also sporulate in the marine environment'. According to a taxonomic review by Jones et al. (2015), more than 700 species of obligate marine fungi and nearly 550 species of facultative and marine-derived fungi have been described. The *Halosphaeriaceae* is the largest family of marine fungi with 141 species and 59 genera; the most common genera in terms of number of species were *Aspergillus*, *Penicillium*, and *Candida* (Jones et al. 2015). However, the richness and diversity of marine fungi in the world seem to be underestimated and more than 10,000 species might exist, based on unidentified species that can be present in habitats and substrates not completely explored, such as those in deep sediments and seawater as well as abyssal organisms (Jones et al. 2009, 2015; Jones and Pang 2012).

Within the ocean ecosystem, marine fungi are important decomposers, which are able to, virtually, colonize all marine substrates, including wood and algae to sediments, muds, soils, sand, corals, calcareous tubes of molluscs, decaying leaves of mangroves, intertidal grasses, living animals, and the guts of crustaceans (Hyde and Goh 1998). Some fungi have the ability to disperse and survive in unusual or hostile environments (Sridhar 2017; Dighton and White 2017), such as those in Antarctica.

10.2 Antarctic Marine Ecosystem

Venter et al. (2004) and Sunagawa et al. (2015) studied the worldwide marine microbial diversity and showed that a large portion is represented by unknown taxa. Among the global oceans, the Southern Ocean, also called Austral Ocean or Antarctic Ocean, starts from 60 °S encircling the border of continental Antarctica (Fig. 10.1). The Antarctic Ocean represents a unique region whose microbiome is practically unexplored, including the microbial genes (Dickinson et al. 2016). Different substrates of the Southern Ocean represent promising microhabitats able to shelter microorganisms like virus, archaea, bacteria, and fungi. Among these substrates, those with availability of organic matter can be considered potentially rich in terms of microorganisms. However, virtually all substrates of Southern Ocean such as sediments, rocks, sea ice, seawater, seaweeds, invertebrates, and vertebrates might shelter microbial life.

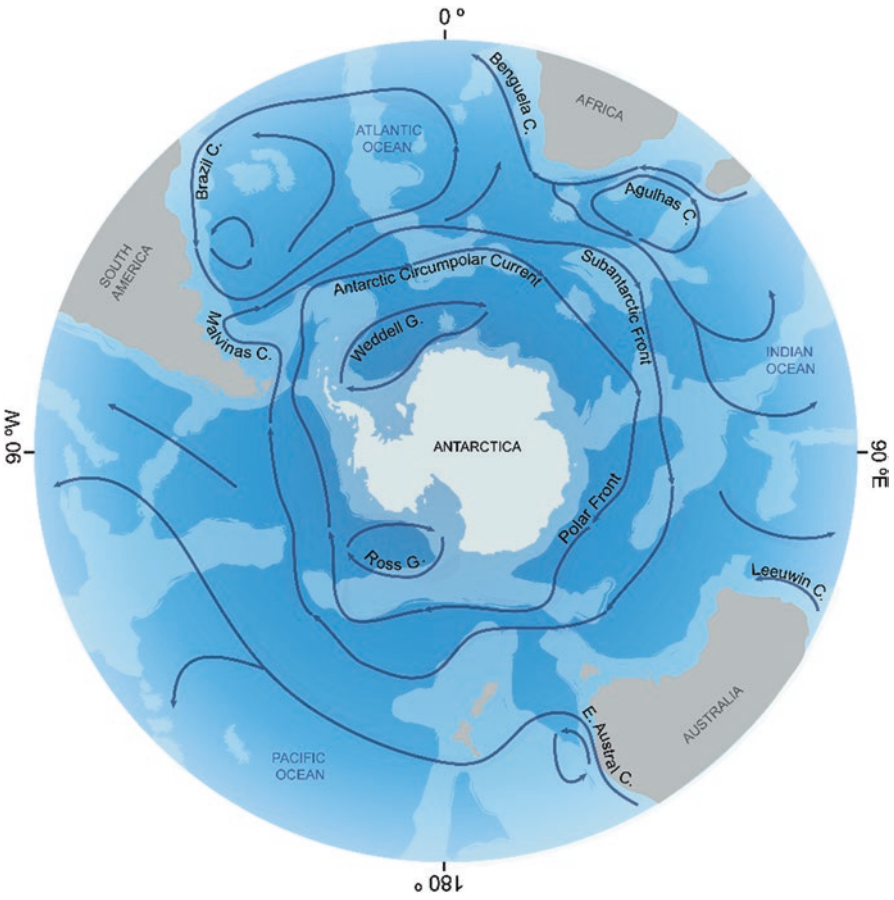

Fig. 10.1 Delimitation of the Antarctic Circumpolar Current and the nearest maritime current

10.3 Fungi in Antarctic Marine Sediments

The seafloor corresponds to two-thirds of the earth's surface and harbours a diversity of microorganisms adapted to live under conditions imposed by the sea depth (Nagano et al. 2010; Rédou et al. 2015). The sea bottom is recognized as an extreme environment, being mostly stable, characterized by the absence of sunlight, low temperatures, high hydrostatic pressure, and low availability of nutrients (Nagano et al. 2010; Raghukumar et al. 2010). Due to its uniqueness (hydrothermal, anoxic, and low temperature), marine environments represent a great potential for the study of the evolution of fungi, mainly the hypothesis that fungal divergence might be initiated in the marine habitats (Zhang et al. 2015).

On the seafloor, fungi act as decomposers of organic matters, parasites, pathogens, and mutualists associated with other marine organisms. Fungi are also

involved in denitrification processes (Cathrine and Raghukumar 2009; Jebaraj et al. 2010), because the major organic matter that is highly resistant to microbial decay is stored in marine sediments (Clarke et al. 2017). Some seagrasses, marsh plants, and mangrove detritus are rich in lignocelluloses; this structural polysaccharide is extremely resistant to decomposition (Clarke et al. 2017). These and other resistant compounds are deposited in sediments, making this substrate a site difficult for colonization by most organisms. However, fungi, present in marine sediments, have some adaptations in their structure and metabolism that demonstrate mesophilic, psychrotolerant, and/or halotolerant profiles (Raghukumar et al. 2010), which make them capable of tolerating the impositions of the deep sea. Among the fungal communities living in the marine sediments, there are some taxa that can produce oxidative and hydrolytic enzymes and can be a source of bioactive metabolites (Vaz et al. 2011; Gonçalves et al. 2013, 2015; Wentzel et al. 2019).

Some reports have demonstrated a significant fungal diversity in marine sediments of the Pacific Ocean (Lai et al. 2007; Nagano et al. 2010; Zhang et al. 2013; Rédou et al. 2015; Ahumada-Rudolph et al. 2016), Indian Ocean (Raghukumar et al. 2004; Cathrine and Raghukumar 2009; Singh et al. 2010; Zhang et al. 2014), Atlantic Ocean (Mouton et al. 2012; Orsi et al. 2013; Nagano et al. 2017), Arctic Ocean (Zhang et al. 2015), and Antarctic Ocean (López-garcía et al. 2001; Gonçalves et al. 2013, 2015) (Fig. 10.2). However, there is not much information on the fungal community in marine sediments of the Polar Regions. The genera *Cladosporium*,

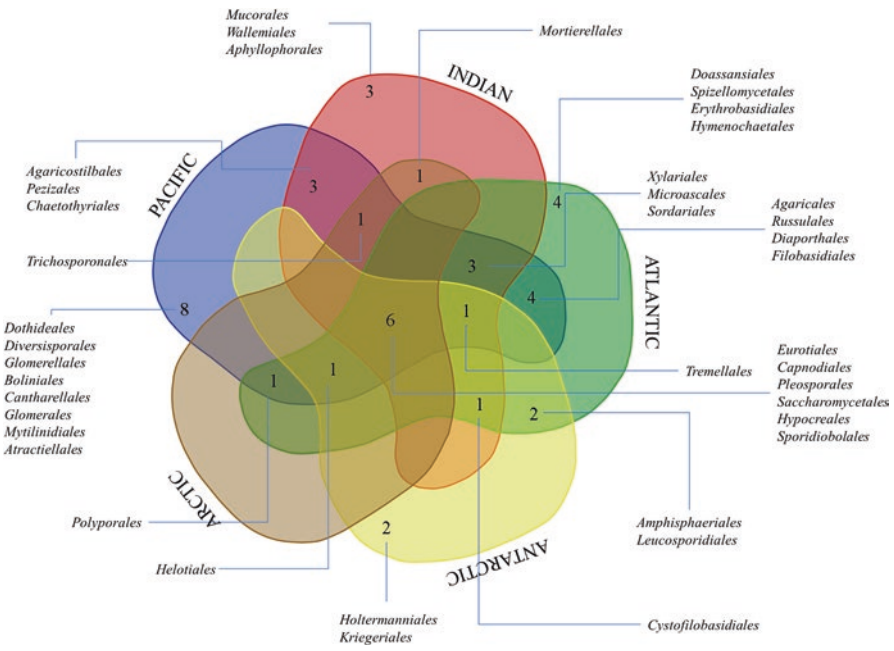

Fig. 10.2 Venn diagram showing fungal orders of isolates obtained from sediment samples of various oceans according to previous reports

Penicillium, and *Rhodotorula* are commonly identified in marine sediments of all oceans. The majority of taxa found in Antarctic marine sediments are shared with Atlantic marine sediments and the marine sediments from other oceans.

Few studies have isolated culturable fungi within the marine sediments of Antarctica, indicating the presence of several known taxa of fungi. Cosmopolitan species of *Aspergillus*, *Cladosporium*, *Penicillium*, *Fusarium*, *Candida*, and *Rhodothorula* seem to be adapted to extreme conditions of the Antarctic seawater (Raghukumar et al. 2004; Cathrine and Raghukumar 2009; Singh et al. 2010, 2012; Zhang et al. 2013). The occurrence of the orders *Kriegeriales* (*Glaciozyma* sp. and *Phenoliferia* sp.) and *Holtermanniales* (*Holtermanniella* sp.) reported by Wentzel et al. (2019) may be more frequent in the Antarctic marine sediments in comparison to other areas, since their occurrence was unique in the reports that were analysed.

To isolate microorganisms from extreme marine environments, like marine Antarctic environments, many specific isolation conditions have to be considered, such as hydrostatic pressure, salinity, nutrient profile, incubation temperature, and oxygen level, in order to simulate the limitations of the environment as much as possible. Due to this challenge, there is a major constraint in the identification of marine Antarctic fungi, and it is estimated that less than 20% of the species present have been isolated and grown in pure culture (Bridge 2007). This may explain the lack of data in the literature regarding obligate marine fungi in the region.

Due to the difficulties in isolation, molecular studies involving uncultured analyses have reported a large number of fungal taxa in marine environments, suggesting that there may be more fungi still unknown in the Antarctic environment, although there is a gap in the knowledge about their ecological role in this habitat, because Polar Regions are complex ecosystems. Some studies investigated fungi associated with marine sediments using molecular methods (clone libraries and pyrosequencing), which allow the identification of uncultivable species (Lai et al. 2007; Nagano et al. 2010; Singh et al. 2011; Xu et al. 2014; Zhang et al. 2014, 2015). Some studies have used cloning libraries to analyse samples from the deep-sea water column of the Drake Passage (Antarctic polar front) (López-garcía et al. 2001; Bass et al. 2007); however, from marine sediments, apparently, only cultivation techniques have been used to identify fungal taxa (Vaz et al. 2011; Laich et al. 2013; Gonçalves et al. 2013; Wentzel et al. 2019), which indicates that much of the fungal assemblages in this substrate remain unknown, mainly the species that are difficult to culture.

10.4 Macroalgae from Antarctic and Sub-Antarctic Areas as Hosts of Marine Fungi

In the Southern Pacific Ocean, the Magellanic biogeographic province (41 °S to 56 °S) is featured by a large extension of channels and fjords with diverse coastal environments arising from the glacial influence by direct exposure to the Pacific Ocean

(Camus 2001; Spalding et al. 2007). Two biogeographic districts have been categorized for this biogeographic province: Austral and Sub-Antarctic. The latter extends from about 52–53 °S to 56 °S (Camus 2001) or from the Magellanic Strait to Cape Horn. This territory is characterized by different environmental conditions and thus is further divided into sub-areas according to its geomorphology, orography, geology, soils, and climates (Pisano 1977).

The Magellanic province presents several ecological singularities that highlight the differences in composition, richness, and structure of macroalgae communities compared with temperate coasts of America (Ojeda 2013; Mansilla et al. 2014). The coastal habitats present high environmental heterogeneity, influencing the Sub-Antarctic macroalgae biodiversity, ruled by several factors such as geomorphology [type of substrate (Ojeda et al. 2014)], oceanographic patterns [salinity variation (Silva and Calvete 2002)], climate [seasonal variation in solar radiation, photoperiod, and temperature (Ojeda et al. 2014; Marambio et al. 2017)], and biological features [diversity of biotopes associated with macroalgae (Soto et al. 2012)]. Figure 10.3 shows macroalgae from the Sub-Antarctic ecosystem potential host of marine fungi.

The concept of macroalgal richness originated from the expeditions of Charles Darwin and Captain Robert Fitz Roy (1834) (Mansilla 2013) and current compilations have shown that the area of Magallanes and Tierra del Fuego afford about 234 macroalgae taxa, not considering the 444 registered for the entire Chilean coast (Ramírez 2010). Extensive Kelp forests cover the Magellanic coast, and *Macrocystis pyrifera* (*Phaeophyceae*) dominate the fjords and channels of the region (Mansilla and Ávila 2011). Its morphological features make this species an important engineering organism in the Magallanes region, as it provides habitat and refuge for

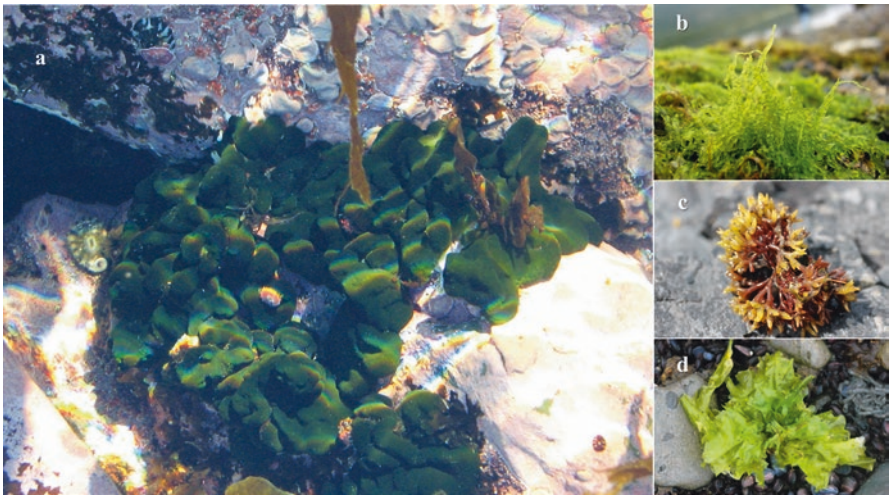

Fig. 10.3 Sub-Antarctic macroalgae (**a**) *Codium dimorphum*, (**b**) *Ulva intestinalis*, (**c**) *Nothogenia fastigiata,* and (**d**) *Ulva lactuca*. (Photos Credits: A Mansilla)

feeding and reproduction to many species (Vanella et al. 2007). The Sub-Antarctic intertidal environment is generally characterized by highly exposed shores situated in the path of the Antarctic Circumpolar Current (ACC).

Mansilla et al. (2016) reported that the Chilean Sub-Antarctic ecoregion of Magallanes hosts a distinct coastal phycobenthic community compared to other temperate continental rocky shores of South America and this could be the result of several factors such as (i) geomorphology generated by the glacial erosion during the advance and retreat of ice in the Quaternary, (ii) oceanographic gradients combining unique current flows, salinity, and thermal gradients, (iii) photoperiod and irradiance regimes, (iv) presence of glaciers and west-to-east winds and rainfall, (v) freshwater coastal discharge, and (vi) distinct substrate types, resulting in unique physical and biogeochemical seawater conditions that generate a peculiar macroalgal structure.

These unique abiotic factors at higher southern latitudes also affect the efficiency of light capture during photosynthesis. Seaweed photosynthesis demands efficient strategies for light utilization and pigment production to maintain algal performance. The environmental heterogeneity of the Sub-Antarctic and Antarctic ecosystems has shaped the evolutionary history and physiological adaptations of the local phycoflora and their associated organisms. Phylogenetic, morphological, and ecophysiological studies using the genus *Desmarestia*, conducted in the Chilean Sub-Antarctic areas, suggested that this species originated from an Antarctic ancestor, then radiated north, eventually reaching the Northern Hemisphere through long-distance natural dispersal (Peters et al. 1997). The representative *Desmarestia* species in Antarctica and the endemic *Himantothallus grandifolius* comprise a large proportion of the macroalgal biomass along the Antarctic Peninsula (Quartino et al. 2005; Pellizzari et al. 2017), both great substrates for marine fungi and yeast.

The differences in diversity, high degree of endemism, and affinities or molecular divergences between macroalgae species from the Antarctic and the Sub-Antarctic ecoregion of Magallanes are evidenced by the complex evolutionary process common in the Southern Ocean and probably due to a high degree of divergence registered between the Antarctic and South American populations of marine organisms (Poulin et al. 2014). Griffiths and Waller (2016) pointed out that the Sub-Antarctic intertidal environment is often characterized by highly exposed shores. The Sub-Antarctic Islands are located in the path of the ACC and are subjected to the force of the Southern Westerly winds. Ice-free coastlines are often dominated by dense beds of kelp *Durvillaea antarctica* (*Phaeophyceae*) and other large macrophytes. Unlike shores further south, there is often a distinct pattern of zonation from extreme high water to low water spring tides. Finally, the high environmental heterogeneity present in the Magallanes Biogeographic Province and the high richness of macroalgae species make the Magallanes region a natural laboratory for further studies.

The Antarctic intertidal environment is also the less well-sampled biome compared to the surrounding deep sea or adjacent terrestrial habitats (Griffiths and Waller 2016). This is in contrast to anywhere else in the world, since being characterized by intense seasonal scouring by ice, winter ice encasement, high UV

radiation, and seasonally large variations in temperature and salinity, it deserves continuous monitoring studies (Pellizzari et al. 2017).

Macroalgae are primary producers that act as a biogenic habitat for several other marine organisms and aid in maintaining the ocean's homeostasis through pH regulation. In addition, seaweeds possess bioactive chemical components of a high nutritional value, which can be applied in several industries, like nutraceutical and pharmaco-cosmetic industries. Recently, compounds such as terpenes, phenols, quinones, macrolides, alkaloids, lipids, chromones, and other related metabolites and extracts from Antarctic macroalgae have been reviewed as potential drugs against antiprotozoal and other neglected diseases (Torres et al. 2014; Falkenberg et al. 2018). Additionally, Antarctic macroalgae also can be a source of anticancer and antimicrobial compounds (Martins et al. 2018; Pacheco et al. 2018). Indeed, as Antarctic macroalgae synthesize large amounts of omega 3 and 6 fatty acids, they can be a source of polyunsaturated fatty acids (PUFAs) and steroids for other animals that consume them (Pereira et al. 2017; Santos et al. 2017). The Antarctic and Sub-Antarctic zones are distinct biogeographical regions, with patterns driven by a small number of widely distributed species. According to Griffiths and Waller (2016) and Sanches et al. (2016), the wide distribution of macroalgae that dominate the biogeographical formation of the intertidal zone along the Southern Ocean is most likely the result of rafting in the ACC.

Antarctic benthic biomes are characterized by winter ice encasement; spring/summer scouring by ice, intense and continuous UV radiation; and seasonal broad variations in temperature and salinity. However, despite the extreme Antarctic intertidal conditions, there are a wide and peculiar diversity of seaweeds and associated fungi/yeast growing in these substrates. In comparison, the Sub-Antarctic intertidal environment is often characterized by highly exposed shores situated in the ACC path. As reported by Astorga-España and Mansilla (2014), the Region of Magallanes, total area of 132,033 km^2, is the largest world representative of Sub-Antarctic environments (Mansilla et al. 2012), where 391 species of macroalgae have been reported (75 *Chlorophyta*, 86 *Phaeophyceae*, and 230 *Rhodophyta*). Many of these are economically crucial species for their alginates, carrageenans, or agarans, besides other compounds that are used as dietary supplements, or directly as food for human consumption (Mansilla et al. 2012).

South Georgia is an isolated island in the Southern Ocean and is the second largest hotspot of macroalgae diversity in the circum-Antarctic zone. The island presents 127 listed seaweed species (Wells et al. 2011), and some sporadic additional records include both endemic and cosmopolitan species. Until now, the origin of these species was unknown and there is lack of knowledge regarding whether they have been present in South Georgia for long periods of time. Moreover, low sampling efforts and low resolution in taxonomic inventories have either masked their presence or deterred the determination of whether they are recent additions to the seaweed flora. It may be speculated that many were not recorded due to the isolation of the area and the inaccessibility of the coastline. However, given the increased tourism and human activity, it is equally plausible that many species have been introduced in recent years through these means.

Studies focusing on seaweeds and their associated microbiota along remote polar areas are limited by logistical and safety challenges. All previous investigators relied on collections from the shore or occasionally by dredging and bottom grab, missing much of the subtidal organisms. The use of remotely operated underwater vehicle (ROV) and scuba diving, besides molecular methods, has improved the taxonomical endeavours, jointly with culturing techniques. Regarding the Maritime Antarctic Peninsula, recently Pellizzari et al. (2017) reported a total of 104 species of benthic marine algae (28 *Phaeophyceae*, 24 *Chlorophyta*, and 52 *Rhodophyta*), representing approximately 82% of all the seaweed taxa present in entire Antarctica, along eight islands of South Shetland Archipelago. The authors also listed six new species records (previously recorded only at lower latitudes), four putative taxa that were confirmed by their biogeographical distribution and two new species that were identified by their morphological and molecular features. Sanches et al. (2016) and Pellizzari et al. (2017) also suggest, using seaweed assemblages as proxy for diversity changes, that the Antarctic intertidal environment is undergoing dramatic changes, and that these sensitive communities are more connected with Sub-Antarctic and South American population than previously reported. Indeed, continuous investigations in these remote places are imperative to enable collection of accurate baseline data to monitor future changes in species composition and distributional shifts particularly due to climate change.

The Antarctic Peninsula is a transitional area and clearly a macroalgal hotspot (Pellizzari et al. 2017); this is followed by the West Antarctic Peninsula (WAP) and then, the East Antarctic Peninsula (EAP). The level of endemism was previously considered high (34% by Wiencke et al. 2014) and mainly involved *Phaeophyceae* and *Rhodophyta*. Figure 10.4 shows some macroalgal species frequently found in the Antarctic Peninsula already studied as hosts of marine fungi. However, a reduction in the endemism level (to ca. of 20%) has been reported since the study conducted

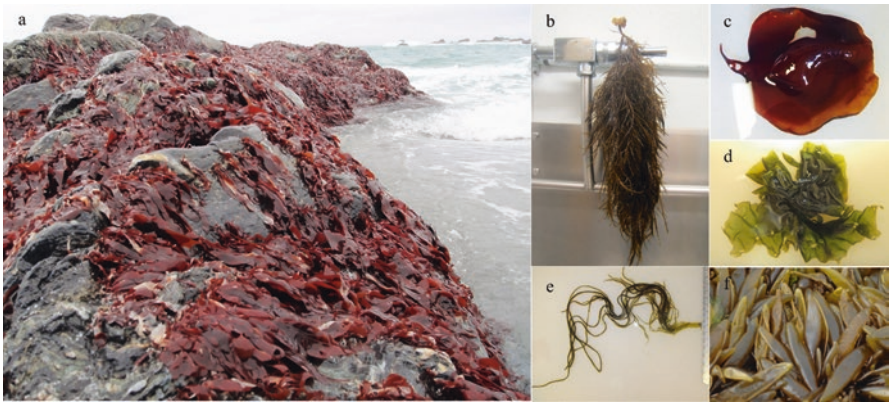

Fig. 10.4 Thalli of macroalgae from Antarctica (**a**) Bed of *Palmaria decipiens*, (**b**) *Desmarestia menziesii*, (**c**) *Iridaea cordata*, (**d**) *Pyropia endiviifolia*, (**e**) *Phaeurus antarcticus*, and (**f**) *Adenocystis utricularis*. (Photos Credits: FM Pellizari)

by Pellizzari et al. (2017), as suggested by several reports of the endemic species from Antarctica, in Patagonian samples (Argentinean and Chilean) and many Sub-Antarctic island samples (Pellizzari et al. 2017). Sanches et al. (2016) have used previously published data to establish a database for monitoring future biogeographical changes in the phycoflora of the Antarctic and Sub-Antarctic areas. The patterns seem to be altered either due to natural dispersion associated with global meteorological and oceanographic changes or due to biological invasions related to anthropogenic activities. The authors also indicate a period of changes in Antarctic diversity, suggesting that Antarctica may not be as isolated as was once thought.

10.5 Ecology and Diversity of Marine Fungi from Antarctica

Fungi occur in marine ecosystems as spores, hypha fragments, or mycelium in active or resistant forms even in unlikely locations like a hot deep-sea volcano (Connell et al. 2009), in deep sea (Damare et al. 2006; Redou et al. 2015), and anoxic hypersaline sediments (Bernhard et al. 2015) to freezing seawater substrates of Antarctica (Godinho et al. 2013; Gonçalves et al. 2015). Marine fungi can occur in harmonic and disharmonic symbiosis, as a commensal, mutualist, or parasite in the oceans (Raghukumar 2017). As in the land ecosystems, in the oceans, fungi play the main ecological role in the decomposition of organic matter including mangrove wood (Raghukumar 2017), seaweeds (Raghukumar 2006), corals, invertebrates, and vertebrates (Jones and Pang 2012). In addition, different chemical and physical environmental factors affect the diversity of marine fungi including seawater temperature, pressure, pH, and salinity (Jones 2000). However, according to Jones and Pang (2012), latitude and seawater temperature represent the limiting factor for fungal species composition in several habitats.

According to their biogeographical distribution, marine fungi are classified as tropical, temperate, polar (Arctic and Antarctic), and cosmopolitan (Jones 1993). When compared with tropical and temperate ecosystems, there are few reports of marine fungi in polar environments, mainly surrounding Antarctica. Marine Antarctic fungi have been recovered from marine animals (Henríquez et al. 2014), driftwood (Pugh and Jones 1986), in coastal waters (Grasso et al. 1997), deep sea (Lopez-Garcia et al. 2001), from macroalgae (Loque et al. 2010; Godinho et al. 2013; Furbino et al. 2014, 2018; Duarte et al. 2016), deep sediments (Gonçalves et al. 2015), and seawater (Gonçalves et al. 2017). However, these reported substrates represent a short portion habitats among those that exist in the Antarctic Ocean. Table 10.1 shows the checklist of Antarctic fungi reported in different substrates in Antarctica.

Some lignicolous marine fungi present in Antarctic seawater were studied using wood baits. In the Sub-Antarctic South Georgia and South Orkney Islands as well as in Antarctic Signy Island, Pugh and Jones (1986) found, as predominant lignicolous fungi, *Monodictys pelagica*, *Ceriosporosis tubulifera*, *Ceriosporosis halina*, and *Remispora maritima*. Grasso et al. (1997) used baits of beech and poplar wood

Table 10.1 List of taxa of marine fungi present in different substrata in Antarctica

Fungal taxa	Substrate	References
Acremonium sp.	*Palmaria decipiens* and seawater	Godinho et al. (2013), Gonçalves et al. (2017), Poveda et al. (2018)
Antarctomyces pellizariae	*Ascoseira mirabilis*	Furbino et al. (2018)
A. psychrotrophicus	*Adenocystis utricularis*, *Ulva intestinalis*, and *Pyropia endiviifolia*	Loque et al. (2010), Godinho et al. (2013), Furbino et al. (2014)
Aspergillus pseudoglaucus	Seawater	Gonçalves et al. (2017)
A. conicus	*Adenocystis* sp.	Godinho et al. (2013)
A. protuberus	*P. endiviifolia*	Furbino et al. (2014)
A. tabacinus	*M. hariotii*	Furbino et al. (2014)
A. terreus	*Phaeurus antarcticus*	Godinho et al. (2013)
Aspergillus sp.	*P. decipiens*, *P. endiviifoli*, and *Monostroma hariotii*	Godinho et al. (2013), Furbino et al. (2014)
Aureobasidium pullulans	*Desmarestia anceps*	Loque et al. (2010)
Beauveria bassiana	*Ascoseira mirabilis*	Furbino et al. (2018)
Cadophora malorum	*P. endiviifolia*	Furbino et al. (2014)
Cadophora sp.	Shallow marine sediment	Wentzel et al. (2019)
Candida sake	*Acrosiphonia arcta* and *Desmarestia menziesii*	Godinho et al. (2013), Duarte et al. (2016)
C. spencermartinsiae	Seawater	Vaz et al. (2011)
C. zeylanoides	Seawater	Vaz et al. (2011)
Ceriosporopsis halima	Wooden panels of balsa	Pugh and Jones (1986)
C. tubulifera	Wooden panels of balsa	Pugh and Jones (1986)
Chaetomium sp.	*P. decipiens*	Godinho et al. (2013)
Cladosporium lignicola	*P. endiviifolia*	Furbino et al. (2014)
C. tenuissimum	*A. arcta*	Godinho et al. (2013)
C. sphaerospermum	Seawater	Gonçalves et al. (2017)
Cladosporium sp.	*A.* mirabili, *A. arcta*, *Georgiella confluens*, *M. hariotii*, *P. endiviifolia,* and Shallow marine sediment	Godinho et al. (2013), Furbino et al. (2014), Furbino et al. (2018), Wentzel et al. (2019)
Comospora sp.	Deep marine sediment	Gonçalves et al. (2015)
Coprinellus radians	*Georgiella confluens*	Furbino et al. (2018)
Cordyciptaceae sp.	*M. hariotii*	Godinho et al. (2013)
Cryptococcus adeliensis	*M. hariotii*	Furbino et al. (2014)
C. albidosimilis	*M. hariotii*	Furbino et al. (2014)
Cryptococcus carnescens	*P. decipiens* and *Himantothallus grandifolius*	Loque et al. (2010), Duarte et al. (2016)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
C. cf. *laurentii*	*M. hariotii*	Godinho et al. (2013)
C. magnus	*P. decipiens*	Duarte et al. (2016)
Vishniacozyma victoriae	*M. hariotii*, *H. grandifolius*, *U. intestinalis,* and shallow sediments	Furbino et al. (2014), Duarte et al. (2016), Godinho et al. (2013), Vaz et al. (2011)
Cryptococcus sp.	*H. grandifolius* and shallow sediment	Duarte et al. (2016), Wentzel et al. (2019)
Cystobasidium slooffiae	Seawater	Gonçalves et al. (2017)
Cystobasidium sp.	Shallow marine sediment	Wentzel et al. (2019)
Cystofilobasidium infirmominiatum	*M. hariotii*	Furbino et al. (2014)
Debaryomyces hansenii	*A. arcta* and *A. utricularis*	Godinho et al. (2013)
Dioszegia athyri	*P. decipiens*	Duarte et al. (2016)
D. xingshanensis	*H. grandifolius*	Duarte et al. (2016)
Dipodascus australiensis	*P. endiviifolia*	Furbino et al. (2014)
Doratomyces sp.	*Iridaea cordata*	Furbino et al. (2018)
Engyodontium sp.	*U. intestinalis*	Godinho et al. (2013)
Eurotium herbariorum	*P. antarcticus*	Godinho et al. (2013)
E. repens	*P. antarcticus*	Godinho et al. (2013)
Exophiala xenobiotica	Seawater	Gonçalves et al. (2017)
Fusarium sp.	*P. decipiens*	Godinho et al. (2013)
Glaciozyma litorale	*Gigartina skottsbergii*, *A. utricularis*, *Desmarestia menziesii,* and *I. Cordata*	Duarte et al. (2016)
G. martinii	*G. skottsbergii*	Duarte et al. (2016)
G. antarctica	Seawater	Gonçalves et al. (2017)
Glaciozyma sp.	Shallow marine sediment	Wentzel et al. (2019)
Graphium rubrum	Seawater	Gonçalves et al. (2017)
Guehomyces pullulans	*M. hariotii*	Furbino et al. (2014)
Helotiales sp.	*M. harioti*, *U. intestinalis*, and deep sediment	Godinho et al. (2013), Gonçalves et al. (2015)
Holtermanniella nyarrowii	*H. grandifolius*	Duarte et al. (2016)
H. festucosa	*H. grandifolius*	Duarte et al. (2016)
Holtermanniella sp.	Shallow marine sediment	Wentzel et al. (2019)
Hyaloscyphaceae sp.	*M. hariotii*	Godinho et al. (2013)
Lecanicillium sp.	*P. endiviifolia*	Furbino et al. (2014)
L. attenuatum	Seawater	Gonçalves et al. (2017)
Leucosporidiella creatinivora	*Tedania* sp. and seawater	Vaca et al. (2013), Vaz et al. (2011)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
L. fragaria	*A. mirabilis* and *H. grandifolius*	Duarte et al. (2016), Furbino et al. (2018)
L. muscorum	*A. mirabilis, H. grandifolius*, *G. skottsbergii,* and shallow sediment	Furbino et al. (2018), Duarte et al. (2016), Vaz et al. (2011)
Leucosporidium scottii	Shallow marine sediment and seawater	Vaz et al. (2011)
Metschnikowia australis	*A. utriculari, D. anceps*, *P. decipien, M. hariotii*, *A. mirabilis*, *A. arcta*, *D. menziesii*, *P. endiviifolia*, *Cystosphaera jacquinotii*, *H. grandifolius*, *I. cordata*, *Curdiea racovitzae*, *G. skottsbergii*, *Curdiea racovitzae*, *Georgiella confluens*, *Dendrilla* sp., *Tedani* sp., *Hymeniacidon* sp., shallow sediment, and seawater	Loque et al. (2010), Vaz et al. (2011), Godinho et al. (2013), Furbino et al. (2014), Duarte et al. (2016), Furbino et al. (2018)
Metschnikowia sp.	Shallow marine sediment	Wentzel et al. (2019)
Meyerozyma caribbica	*A. utricularis*	Godinho et al. (2013)
M. guilliermondii	*M. hariotii* and *P. endiviifolia*	Furbino et al. (2014), Godinho et al. (2013)
Meyerozyma sp.	Shallow marine sediment	Wentzel et al. (2019)
Monodictys pelagica	wooden panels of vessel	Pugh and Jones (1986)
Mortierella antarctica	*P. endiviifolia*	Furbino et al. (2014)
Mortierella sp.	*A. arcta*	Godinho et al. (2013)
Mrakia sp.	*C. jacquinotii*, *G. skottsbergii*, *H. grandifolius*, *M. hariotii*, *D. menziesii*, and shallow sediment	Duarte et al. (2016), Wentzel et al. (2019)
Mycoarthris cf. *corallines*	*U. intestinalis*	Godinho et al. (2013)
Oidiodendron sp.	*A. utricularis*	Loque et al. (2010)
O. truncatum	*P. endiviifolia*	Furbino et al. (2014)
Paraconiothyrium sp.	Shallow marine sediment	Wentzel et al. (2019)
Penicillium chrysogenum	Seawater and *A. mirabilis*	Gonçalves et al. (2017), Furbino et al. (2018)
P. citreosulfuratum	Seawater	Gonçalves et al. (2017)
P. citrinum	*Adenocystis utricularis* and *M. hariotii*	Godinho et al. (2013), Furbino et al. (2014)
P. crustosum	*M. hariotii*	Furbino et al. (2014)
P. discolor	*U. intestinalis*	Godinho et al. (2013)
P. solitum	Shallow to deep marine sediment	Gonçalves et al. (2013)
P. spinulosum	*P. decipiens*	Godinho et al. (2013)
P. steckii	*M. hariotii* and *P. antarcticus*	Furbino et al. (2014), Godinho et al. (2013)
*P.*cf. *rubens*	Seawater	Gonçalves et al. (2017)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
Penicillium sp.	*A. utricularis*, *P. decipiens*, *A. arcta, D. menziesii*, *P. endiviifolia*, *M. hariotii*, *Adenocystis* sp., *I. cordata*, *U. intestinalis*, *P. decipiens*, *P. antarcticus*, *G. skottsbergii*, *G. confluens*, seawater, and shallow sediment	Loque et al. (2010), Godinho et al. (2013), Furbino et al. (2014), Duarte et al. (2016), Furbino et al. (2018), Gonçalves et al. (2017), Wentzel et al. (2019)
Pestalotiopsis sp.	Shallow marine sediment	Wentzel et al. (2019)
Phaeosphaeria herpotrichoides	*A. utricularis*	Loque et al. (2010)
Phenoliferia sp.	Shallow marine sediment	Wentzel et al. (2019)
Phoma sp.	*A. arcta*	Godinho et al. (2013)
Pleosporaceae sp.	Deep marine sediment	Gonçalves et al. (2015)
Pseudocercosporella sp.	Shallow marine sediment	Wentzel et al. (2019)
Pseudogymnoascus destructans	*M. hariotii*	Godinho et al. (2013)
P. luteus	*U. intestinalis*	Godinho et al. (2013)
P. pannorum	*A. utricularis* and *D. anceps*	Loque et al. (2010)
Pseudogymnoascus sp.	*M. hariotii*, *P. endiviifolia*, *I. cordata*, *A. arcta*, *Adenocystis* sp., *P. decipiens*, *P. Antarcticus,* and shallow sediment	Furbino et al. (2014), Godinho et al. (2013), Gonçalves et al. (2015)
Pseudozyma tsukubaensis	*A. utricularis*	Duarte et al. (2016)
Purpureocillium lilacinum	Seawater	Gonçalves et al. (2017)
Phenoliferia glacialis	*G. skottsbergii*, *H. grandifolius*, and shallow sediment	Duarte et al. (2016), Vaz et al. (2011)
R. laryngis	*M. hariotii* and seawater	Furbino et al. (2014), Vaz et al. (2011)
R. marina	*P. decipiens*	Duarte et al. (2016)
R. minuta	*M. hariotii*	Furbino et al. (2014)
R. mucilaginosa	*A. utricularis*, *P. decipiens*, *M. hariotii,* and *G. confluens*	Loque et al. (2010), Furbino et al. (2014, 2018)
R. pinicola	*Hymeniacidon* sp.	Vaca et al. (2013)
Rhodotorula sp.	Shallow sediment	Wentzel et al. (2019)
Schizophyllum commune	Deep marine sediment	Gonçalves et al. (2015)
Simplicillium lamellicola	Deep marine sediment	Gonçalves et al. (2015)
S. aogashimaense	Seawater	Gonçalves et al. (2017)
Sporidiobolus pararoseus	*A. utricularis* and *Curdiea racovitzae*	Duarte et al. (2016)

(continued)

Table 10.1 (continued)

Fungal taxa	Substrate	References
Thelebolus globosus	*A. arcta* and *U. intestinalis*	Godinho et al. (2013), Furbino et al. (2014)
Tilletiopsis washingtonensis	*P. decipiens*	Duarte et al. (2016)
Toxicocladosporium sp.	Shallow marine sediment	Wentzel et al. (2019)
Ustilaginaceae	*A. utricularis*, *D. anceps*, and *P. decipiens*	Duarte et al. (2016)
Verticillum sp.	*P. endiviifolia*	Furbino et al. (2014)
Yamadazyma mexicana	*P. decipiens*	Godinho et al. (2013)

at a depth of 50 m, and after 1 year, they processed the baits and detected the presence of 14 *Ascomycota* species, with *Phoma* sp., *Trichocladium achrasporum*, *Trichocladium constrictum*, and *Trichocladium lignincola* being the dominant species/lineages.

Antarctic macroalgae represent the most studied marine substrate/host of marine fungi. The first study was conducted by Loque et al. (2010), who selected the macroalgae *Adenocystis utricularis*, *Desmarestia anceps*, and *Palmaria decipiens* to recover associated fungi. The authors recovered 75 fungal isolates, represented by 27 filamentous fungi and 48 yeasts of the genera *Pseudogymnoascus*, *Antarctomyces*, *Oidiodendron*, *Penicillium*, *Phaeosphaeria*, *Aureobasidium*, *Cryptococcus*, *Leucosporidium*, *Metschnikowia*, and *Rhodotorula*. After this pioneer study, Godinho et al. (2013), Furbino et al. (2014), Furbino et al. (2018), and Duarte et al. (2016) confirmed that Antarctic macroalgae shelter a rich and diverse fungal community, which include taxa with different ecological roles and possibly those which contribute to cycling organic matter in the Southern Ocean.

Fungi have already been detected in deep-sea sediments of the Atlantic, Pacific, and China Oceans. However, studies of the presence of fungi in deep sediments of the Southern Ocean surrounding Antarctica are in initial stages. Vaz et al. (2011) identified few yeast species (*Candida glaebosa*, *Vishniacozyma victoriae*, *Leucosporidiella muscorum*, *Metschnikowia australis*, *Nadsonia commutate*, and *Phenoliferia glacialis*) from the shallow marine sediments of Antarctica. Wentzel et al. (2019) processed 5 cm deep-sea sediment samples from the Admiralty Bay of the King George Island and obtained 226 fungal isolates containing species from 17 genera.

Apparently, Gonçalves et al. (2013) represented the first study focusing on cultivable fungi present in deep-sea sediments of Antarctica. They explored marine sediments from 100, 500, 700, and 1100 m depths and obtained 52 fungal isolates using the USNEL-type box corer, one of the most common devices utilized to sample deep marine sediments (Fig. 10.5). In this study, all fungal isolates were identified by polyphasic taxonomy as *Penicillium solitum*. The authors showed that conidial germination of *P. solitum* occurred at low temperatures, high salinities, and extracellular amylasic and esterasic activities, demonstrating its adaptability to extreme conditions of Southern Ocean.

Fig. 10.5 Details of the USNEL-type box corer utilized to sample deep marine sediments (**a**) General view of the process of sampling marine sediments in Antarctica using the box corer, (**b**) box corer with Antarctic marine sediments, (**c**) preparation of marine sediment corers, and (**d**) corer of Antarctic marine sediment. (Photos Credits: LH Rosa)

This pioneer study performed by Gonçalves et al. (2013) suggested that Antarctic marine sediments, mainly those from deep sea, might represent an interesting microhabitat to recover and study the biology of barophilic/psychrophilic fungi. However, to recover and better characterize these fungal communities, new studies using different culture media, pressure conditions, and metagenomic techniques are necessary to understand the complexity, ecological role, and biotechnological potential of these extremophilic fungi. An interesting result was that the sequences of *P. solitum* (Fig. 10.6) isolated from the Southern Ocean showed high query coverage and similarities with sequences of *Penicillium* taxa detected in different parts of the world, mainly those obtained from marine ecosystems like sediments of the Yellow Sea and the tropical Pacific Ocean, seawater of the East Pacific Ocean (both in China), macroalgae from the Mediterranean Sea along the coast of Spain, and marine air in Germany. These results might suggest that fungi with high genetic and physiological plasticity (like *P. solitum*) may use the marine current to disperse from Antarctica to other parts of the world and vice versa.

Lopez-Garcia et al. (2001) suggested that fungi from deep-sea environment are some of the few eukaryotes in the aphotic zone between 250 and 3000 m below the Southern Ocean. In a preliminary study on seawater samples of the Antarctic Peninsula, Vaz et al. (2011) detected the yeast species *Candida spencermartinsiae*, *Candida zeylanoides*, *Leucosporidiella creatinivora*, *Leucosporidium scottii*, *Metschnikowia australis,* and *Rhodotorula laryngis*. Further, from seawater at different depths and sites across the Gerlache and Bransfield Straits of the northern Antarctic Peninsula, using a combined Sea-Bird CTD equipped with 24 5L Niskin Sampling Bottles (Fig. 10.7), Gonçalves et al. (2017) recovered species of *Acremonium*, *Aspergillus*, *Cladosporium*, *Cystobasidium*, *Exophiala*, *Glaciozyma*, *Graphium*, *Lecanicillium*, *Metschnikowia*, *Penicillium*, *Purpureocillium*, and

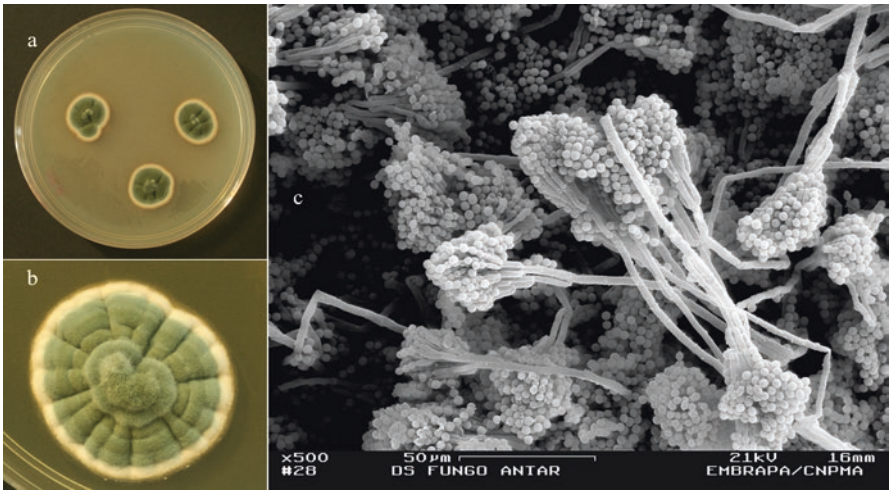

Fig. 10.6 *Penicillium solitum* isolated from marine sediments of Antarctica (**a**) Colonies on malt extract agar, (**b**) colony details, and (**c**) scanning electron microscopy details of its asexual structures. (Photos Credits: LH Rosa)

Fig. 10.7 Sequential process of seawater sampling using a combined Sea-Bird CTD equipped with 24 5L Niskin Sampling Bottles. (Photos Credits: LH Rosa)

Simplicillium. Among these, *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, and *Graphium rubrum* were obtained at high densities, suggesting their ability to survive in the extreme conditions of seawater. These authors hypothesized that the marine fungal web of seawater is complex and includes species cited as barophiles, symbionts, weak and strong saprobes, parasites, pathogens, and some taxa with genetic similarities with those present in polluted environments around the world.

10.6 Conclusions and Perspectives

The different substrates and ecological niches of the Southern Ocean surrounding Antarctica represent the few unexplored frontiers of the planet that can be used to discover different extremophile fungal communities. In the Antarctic marine ecosystems, seawater, sea ice, deep-sea sediments, macroalgae, invertebrates, and vertebrates remain practically unexplored as microhabitats of Antarctic fungi. However, some abiotic factors, such as high pressure, affect access to the marine fungi for sampling purposes. Probably, there are new species and taxa able to produce bioproducts useful in biotechnological processes yet unknown in the Southern Ocean. Further interdisciplinary studies involving microbiology, phycology, oceanography, and geology have to be conducted to understand the total richness, diversity, and biotechnological potential of Antarctic marine fungi.

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Chapter 11
The Use of Psychrophilic Antarctic Yeast in the Biological Control of Post-harvest Diseases of Fruits Stored at Low Temperatures

Eskálath Morganna Silva Ferreira, Deyse Alencar Resende, Silvana Vero, and Raphael Sanzio Pimenta

11.1 Introduction

The current primary strategy used to control the post-harvest losses of fruits and vegetables is the utilization of massive amounts of chemical fungicides (Pimenta et al. 2009; Zhou et al. 2014; Spadaro and Droby 2016; Usall et al. 2016a; Ferreira et al. 2018). However, there is now a global trend towards a reduction in the use of toxic chemicals, mainly due to an increased concern with their negative effects on public health and the environment. Thus, the need for alternative strategies such as biological control is becoming more evident (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a). Research on the biological control of post-harvest diseases, using antagonistic yeasts, has received considerable attention from researchers in the last decades and represents one of the approaches used to guarantee the quality and safety of vegetables, grains, and mainly fruits (Droby et al. 2016). This chapter discusses how, among the different groups of microorganisms used as biological control agents, yeasts have been the most used mainly for promoting the control of several phytopathologies, both under pre-harvest and post-harvest conditions. The use of these microorganisms is a promising alternative for the total or partial replacement of the use of chemical pesticides (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a).

Yeasts that are naturally found colonizing plant surfaces are termed epiphytic and represent the main group of yeasts used to manage post-harvest diseases.

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However, other sources, such as seawater, soil samples, and plant exudates, are being studied to obtain antagonistic microorganisms. The main intention in exploring new sources of isolation is to identify yeasts that may develop in stressful environments and have different mechanisms of action (Liu et al. 2013). This study aims to better adapt the adverse conditions to which they can be subjected when used as a biocontrol agent (Liu et al. 2013).

The use of microorganisms isolated from cold environments such as those from the Antarctic, Tibetan, polar sea, and other low temperature regions may favour the development of biocontrolled yeasts previously adapted to cold which present a higher tolerance to this stress abiotic. This feature favours their use as biocontrollers in fruits that are stored and/or transported in cold chambers (Wang et al. 2010a, b; Lutz et al. 2012; Vero et al. 2013; Hu et al. 2017).

Several investigations have already been carried out to identify and to evaluate potential biocontrol agents isolated from cold environments. However, the work in this line of research is still limited, especially in the Antarctic region (Wang et al. 2010a; Lutz et al. 2012; Vero et al. 2013; Arrarte et al. 2017; Hu et al. 2017). Therefore, the purpose of this chapter is to discuss the use of psychrophilic yeasts as biological control agents for the management of post-harvest diseases of fruits stored at low temperature.

11.2 Post-harvest Diseases of Fruits Stored at Low Temperature

Post-harvest losses of fruits and vegetables caused by fungi are the main causes of economic losses in food production. It is estimated that about 20–25% of these products are lost during storage in developed and developing countries; these losses may be even higher and exceed 50% (Lima et al. 2015; Spadaro and Droby 2016; Usall et al. 2016a). Strategies to reduce these losses are very important because there is an increase in the global demand for food and areas of agricultural expansion are becoming limited. Fungal degradation of food takes a toll not only on the economy but also on the health of the population, since many pathogens can release toxic substances in food or directly cause infections in consumers (Wilson 2013; Dukare et al. 2018).

Rigorous sanitation, fungicide use, cold storage, modified atmosphere application, and other physical and/or chemical tools are used to reduce or delay the development of pathogens (Sangorrín et al. 2014). Most fruits have a very limited shelf-life after harvest if they are kept at normal storage temperatures. For this reason, refrigeration is one of the most used techniques for the preservation of fresh fruits, since it reduces metabolism, decreases weight loss, slows senescence, and delays development of some deteriorating fungus species (Usall et al. 2016b).

Some species of phytopathogenic fungi can grow rapidly under low temperatures, such as *Alternaria alternata*, which can degrade papaya, apple, and various horticultural crops (Troncoso-Rojas et al. 2014). *Penicillium expansum* is a psy-

chrophilic fungus able to grow at 0 °C, which decays mainly apples, grapes, and pears during cold storage (Morales et al. 2010; Zoffoli and Latorre 2011). *Penicillium digitatum* and *Penicillium italicum* are the main agents of blue rot and green rot, post-harvest diseases affecting citrus, and can grow during refrigerated storage of fruits (Plaza et al. 2003; Pimenta et al. 2008; El-Otmani et al. 2011). *Cladosporium herbarum* and *Cladosporium cladosporioides* cause post-harvest disease in grapes stored at 0 °C (Zoffoli and Latorre 2011). *Botrytis cinerea fungus* is capable of degrading strawberries at low temperatures (0–5 °C) (Feliziani and Romanazzi 2016).

However, other fungi causing post-harvest diseases can grow at temperatures just above 0 °C, such as *Monilinia fructicola* (1 °C), the most economically important pathogen for stone fruit such as plums and peach; *Aspergillus niger* (11 °C); *Colletotrichum gloeosporioides* (9 °C), which causes rot in papaya, mango, and citrus; *Colletotrichum musae* (9 °C), anthracnose agent in bananas; and *Botryodiplodia theobromae* (8 °C) and *Ceratocystis paradoxa* (5 °C), disease agents in bananas and pineapples (Sommer 1985; Usall et al. 2016b). In the case of mesophilic phytopathogenic fungi, refrigeration is able to limit growth and prevent disease development. However, for those who are called psychrophiles, cold storage is not sufficient to ensure satisfactory fruit preservation (Barkai-Golan 2001). In view of this, control alternatives that are complementary to or that increase the efficiency of the technologies already used to reduce or to delay the development of post-harvest pathogens can contribute to a more efficient way to the integrated control of these phytopathologies.

11.3 Biological Control

Chemical control methods using fungicides are conventionally the most widely used treatment for the prevention and control of fungal decomposition in fruits during field and post-harvest handling. They are consistently used with systems management practices and storage at low temperatures. However, the use of many synthetic fungicides has been reduced in the last decade due to several factors such as environmental pollution, selection of resistant pathogens, occurrence of outbreaks of diseases considered as secondary, reduction of populations of beneficial microorganisms, and presence of toxicity to human and other animals (Pimenta et al. 2009; Spadaro and Droby 2016; Usall et al. 2016a; Zhou et al. 2014; Dukare et al. 2018; Ferreira et al. 2018).

As a result, the management of fruits has been changing, due to growing consumer concern with impacts on public health and the environment. Moreover, the legislation has become more restrictive and rigorous regarding the registration and application of chemicals and minimum residues tolerated in food, especially at the post-harvest stage. For this reason, there is an ongoing search for alternative control methods, such as biological approaches that include the use of antagonistic microorganisms (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a). In con-

trast to chemical treatments, the use of biological control through the use of microbial antagonists does not leave toxic residues in the fruits, but generally requires integrated action with other control methods (Lima et al. 2015; Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a).

According to Cook and Baker (1983), biological control involves a reduction in the inoculum and disease-causing activities caused by a pathogenic organism, using one or more other organisms. According to Lima et al. (2000), the basic premise of biological control is the maintenance of the population density of pest or pathogen species associated with agriculture at economically and ecologically acceptable levels. During the post-harvest period, biological control can completely replace or act in association with chemical control, making food storage more sustainable and safe and reducing the pathogen inoculum or the intensity of disease symptoms (Mondino and Vero 2006).

There are two basic sources of microorganisms that can be used as microbial antagonists in the control of post-harvest diseases. First is the use of microorganisms that occur naturally in the product (fruit and vegetable surface), and second is the artificial introduction of antagonistic microorganisms to the pathogens obtained from other substrates (Sharma et al. 2009). Numerous microorganisms, such as yeast and bacteria, have been used in laboratory, semi-commercial and commercial studies as antagonists. However, the potential of yeasts as post-harvest biocontrol agents has been widely demonstrated in the scientific literature (Droby et al. 2009, 2016).

Another aspect associated with traditional biological control is integrated biological control, which involves the use of an organism antagonistic to the pathogen to be controlled using Generally Regarded As Safe (GRAS) compounds, which contain micro-biostatic or microbiocidal chemicals. Strategies combining microbiostatic and microbiocidal approaches can produce a synergistic effect and potentiate the results in comparison to the classical biological control and have, therefore, also been widely explored by researchers in the field (Pimenta et al. 2012).

11.4 Yeasts as Biocontrol Agents

Yeasts are defined as fungi belonging to *Ascomycota* or *Basidiomycota* phyla, whose sexual state has no fruiting bodies and vegetative growth occurs by budding or fission. They are predominantly unicellular, immobile microorganisms. Most of them are saprobic and some are opportunistic parasites (Miller 1979; Boekhout and Kurtzman 1996; Lachance and Starmer 1998; Kurtzman et al. 2011).

The ability of these microorganisms to assimilate a wide variety of organic compounds expands their dispersal and survival capacity in different ecological niches in terrestrial (plants, soil, animals), air, and aquatic environments (lakes, rivers, seas) (Phaff and Starmer 1987). They have several characteristics that make them good candidates as biocontrol agents, such as a high nutrient utilization capacity, which allows them to proliferate rapidly (Lima et al. 1997, 2000; Spadaro et al.

2004); the production of extracellular polysaccharides that increase their ability to survive in several environments, thereby restricting space for the development of phytopathogenic agents (Mendéz an Mondino 1999); and tolerance for the fungicides frequently used during post-harvest (Spadaro et al. 2004). There are two basic sources of microorganisms that can be used as microbial antagonists in the control of post-harvest diseases. First is the use of microorganisms that occur naturally in the product (fruit and vegetable surface), and second is the artificial introduction of antagonistic microorganisms to the pathogens obtained from other substrates yeasts in the control of post-harvest diseases due to the fact that these organisms are the major components of the microbial community on the leaf and fruit surfaces (Wilson et al. 1993). Yeasts may be effective control agents because they are phenotypically more adapted to these niches and are skilled in the colonization and competition for space and nutrients (Filonow 1998). Another advantage of the use of yeasts in the biocontrol of plant diseases is consumer acceptance, which is due to the fact that yeasts are widely used for food and beverage production (Wisniewski et al. 2016).

In the case of epiphytic microorganisms, due to the physical and environmental conditions (humidity, temperature, luminosity) of the phylloplane being quite variable, the colonization process is influenced by a series of specific factors. In the leaves, for example, the important factors are the thin layer of air that covers the surface of the leaves and retains vapours of water emitted from the stomata and the veins (Axtell and Beattie 2002), release of simple sugars 'leached' from the interior of the plant by means of small lesions or glandular trichomes (Mercier and Lindow 2000; Lindow and Brandl 2003), and the deposition of exogenous nutrients such as pollen and insect secretions (Warren and Dias 2001; Fokkema et al. 1983). The production of surfactants by some microorganisms reduces the hydrophobic effects of plant cuticles on the water scattering and diffusion of substrates (Bunster et al. 1989; Hutchison et al. 1995). Other microorganisms are producers of substances that affect the transport of ions in plant cell membranes (Hutchison et al. 1995). For these reasons, epiphytic microorganisms are also plant growth promoters and have been extensively studied for use in the biocontrol of post-harvest diseases in fruits and vegetables (Wilson and Wisniewski 1989) and in the control of foliar diseases (Lee et al. 2017). Yeasts are known to efficiently colonize the epiphytic environment, which could antagonize the introduction and development of plant pathogens (Buck and Burpee 2002; Fonseca and Inácio 2006).

Yeasts are particularly interesting microorganisms for use in a Biological Control program because they are relatively easy to grow and have several characteristics that can be manipulated to improve their use and efficiency (Pimenta et al. 2009; Sharma et al. 2009; Janisiewicz et al. 2011). Yeast species have been used as biological control agents to control *B. cinerea*, causal agent of grey mould in grapes and strawberries; *P. digitatum* in grapes; *P. italicum* and *P. digitatum* in citrus; *Botrytis*, *Rhizopus*, *Penicillium,* and *Alternaria* in tomato; and *B. cinerea* and *Rhizopus* fungi, which cause post-harvest diseases in apples (Mehrotra et al. 1996; Jijakli and Lepoivre 1998; Guetsky et al. 2001; Masih et al. 2001; Jijakli and Kupper et al. 2013) (Table 11.1). Despite the knowledge about some functions of yeasts in the environment, much remains to be discovered, especially on the mechanisms of

Table 11.1 Yeasts used as a biocontrol agent obtained from several sources of insulation

Antagonist yeast	Disease (pathogen)	Fruits	References
Aureobasidium pullulans	Grey mould (*Penicillium expansum*)	Apple	Vero et al. (2009)
Candida sake	Grey mould (*Botrytis cinerea*), Penicillium rot (*Penicillium expansum*)	Apple	Wilson et al. (1993)
	Penicillium rot (*Penicillium expansum*)	Apple	Vinas et al. (1996)
	Rhizopus rot (*Rhizopus nigricans*)	Apple	Vinas et al. (1998)
	Penicillium rot (*Penicillium expansum*)	Apple	Usall et al. (2001)
	Penicillium rot (*Penicillium expansum*)	Apple	Torres et al. (2006)
	Penicillium rot (*Penicillium expansum*)	Apple	Morales et al. (2008)
Candida oleophila	Penicillium rot *(Penicillium expansum*)	Apple	El-Neshawy and Wilson (1997)
	Anthracnose (*Colletotrichum gloeosporioides*)	Papaya	Gamagae et al. (2003)
	Grey mould (*Botrytis cinerea*)	Peach	Karabulut and Baykal (2004)
	Penicillium rots (*Penicillium digitatum*, *Penicillium italicum*)	Citrus	Lahlali et al. (2004, 2005)
Candida guilliermondii	Grey mould (*Botrytis cinerea*)	Nectarine / peach	Tian et al. (2002)
	Grey mould (*Botrytis cinerea*)	Tomato	Saligkarias et al. (2002)
Candida ciferrii	Blue mould (*Penicillium expansum*)	Apple	Vero et al. (2002)
Cystofilobasidium infirmominiatum	Blue mould (*Penicillium italicum*)	Citrus	Vero et a. (2011)
Cryptococcus laurentii	Bitter rot (*Glomerella cingulata*)	Apple	Blum et al. (2004)
	Grey mould (*Botrytis cinerea*)	Peach	Zhang et al. (2007a)
	Rhizopus rot (*Rhizopus stolonifer*)	Strawberry	Zhang et al. (2007b)
Debaryomyces hansenii	Green and blue mould (*Penicillium digitatum*, *Penicillium italicum*)	Citrus	Singh (2002)
	Rhizopus rot (*Rhizopus stolonifer*)	Peach	Mandal et al. (2007)
	Blue rot (*Penicillium italicum*)	Lemon	Hernández-Montiel et al. (2010)
	Anthracnose (*Colletotrichum gloeosporioides*)	Papaya	Hernández-Montiel et al. (2018)

(continued)

Table 11.1 (continued)

Antagonist yeast	Disease (pathogen)	Fruits	References
Metschnikowia fructicola	Blue mould (*Penicillium expansum*) and grey mould (*Botrytis cinerea*)	Apple	Spadaro et al. (2002, 2004)
	Botrytis rot (*Botrytis cinerea*)	Grapes.	Kurtzman e Droby (2001)
Pichia guilliermondii	Blue mould (*Penicillium expansum*)	Apple	McLaughlin et al. (1990)
	Grey mould (*Botrytis cinerea*)	Apple	Janisiewicz et al. (1998)
	Green mould (*Penicillium digitatum*)	Citrus	Chalutz and Wilson (1990)
	Rhizopus rot (*Rhizopus stolonifer*)	Grape	Chalutz et al. (1988)
Pichia guilliermondii	Grey mould (*Botrytis cinérea*)	Tomato	Chalutz et al. (1988)
	Rhizopus rot (*Rhizopus nigricans*)	Tomato	Zhao et al. (2008)
Saccharomyces cerevisiae	Athracnose (*Colletotrichum musae)*	Banana	Zhimo et al. (2016)
Saccharomycopsis schoenii	Blue mould (*P. expansum* and *P. italicum*)	Citrus	Pimenta et al. (2008)
Rhodotorula glutinis	Blue mould (*Penicillium expansum*)	Apple	Calvo et al. (2007)
	Grey mould (*Botrytis cinerea*)	Apple	Zhang et al. (2009)
	Blue rot (*Penicillium expansum*)	Pear	Zhang et al. (2008)
Rhodotorula mucilaginosa	Grey mould (*Botrytis cinerea*)	Strawberries	Zhang et al. (2013)

action of most biocontrol agents (Rosa-Magri et al. 2011). These biological agents can act in complex interactions between the host (fruit), pathogen, antagonist, and environment (Fig. 11.1). The main interaction processes are: mycoparasitism, lytic enzyme production, predation, resistance induction, competition for space and/or nutrients, among others. Often more than one mechanism of antagonism is involved in the process of action of biological control agents (Pimenta et al. 2008; Di Francesco et al. 2015; Lima et al. 2015; Droby et al. 2016; Spadaro and Droby 2016).

Among the mechanisms of action, competition for space and nutrients (e.g. carbohydrates and nitrogen sources) is considered the main way in which yeasts suppress the development of pathogens. For this reason, the biocontrol agent must have the ability to grow rapidly and efficiently and remain viable in the substrate to be protected in order to limit the establishment of the pathogen in the fruits (Nunes et al. 2001). These mechanisms have already been demonstrated in several studies as developed by Zhang et al. (2010) when using *P. guilliermondii* against *B. cinerea* in apples and Lutz et al. (2012) when evaluating the mechanisms of action

Fig. 11.1 Improved strategy for the selection of Antarctic antagonist yeasts to control post-harvest strawberry diseases and the mechanism of their action**.** (**1**) and (**2**) Isolation of antagonist yeasts of angiosperms from Antarctica; (**3**) Biological control test in strawberries containing the antagonistic yeast and the pathogen; (**4**) Pathogen developing the disease without the presence of yeast; (**5**) Yeasts promoting biological control in strawberry. (Source: authors)

associated with the biocontrol capacity of four yeast strains (*Cryptococcus albidus* strains NPCC 1248 and NPCC 1250, *Pichia membranifaciens,* and *Vishniacozyma victoriae*) against *Penicillium expansum* and *Botrytis cinerea* in pears; and the application of *Debaryomyces hansenii* yeast in the control of *Penicillium digitatum* in grapes (Droby et al. 1989) and *Aureobasidium pullulans* (Janisiewicz et al. 2000).

Mycoparasitism was a mechanism of action observed in the studies conducted by Zhang et al. (2010); Arras et al. (1998) found that *P. guilliermondii* caused changes in the hyphae of the fungi *Botrytis cinerea* and *Penicillium digitatum*. Predation was observed by Pimenta et al. (2008) when studying the activity of the yeast *Saccharomycopsis schoenii* against the fungus *P. digitatum*, which is a pathogenic agent in oranges; the authors observed that hyphae and spores of the pathogen were predated by the yeast.

Antibiosis is the production of metabolites that are generated by the yeast antagonists which have inhibitory effects on the growth or germination of the pathogen through the production of antifungal compounds such as peptides and volatile metabolites. Some yeast strains may produce extracellular proteins or toxins called killer toxins, which are lethal to sensitive microbial cells (Spadaro and Droby 2016). Kupper et al. (2013) evaluated the antagonistic potential of *Saccharomyces cerevisiae* against *P. digitatum* and observed inhibition of fungus growth by the production of inhibitory substances. The production of volatile compounds was observed by Arrarte et al. (2017) who demonstrated that the psychrophilic yeast *Candida sake* played a significant role in reducing the growth of *P. expansum* in apples stored at low temperatures. Rosa-Magri et al. (2011) investigated the production of killer toxins as control mechanisms and verified that the yeasts *Torulaspora globosa* and *Candida sublineola* controlled the growth of *Colletotrichum graminicola*.

The production of lytic enzymes may also play an important role in biocontrol activity. The production of chitinase, β-glucanase, and chitosanases, which are the main components of the wall of fungal cells, antagonistic yeasts that have high enzymatic activity related to β-1,3-glucanase and chitinases (endo and exo), could cause lysis of the cell wall of phytopathogenic agents (Di Francesco et al. 2015). However, there is also evidence that biocontrol agents have the ability to induce disease resistance by activation of fruit defence enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase, and polyphenoloxidase (Di Francesco et al. 2015; Liu et al. 2013). In works by Wang et al. (2010a, b), inhibitory actions promoted by enzymes of the β-1,3-glucanase (GLU), phenylalanine ammonia (PAL), peroxidase (POD), and polyphenoloxidase (PPO) were observed to be responsible for the control of pathogens. Hernandez-Montiel et al. (2010) also found that strains of *Debaryomyces hansenii* inhibited the growth of *Penicillium italicum* by detecting β-1,3-glucanase production and protease activity. In a more recent work, Hernandez-Montiel et al. (2018) also observed that the same enzymes were associated with the biocontrol capacity of *D. hansenii* against *Colletotrichum gloeosporioides*.

Biological control using cold-adapted yeasts has been cited as an important alternative to the use of synthetic chemical fungicides for the management of post-harvest fruit rot (Santos et al. 2004; Nally et al. 2012; Sriram and Poornachandra 2013; Sukorini et al. 2013; Lopes et al. 2015; Zhimo et al. 2016).

11.5 Antarctic Psychrophilic Yeasts as Biocontrol Agents in Post-harvest of Fruits

Antarctica is a continent that presents a set of characteristics that make it inhospitable for the survival of many organisms, because it has the coldest and driest climate on the planet. Despite this, Antarctica presents enormous biological diversity (Ruisi et al. 2007; Yergeau and Kowalchuk 2008; Onofri et al. 2008; Rosa et al. 2010; Margesin and Miteva 2011). Among the organisms adapted to these environmental conditions are microorganisms and among them the yeasts which may present great potential for use as biocontrol agents (Rosa et al. 2010; Nascimento et al. 2015). In addition, the microbial diversity in this environment is at an early stage of discovery. (Ruisi et al. 2007).

The yeast communities that inhabit the Polar Regions can be classified as endemic or cosmopolitan. Although some endemic species have psychrophilic behaviour, most of them are psychro-tolerant yeasts that can adapt and grow over a wide temperature range. Connell et al. (2008) studied the diversity of yeasts isolated from the soil in the Antarctic region and found that 89% of the isolates were composed of basidiomycetes. De garcia et al. (2007) observed that the genus *Cryptococcus* was the most frequently isolated, followed by cold-adapted *Leucosporidium and Rhodotorula*. In a study conducted in glacial rivers of melted water in Argentine Patagonia, Buzzini et al. (2017) obtained different genera of yeasts in the Antarctic region, the most prevalent being: *Debaryomyces*, *Metschnikowia*, *Pichia*, *Cystobasidium*, *Dioszegia*, *Filobasidium*, *Glaciozyma*, *Holtermanniella*, *Malassezia*, *Mrakia*, *Naganishia*, *Papiliotrema*, *Phenoliferia*, *Sporidiobolus*, *Tausonia*, and *Vishniacozyma.*

Yeasts that develop in extremely cold environments are interesting as biological control agents for post-harvest diseases because they naturally produce compounds capable of allowing them to grow under extremely low temperature conditions such as those observed in cold storage chambers (Buzzini et al. 2012). Many species of psychrophilic yeasts were considered to be good antagonists against pear post-harvest pathogens (Lutz et al. 2012; Hu et al. 2015). An isolate of soil samples collected in Tibet identified as *Rhodotorula mucilaginosa* have presented biocontrol potential against fungi *Penicillium expansum* in pear (Hu et al. 2015). Using different criteria, *Rhodosporidium paludigenum*, obtained from cold sea water, showed remarkable activity against *Alternaria* rot in cherry tomatoes (Wang et al. 2008).

The knowledge of the diversity of yeasts in the Antarctic environment is recent (Connel et al. 2010; Carrasco et al. 2012; Godinho et al. 2013; Furbino et al. 2014; Zhang et al. 2014). Vero et al. (2013) examined the potential of cold-adapted yeasts isolated from Antarctic soils to manage post-harvest disease of fruits. *Leucosporidium scottii* isolate (At17) was identified as a good biocontrol agent producing volatile antifungal substances that inhibit apple pathogens (Vero et al. 2013). Arrarte et al. (2017) demonstrated that the production of volatile compounds (VOCs) by a psychrophilic *Candida sake* strain isolated from water and soil samples from King George Island in the subantarctic region showed potential use as post-harvest

biocontrol agents in apples stored at low temperature. In a previous work, Robiglio et al. (2011) showed that the microbiota associated with pears stored in the cold showed better biocontrol performance against post-harvest deterioration fungi than conventional yeast. Vero et al. (2011) demonstrated that *Cystofilobasidium infirmo-miniatum* (PL1), selected as cold-adapted yeast, was able to control blue and green mould in oranges during storage at 5 °C. Lutz et al. (2012) successfully used the yeasts *C. albidus*, *P. membranifaciens,* and *V. victoriae* on *P. expansum* and *B. cinerea* in pears when fruits were stored at 0 °C for 100 days.

Thus, several investigations using yeasts isolated from the Antarctic region, as well as in other cold environments, have demonstrated that this approach may present a great potential for prevention against the deterioration of fruits by phytopathogenic fungi during post-harvest and storage (Sangorrín et al. 2014).

11.6 Strategies for Success of Yeasts as Biocontrol Agents

Tests for the use of biocontrol agents still focus mainly on research laboratories. The success of formulations based on these microorganisms depends mainly on the level of control performed for the target disease. Thus, the agents' efficacy needs to be evaluated, first in a pilot, then in a semi-commercial scale, and finally in commercial studies under different conditions of storage and packaging (Nunes 2011; Dukare et al. 2018). Once the previous stages have been successful, the next step involves regulatory licensing and regulatory approval. Regulatory approval of biofungicide formulations is generally based on the effectiveness of disease control and safety assessment of the formulated product and involves numerous research and development stages which should include the following steps (Dukare et al. 2018):

- Proof of post-harvest rot or disease;
- Isolation and characterization of native strains of pathogens involved;
- Isolation and selection of microorganisms/yeasts that are possible biocontrol agents;
- Testing of the ability to control the site of action;
- Identification of the biocontrol agent;
- Determination of the mechanisms of action;
- Toxicity test;
- Large-scale production.

In the last decades, many microorganisms have been identified for use as agents of biological control of post-harvest diseases in fruits. However, only few have been formulated and marketed effectively (Droby et al. 2009; Abano and Sam-Amoah 2012). This scarcity in products has been the focus of much discourse, and among the main reasons identified for this limitation are the small number of companies involved in the development of organic products, the small size of the post-harvest market, and the necessary expenses and time required for the selection and registration of products (Droby et al. 2009; Abano and Sam-Amoah 2012).

Table 11.2 Examples of biocontrol products based on yeast antagonists

Biocontrol products	Yeast base	Fruit	Target pathogens	Country	In use
Shemer	*Metschnikowia fructicola*	Table grape, strawberry, sweet potato	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Netherlands	Yes
Boni protect	*Aureobasidium pullulans*	Pome fruit	*B. cinerea*, *Penicillium* sp., *Monilinia* sp.	United States	Yes
Nexy	*Candida oleophila*	Pome fruit	*B. cinerea*, *Penicillium* sp.	Belgium, USA	Yes
Aspire	*Candida oleophila*	Citrus	*B. cinerea*, *Penicillium* sp.	United States	No
Candifruit	*Candida sake*	Pome fruit	*B. cinerea*, *Penicillium* sp.	Spain	No
Yieldplus	*Cryptococcus albidus*	Apples and pears	*B. cinerea*, *Penicillium* sp., *Mucor* sp.	South Africa	No
Pro Yeast-ST	*Metschnikowia fructicola*	Strawberries, grapes, and citrus	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp.	USA	Yes
Pro Yeast-ORG	*Metschnikowia fructicola*	Strawberries, grapes, and citrus	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp.	USA	Yes
Biocoat	*Candida saitoana* + chitosan	Grapes and sweet cherries	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Israel	Yes
Biocure	*Candida saitoana* + lysozyme	Grapes and sweet cherries	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Israel	Yes

Source: Wisniewski et al. (2016), Spadaro and Droby (2016)

However, several yeast-based products have already reached advanced stages of development and are available on the market (Table 11.2). These products were registered for use in post-harvest against various fungal pathogens in fruits and vegetables. For example, the Shemer product was developed based on the yeast *Metschnikowia fructicola* NRRL Y-27328 (Droby et al. 2009; Dukare et al. 2018), initially registered in Israel for both pre-harvest and post-harvest application. It is used to control deterioration caused by fungal genera such as *Aspergillus*, *Botrytis*, *Penicillium,* and *Rhizopus* (Blachinsky et al. 2007). This product was acquired later by Bayer CropScience (Germany) and licensed to Koppert (Holland). BoniProtect, a product developed in Germany and produced by Bio-Ferm (Australia), is based on two antagonistic strains of *Aureobasidium pullulans* and is used for the control of developing pathogens in lesions of apples during storage (Spadaro and Droby 2016).

Nexy, based on a strain of *Candida oleophila* which was developed in Belgium and registered throughout the European Union, is produced by Lesaffre Company (Spadaro and Droby 2016). Aspire is made from another strain of *Candida oleophila*. It is among the first products made using yeast. Aspire was registered as a commercial formulation in 1995, and it is commercially developed by the Ecogen Corporation for use in the United States and Israel (Droby et al. 1998). Candifruit is produced from the *Candida sake* yeast; it was registered in Spain by the company IRTA/Sipcam-Inagra. Another bioproduct, Yieldplus, based on the yeast *Cryptococcus albidus,* was registered in South Africa and is being produced by Lallem Company (Janisiewicz and Korsten 2002; Dukare et al. 2018).

However, Aspire, Candifruit, and Yieldplus have been withdrawn from the market due to a number of reasons, including low and inconsistent trading efficiency, low profitability, and difficulties in market penetration because of the fact that small companies do not have the resources available to sustain product development and marketing (Spadaro and Droby 2016).

Pro Yeast-ST and Pro Yeast-ORG, both developed from *M. fructicola* yeast, are products developed by BASF and licensed by Inova Technology for use in the United States (Sharma et al. 2012). A new generation of biofungicides such as Biocoat and Biocure has also been developed for some years, being produced by AgroGreen. They present *Candida saitoana* yeast in their composition, but also chemical substances of natural origin such as chitosan and lytic enzymes are added in order to potentiate the protective action and to optimize the control effects. The combination of yeasts with other methods of control may be a promising approach to overcome disadvantages in the activity of the biological control agent, increasing its effectiveness. The combination of biocontrol agents with heat treatments, cooling, use of GRAS substances, and other conservation treatments can produce a synergistic effect and increase levels of control of pathogens at levels similar to those achieved with chemical fungicide treatments (Droby et al. 2009; Usall et al. 2009; Sharma et al. 2012).

The commercial use of biofungicides is limited and represents only a very small fraction of the potential market (Usall et al. 2001). To expand the use of biocontrol products, Sangorrín et al. (2014) suggest that research must evolve to integrate the use of agents into a production approach with greater efficiency and to obtain a greater quantity of effective microorganisms. Thus, the development of new commercial products will be more feasible if the microbial antagonists have a wide scope of application, perform reliably under a variety of conditions, and are adaptable to different packaging and processing systems. In addition, evaluation using different post-harvest practices, modes of application, and storage conditions is also required (Janisiewicz and Korsten 2002).

Another important consideration would be the selection of yeasts adapted to cold temperatures for the development of new bioproducts. Because these yeasts are adapted to extreme conditions, they may present greater resistance and adaptation compared to species present in tropical environments. Since the determination of cardinal temperatures (minimum, optimal, and maximum) for the growth of a specific biocontrol agent should be considered, psychrophilic or psychro-tolerant

microorganisms present potential for use as biocontrol agents, especially in cold rooms (Sangorrín et al. 2014).

This strategy has still been little explored and no biofungicide has been developed or registered for this purpose so far. Thus, if more species of yeast adapted to cold are identified, their use as biological control agents for the management of post-harvest diseases should be expanded and more successful strategies may be achieved. Refrigeration is still the main method of preserving and prolonging the shelf-life of fresh food. This fact makes evident the potential of using yeasts adapted to cold as possible biological control agents to optimize the production and conservation of fresh fruits (Sangorrín et al. 2014).

11.7 Conclusions and Perspectives

Prolonging the availability and shelf-life of post-harvest of fruits stored under refrigeration often requires application of a chemical fungicide to prevent decompositions caused by fungi growing under this condition. An alternative approach that has not been explored yet is the use of yeasts from extreme environments such as Antarctica or other cold environments. Thus, because they are adapted to such conditions, these yeasts can present a greater resistance and adaptation and facilitate the process of development of biological control agents for use under storage conditions in cold rooms.

According to Sangorrín et al. (2014), not only the control by psychrophiles biocontrolled yeasts of post-harvest diseases of fruits should be investigated but also the potential to prevent diseases during the storage of tubers, and other nonedible species such as flowers and ornamental plants should be explored. It is also important to highlight other possible areas of application such as the use in supermarkets and domestic refrigerators in order to extend the life of a greater diversity of products.

The psychrophiles yeast selection system for application in biocontrol is still a vast field, and it is likely that several new studies will be developed using only strains adapted for cold conditions. However, although there are some products of yeast-based biological control on the market, no product developed with yeasts adapted to cold is available. This methodology should become a practice adopted in the future for the development of new biofungicides suitable for the refrigerated fruit market.

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Chapter 12
Bioactive Compounds Produced by Antarctic Filamentous Fungi

Inmaculada Vaca and Renato Chávez

12.1 Introduction

Filamentous fungi are eukaryotic microorganisms with several interesting biological properties, among them, a great ability to synthesize secondary metabolites. Secondary metabolites are small organic molecules that are not essential for the growth, development, and reproduction of fungi but provide them competitive advantages over other microorganisms in the natural environment (Brakhage 2013; Keller et al. 2005). Thus, fungal secondary metabolites have several ecological roles, such as chemical communication, chemical defense, and virulence factors for plants and animals (Macheleidt et al. 2016; Spiteller 2015).

From the human point of view, fungal secondary metabolites have been historically important. Some of these metabolites, such as mycotoxins, are toxic and represent important contaminants of foods. On the other hand, filamentous fungi have provided mankind with important pharmaceuticals, such as antibiotics, immunosuppressors, and other chemicals (Macheleidt et al. 2016; Yu and Keller 2005). However, over the years, researchers have been experiencing increasing difficulties to find new bioactive compounds from fungi by using classic methodologies, because these methodologies usually yield the re-isolation of already known compounds. To overcome these difficulties, currently researchers are using different strategies such as cultivation-based approaches (Chávez et al. 2015) or dereplication of fungal extracts (Nielsen and Larsen 2015). Another interesting strategy is

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searching for new chemical compounds in fungi isolated from underexplored extreme environments. Fungi from these habitats have adopted singular strategies to survive in harsh conditions, including the production of active organic molecules, which usually differ from those found in their counterparts from non-extreme environments. In addition, extreme habitats are an interesting source of new fungal species, which could contain unique secondary metabolites. Therefore, the chemical study of fungal species from extreme environments, either new species or known species, may increase the possibility of finding new metabolites of pharmaceutical importance.

Antarctica is one of the most unexplored and harsh environments on the Earth. Its geographical isolation and severe climatic conditions turn Antarctica to an excellent place to find fungal species with novel biochemical adaptations to survive in this polar region. In fact, chemical studies performed on fungi isolated from different Antarctic environments show that, compared with mesophilic fungi, they produce metabolites with atypical structures, supporting the notion that Antarctic fungi have evolved particular genetic and metabolic mechanisms to produce new metabolites.

Many studies have described the fungal diversity inhabiting different Antarctic niches, such as rocks, macroinvertebrates, soils, snow, and others (for examples see Coleine et al. 2018; Furbino et al. 2014; Held and Blanchette 2017; Godinho et al. 2015). However, comparatively, there are few studies reporting the bioactive potential of chemical compounds from these fungi. More specifically, since 2007 to date, just 17 articles have described the characterization of chemical molecules from Antarctic fungi. Importantly, and despite some of these studies being included in previous reviews articles (Tian et al. 2017; Chávez et al. 2015; Lebar et al. 2007), to the best of our knowledge, a comprehensive description of natural products from Antarctic fungi has not been reviewed yet.

12.2 Studies Prospecting Bioactivities of Crude Extracts from Filamentous Fungi Isolated from Antarctica

In general, studies prospecting bioactivities begin with the collection of samples from different environments and the isolation of fungi from such samples. Once axenic fungal cultures have been obtained, they are analyzed for the presence of specific biological activities such as antimicrobial, antifungal, antiviral, antiparasitic, antitumoral, and others. For this purpose, fungi are grown on different solid or in liquid media, and metabolites from culture supernatants and mycelia are extracted with organic solvents. These extracts, usually concentrated, are tested by bioassays techniques (Kjer et al. 2010). In the following paragraphs, we will summarize the results of bioprospection analyses using extracts obtained from filamentous fungi isolated in aquatic and terrestrial environments from Antarctica.

12.2.1 Bioactivities Detected in Filamentous Fungi from Antarctic Aquatic Environments

12.2.1.1 Filamentous Fungi from Antarctic Benthic Mats

The benthic mats at the bottom of Antarctic lakes are dense microbial communities, which have accumulated for thousands of years virtually undisturbed (Biondi et al. 2008). To the best of our knowledge, Brunati et al. (2009) have performed the only study about the bioactivity of fungi isolated from these environments. Brunati et al. isolated 160 filamentous fungi from benthic environments from 11 Antarctic lakes located in three distinct regions of the Antarctic continent. These fungi belong to 15 fungal genera, *Thelebolus* being the predominant genus. The extracts of the culture broths of these fungi were screened for antimicrobial and cytotoxic activity. Twenty-nine percent of them showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Cryptococcus neoformans*. According to the authors, the number of extracts that inhibit the growth of Gram-negative bacteria and yeasts is higher than that observed against the same pathogens in other similar screenings. Thus, and taking into account that Antarctic microbial mats are dominated by Gram-negative bacteria and yeasts, Brunati et al. (2009) speculated that the ability of filamentous fungi to inhibit Gram-negative and yeasts growth may confer them competitive advantage to survive in this particular environment.

12.2.1.2 Filamentous Fungi from Antarctic Marine Macroalgae

In general, marine fungi associated with seaweeds produce antioxidants, antialgal, antifungal, and anti-insect metabolites, which may help in deterring colonization of algal thalli by other microbes or in warding off herbivores (Suryanarayanan 2012). Moreover, the marine macroalgal communities from Antarctica are characterized by a high degree of endemism and the presence of cold-adapted species (Oliveira et al. 2009). And importantly, Antarctic macroalgae shelter a large number of associated fungal species (Loque et al. 2010). Thus, the bioprospecting of fungi from Antarctic seaweeds looks promising. Strikingly, despite these interesting characteristics, only two studies have addressed the potential of fungi isolated from Antarctic macroalgae as producers of bioactive compounds.

In the first study, Godinho et al. (2013) collected fresh thalli from eight macroalgae species along the rocky coastline of Elephant, King George, and Deception Islands, in the Antarctic Peninsula. They recovered 148 fungal strains which were identified as members of 21 different genera. The most frequent fungal taxa identified were *Penicillium* (35.8%), *Geomyces* (24.3%), and the yeast *Mestchnikowia australis* (4.7%). All these isolates were grown using solid-state fermentation techniques to obtain crude extracts, which were screened against bacteria (*E. coli* ATCC 11775, *S. aureus* ATCC 12600, *Pseudomonas aeruginosa* ATCC 10145), fungi (*C. albicans* ATCC 18804, *Candida krusei* ATCC 6258, and *Cladosporium sphaerospermum* CCT1740), and parasites (*Trypanosoma cruzi*). The best results were

obtained with extracts from two *Penicillium* sp., which were recovered from endemic macroalgae *Palmaria decipiens* and *Monostroma hariotii*. These extracts displayed high and selective antifungal and trypanocidal activities, with low MIC and IC_{50} values.

In the second study, Furbino et al. (2014) estimated the bioactive potential of fungi associated with Antarctic endemic macroalgae *M. harioti* and *Pyropia endiviifolia* against the same targets screened by Godinho et al. (2013), and also against the yellow fever virus. The extracts of *Pseudogymnoascus* species, *Guehomyces pullulans*, and *M. australis* showed antifungal activities against *C. albicans*, *C. krusei*, and *C. sphaerospermum*. In particular, a strain of *Pseudogymnoascus* displayed 95% antifungal activity against *C. sphaerospermum*, similar value to that showed by the control drug benomyl. Additionally, the extract of an isolate identified as *Penicillium steckii* showed 96% of inhibition of yellow fever virus, better than interferon alpha used as control. The extracts did not display antibacterial or trypanocidal activities.

12.2.1.3 Filamentous Fungi from Antarctic Marine Sponges

Sponge-derived fungi from temperate latitudes are prolific producers of novel natural products (Bugni and Ireland 2004). Although sponges are the dominant macroinvertebrate organisms in Antarctic benthic communities (McClintock et al. 2005; Avila et al. 2007), the bioactivity potential of Antarctic sponge-derived fungi has received little attention. To the best of our knowledge, Henríquez et al. (2014) performed the only study about this issue. These authors obtained 101 phenotypically different fungal isolates from 11 sponge samples collected in Fildes Bay, King George Island. Sixty-five isolates belong to genera *Geomyces*, *Penicillium*, *Epicoccum*, *Pseudoeurotium*, *Thelebolus*, *Cladosporium*, *Aspergillus*, *Aureobasidium*, *Phoma*, and *Trichocladium*. However, the rest of isolates could not be identified as member of any known genus. In order to estimate the bioactive potential of these isolates, antimicrobial, antitumoral, and antioxidant activities were tested. Fifty-one percent of the extracts, mainly from the genus *Geomyces* and non-identified relatives, showed antimicrobial activity against some of the bacteria tested. In general, these fungal extracts were most active against Gram-positive than Gram-negative bacteria (Henríquez et al. 2014). On the other hand, 42% of the extracts also showed potent antitumoral activity, but just moderate antioxidant activity (Henríquez et al. 2014). These results suggest that fungi associated with Antarctic sponges, particularly *Geomyces* and their relatives, would be valuable sources of antimicrobial and antitumoral compounds.

12.2.1.4 Filamentous Fungi from Marine and Lake Sediments

The sedimentation of terrigenous material on the Antarctic continental shelf is a function of the mean ocean currents transporting icebergs and the melt rates at the base of the floating ice (Hemer 2003). Thus, these sediments are interesting habitats

that may provide fungi with novel bioactivities. Despite this, the bioactivity of the fungi from the Antarctic marine sediments has not received much attention. In the same way, lake sediments have been poorly studied. The sediments of Antarctic lakes are of particular interest because they have different types and amount of organic matter deposited at their bottoms (Mahesh et al. 2015).

Purić et al. (2018) collected marine sediments in different points at Admiralty Bay. From these samples, they obtained 47 fungal strains. The extracts of these fungi were used to test antibacterial activity against *Xanthomonas* species. Extracts of 23 fungi (belonging to the genera *Pseudogymnoascus*, *Penicillium*, *Cadophora*, *Paraconiothyrium*, and *Toxicocladosporium*) produced secondary metabolites that are able to inhibit the growth of *Xanthomonas passiflorae*. The average of inhibition was 96%, with one strain (*Pseudogymnoascus* 5A-1C315IIII) showing the highest inhibition (99%). In addition, 19 extracts produced secondary metabolites that inhibited the growth of *Xanthomonas euvesicatoria*. Again, *Pseudogymnoascus* 5A-1C315IIII showed the highest inhibition percentage (98%).

In a further study (Vieira et al. 2018), the extracts of the same strains described above were tested against *Xanthomonas citri*, the agent of citrus canker. In general, extracts previously showing activity against *X. euvesicatoria* and *X. passiflorare* also were active against *X. citri*. Again, the highest inhibition was shown by the strain *Pseudogymnoascus* 5A-1C315IIII.

Finally, Gonçalves et al. (2015) conducted a survey of antimicrobial (*E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, *C. krusei*, *C. sphaerospermum*, and *Paracoccidioides brasiliensis*), cytotoxic (against breast MCF-7 and renal TK-10 human tumoral cells), and antiprotozoal (against *Leishmania amazonensis* and *T. cruzi*) activities of fungal communities present in different substrates from Antarctica. Among these substrates, marine and lake sediments from Deception Island were assayed. The authors found three strains from the genera *Penicillium*, *Pseudogymnoascus*, and *Schizophyllum* with high and selective antifungal activity against *P. brasiliensis*.

12.2.2 Bioactivities Detected in Filamentous Fungi from Antarctic Terrestrial Environments

12.2.2.1 Filamentous Fungi from Terrestrial Soils

Antarctica harbors an interesting variety of soil habitats, ranging from relatively warm, moist soils with high organic carbon, to cold arid oligotrophic soils (Connell et al. 2014). So far, the bioprospecting of fungi from Antarctic soils has been intensively addressed, probably due to the relatively easier accessibility to these samples.

As described above, Gonçalves et al. (2015) collected different samples from Antarctic environments. Among them, they collected soil samples from Admiralty Bay, King George Island, and Deception Island. Gonçalves et al. (2015) isolated

several fungal strains from these samples, which were used to detect antimicrobial, cytotoxic, and antiprotozoal activities. They found that eight strains from the genera *Bauveria*, *Penicillium*, *Phanerochaete*, *Pseudoeurotium*, *Pseudogymnoascus*, *Purpureocillium*, and *Trichoderma* showed moderate (70% of inhibition) to high (100%) selective antifungal activity against *P. brasiliensis*. Interestingly, the extract of *Purpureocillium lilacinum* completely inhibited the growth of *T. cruzi* (in its amastigote intracellular forms) and *S. aureus*.

In another study, Godinho et al. (2015) performed an extensive screening of the bioactivity of fungi from Antarctic oligotrophic soils. They collected samples from ice-free areas at Union Glacier, in the southern Heritage Range, and isolated a fungal community with poor diversity, where *Hypocreales* sp. and *Penicillium brevicompactum* were the prevalent fungi. They obtained extracts from all these fungi and investigated their ability to produce antimicrobial (*E. coli* ATCC 11775, *S. aureus* ATCC 12600, *P. aeruginosa* ATCC 10145, *C. albicans* ATCC 18804, *C. krusei* ATCC 6258, and *C. sphaerospermum* CCT 1740), antiviral (dengue virus 2), antitumoral (human cancer cell lines MCF-7 (breast) and TK-10 (renal)), antiprotozoal (*L. amazonensis* and *T. cruzi*), herbicidal (*Lactuca sativa* (lettuce) and *Agrostis stolonifera* (bentgrass)), and insecticidal (larvae of *Aedes aegypti* L.) activities. Under the conditions used by Godinho et al. (2015), 17 fungi produced extracts with some of these biological activities. Among them, extracts from *A. sydowii*, *P. alliisativi*, *P. brevicompactum*, *P. chrysogenum*, and *P. rubens* showed antibacterial, antifungal, antitumoral, antiprotozoal, and herbicidal activities equivalent or even better than that showed by control drugs. In particular, *P. brevicompactum* strain UFMGCB 9480 showed a high antiviral activity against Dengue virus 2, high antiprotozoal activity against *T. cruzi*, the agent of the Chagas disease, strong antifungal activity against the plant pathogen *Colletotrichum gloesporioides*, as well as herbicidal activity. None of the extracts showed activity against *Aedes aegypti* larvae. An important conclusion obtained by Godinho et al. (2015) is that conspecific isolates produce distinct secondary metabolites, so the low interspecific diversity of the fungal community present in the oligotrophic soil of continental Antarctica may be compensated by a high intraspecific diversity.

In another study, Ding et al. (2016) evaluated the cytotoxic (using P388 lymphocytic leukemic cell line) and antimicrobial (*E. coli*, *Myobacterium ohlei*, *Proteus mirabilis*, *S. aureus*, and *C. albicans*) activities of 47 fungal strains obtained from soil samples collected near Chinese Antarctic station at Fildes Bay, King George Island. By using two different fermentation conditions, they determined that 18 isolates (38%) produced biologically active compounds. Ten of them exhibited cytotoxic activity, whereas 14 exhibited antimicrobial activities.

Gomes et al. (2018) obtained fungi from superficial soil samples collected in Robert, Nelson, King George, and Penguin Islands at South Shetland archipelago. All fungal isolates were cultivated using solid-state fermentation to obtain crude extracts which were assayed to detect the presence of antiviral activity against dengue and Zika virus (DENV-2 and ZIKV), antiparasitic activity against *T. cruzi* and *L. amazonensis*, and herbicidal activity against *L. sativa* (lettuce) and *Allium schoenoprasum* (chive). *Pseudogymnoascus destructans*, *Mortierella parvispora*,

and *P. chrysogenum* displayed antiparasitic activities. In addition to its tripanocidal activity, the extract from *P. destructans* UFMGCB10312 also showed strong and selective herbicidal activity against *A. schoenoprasum*. Six extracts of *P. destructans*, *Mortierella* sp., and *P. tardochrysogenm* showed high herbicidal activity. None of the fungal extracts tested showed any antiviral activity against Zika virus and Dengue virus 2.

Purić et al. (2018) tested the activity of 33 filamentous fungi obtained from soil under rotten wood in Deception Island against two *Xhantomonas* bacteria. Among the soil fungi, six representatives of *Pseudogymnoascus* produced secondary metabolites able to inhibit the growth of *X. euvesicatoria* and *X. passiflorae.* Further research using these same 33 fungal strains found that the same six *Pseudogymnoascus* isolates which produced extracts with antibacterial action against *X. euvesicatoria* and *X. passiflorae* also were active against *X. citri* (Vieira et al. 2018).

12.2.2.2 Endophytic Fungi

In Antarctica, there are only two native angiosperms plants, the grass *Deschampsia antarctica* Desv. (*Poaceae*) and *Colobanthus quitensis* (Kunth) Bartl (*Caryophyllaceae*). These plants harbor endophytic fungi, which produce interesting bioactive molecules. Santiago et al. (2012) obtained 313 fungal isolates from *D. antarctica* and 251 isolates from *C. quitensis*. These isolates were screened against parasites *L. amazonensis* and *T. cruzi*, and against different human tumor cell lines. As a result, 25 isolates (4.43%) displayed at least one biological activity. In particular, 19 extracts showed over 70% of inhibition of growth of *L. amazonensis*. The fungus *Phaeosphaeria herpotrichoides* displayed leishmanicidal activity with an IC_{50} equivalent to the inhibitory value of amphotericin B. On the other hand, six fungal extracts displayed cytotoxic activity and inhibited the growth of at least one tumor cell line. None of the extracts displayed trypanocidal activity. All leishmanicidal extracts were obtained from endophytic fungi recovered from *D. antarctica*, while the majority (five) of the cytotoxic extracts were obtained from fungal endophytes isolated from *C. quitensis.*

In other study, Gonçalves et al. (2015) analyzed the activity of 21 fungal strains isolated from *D. antarctica*. They found that 7 strains (*Fusarium avenaceum*, *Penicillium coralligerum*, *Peniophora* sp., *Pestalotiopsis microspora*, *Peniophora lycii*, *Simplicillium lamellicol*, and *Geomyces* sp.) showed a high antifungal activity against *P. brasiliensis*.

A particular case is the work of Melo et al. (2014). They obtained a single endophytic fungus belonging to the species *Mortierella alpine*, from the moss *Schistidum antarctici* found in Admiralty Bay, King George Island. Extracts from this fungus demonstrated strong antioxidant activity and strong antibacterial activity, mainly against *E. coli*, *P. aeruginosa*, and *E. faecalis*.

12.3 Novel Chemical Compounds Isolated from Antarctic Filamentous Fungi

In general, chemical studies suggest that the fungi isolated from extreme environments are an excellent source of structurally unprecedented compounds (Chávez et al. 2015). So far, 17 fungal strains isolated from different Antarctic environments have been chemically examined, and in 14 of them, new compounds have been found (Table 12.1). In this section, we describe chemical compounds that have been isolated from Antarctic filamentous fungi. The chemical compounds are described in function of the species or genus of the fungal strain from which they were isolated. As it can be seen, the largest number of fungi studied belongs to the genus *Penicillium*, whose chemistry has been extensively studied in strains isolated from other latitudes. However, species of *Penicillium* isolated from Antarctic environments show a remarkable ability to produce new metabolites. On the other hand, *Geomyces* and *Pseudogymnoascus*, the other majority genera besides *Penicillium* found in Antarctic environments, have not received as much attention. According to the screening analyses shown in the previous section, these fungal genera have a great potential as producers of biological activities. Despite this, only two isolates from these genera have been chemically analyzed.

12.3.1 Chemical Compounds from Tritirachium *sp.*

To the best of our knowledge, *Tritirachium* sp. HKI0317, isolated from the lichen *Neuropogon* sp. collected in Livingston Island, is the only filamentous fungus from the phylum *Basidiomycota* isolated in Antarctica that has been studied at chemical level (Ivanova et al. 2007). From fermentation broths of this fungus, three known compounds were isolated, namely 4-carboxy-5,5′-dihydroxy-3,3′-dimethyldiphenylether and macrosphelides A and J. Although Ivanova et al. did not perform any bioactivity test using these compounds, other previous studies demonstrated that macrosphelides displayed interesting activities as cell adhesion inhibitors and moderately cytotoxic agent (Hayashi et al. 1995). Currently, biological activities of the dimethyldiphenylether (if any) are not known.

12.3.2 Chemical Compounds from Trichoderma asperellum

Six new peptaibols, named asperelines A-F, were isolated from fermentation broths of *Trichoderma asperellum* collected from the sediment of the Antarctic Penguin Island (Ren et al. 2009). These peptaibols have an acetylated N-terminus and a C-terminus containing an uncommon prolinol residue. Regarding their activity, these compounds showed very weak inhibitory activity against fungi and bacteria

Table 12.1 New chemical compounds isolated from fungi from different Antarctic environments

Fungal strain	Sample and location	Compounds	Estructural family	Bioactivity detected	References
Tritirachium sp. HKI 0317	*Neuropogon* sp. (lichen), Livingston Island	4-carboxy-5,5′-dihydroxy-3,3′-dimethyldiphenylether	Diphenylether	Antimicrobial	Ivanova et al. (2007)
Geomyces sp.	Soil, King George Island	Ethyl asterrate; n-butyl asterrate; geomycin A	Asterric acid derivatives	–	Li et al. (2008)
		Geomycin B	Asterric acid derivative	Antifungal	
		Geomycin C	Diphenyl ether and spirocoumaranone derivative	Antibacterial	
Pseudogymnoascus sp. F09-T18-114	Sponge, King George Island	Pseudogymnoascins A–C and 3-nitroasterric acid	Nitro asterric acid derivatives	–	Figueroa et al. (2015)
Trichoderma asperellum Y19-07	Sediment, Penguin Island	Asperelines A–F	Peptaibols	–	Ren et al. (2009)
		Asperelines G–Z_{13}	Peptaibols	na	Ren et al. (2013)
Oidiodendron truncatum GW3-13	Soil, near Great Wall Station	Chetracins B and C	Epipolythiodioxopiperazines	Cytotoxic	Li et al. (2012)
		Chetracin D	Diketopiperazin	Cytotoxic	
		Oidioperazines A–D	Diketopiperazines	–	
Aspergillus sydowii SP-1	Marine sediment, King George Island	Acremolin C	Alkaloid	Antibacterial	Li et al. (2018)
Aspergillus ochraceopetaliformis SCSIO 05702	Soil, near Great Wall Station	Ochraceopones A	α-pyrone merosesquiterpenoid	Antiviral	Wang et al. (2015a)
		Ochraceopones B–E	α-pyrone merosesquiterpenoids	–	
		Isoasteltoxin	Polyenic α-pyrone	Antiviral	
		Ochracene A and ochracenes D–I	Humulane-derived sesquiterpenoids	–	Wang et al. (2017)
		Ochracenes B and C	Humulane-derived sesquiterpenoids	NO inhibitory	

(continued)

Table 12.1 (continued)

Fungal strain	Sample and location	Compounds	Estructural family	Bioactivity detected	References
Cadophora luteo-olivacea UMN PL12-3	Wood, Port Lockroy	Spiciferone F, colomitides C and D, cadopherones A–D, similin C, and spicifernin B	Hexaketides	–	Rusman et al. (2018)
Penicillium crustosum PRB-2	Deep-sea sediment, Prydz Bay	Penilactone A	Highly oxygenated polyketide	Inhibitory activity of the nuclear factor NF-kB	Wu et al. (2012)
		Penilactone B	Highly oxygenated polyketide	–	
Penicillium sp. PR19N-1	Deep-sea sediment, Prydz Bay	1-chloro-3β-acetoxy-7-hydroxy-trinoreremophil-1,6,9-trien-8-one	Chloro-trinoreremophilane sesquiterpene	Cytotoxic	Wu et al. (2013)
		1α-chloro-2β-hydroxyeremophil-7(11),9-dien-8-one	Chlorinated eremophilane sesquiterpene	–	
		Two eremofortine C analogues	Chlorinated eremophilane sesquiterpenes	–	
		7βH-eremophil-1(10),11(12)-dien-2β,8β-diol	Eremophilane-type sesquiterpene	Cytotoxic	Lin et al. (2014)
		3β-hydroxy-7βH-eremophil-1(2),9(10),11(12)-trien-8-one	Eremophilane-type sesquiterpene	–	
		1α-hydroxy-7βH-eremophil-9,11-dien-8-one	Eremophilane-type sesquiterpene	–	
		One analogue of eremofortine C	Eremophilane-type sesquiterpene	Cytotoxic	
		One lactam-type eremophilane	Eremophilane-type sesquiterpene	–	
Penicillium sp. SCIO 05705	Soil, near Great Wall Station	Penillines A and B, isopenilline A	Indolyl diketopiperazine derivatives	–	Wang et al. (2015b)

Penicillium funiculosum GWT2–24	Moss, around the Great Wall Station	Chrodrimanins I and J	Meroterpenoids	–	Zhou et al. (2015)
		Penipyridones A, B, and E	Pyridone alkaloids	Lipid-lowering activity in HepG2 hepatocytes	Zhou et al. (2016)
		Penipyridones C and D	Pyridone alkaloids	–	
Penicillium sp. S-1-18	Seabed sediments	Butanolide A	Furanone derivative	Inhibitory effect against protein tyrosine phosphatase 1B	Zhou et al. (2017)
		Guignarderemophilane F	Sesquiterpene	–	
Penicillium crustosum HDN153086	Sediment, Prydz Bay	(2*E*,4*E*,6*E*,8*E*)-10-hydroxyundeca-2,4,6,8-tetraenoic acid	Polyene	–	Liu et al. (2019)
		Fusaperazine F	Diketopiperazine	Cytotoxic	

– = compounds that do not have the activities tested; na = bioactivity was not assayed

(Ren et al. 2009). A further study on the same fungal strain identified 32 peptaibols, namely, asperelines G-Z_{13}, which had the C-terminus bonded to proline or hydroxyprolinil, a very rare association in nature (Ren et al. 2013). Biological activities of these compounds were not assayed.

12.3.3 Chemical Compounds from Oidiodendron truncatum

Chemical investigation of the fungus *Oidiodendron truncatum* GW3-13 collected from soil near the Chinese Antarctic station resulted in the isolation of two new epipolythiodioxopiperazines (ETPs), named chetracins B and C, and five new diketopiperazines, named chetracin D and oidioperazines A–D (Li et al. 2012). In addition, the previously known natural products melinacidin IV, T988 B, T988 C, T988 A, chetoseminudin C, and cyclo-L-Trp-L-Ser were also isolated. Interestingly, the oidioperazines B–D have OH/OR groups at the α-carbon of the amino acid residues, which is unusual within the diketopiperazines group.

An in vitro MTT cytotoxicity assay performed by the same authors revealed potent biological activity (in the nanomolar range) for chetracin B and melinacidin IV against a panel of five human cancer lines. On the other hand, new metabolites chetracin C and chetracin D along T988 A and T988 C displayed significant cytotoxicity at micromolar concentration. The structure-activity analysis of these molecules suggests that the sulfide bridge is a key factor for their cytotoxicity (Li et al. 2012).

12.3.4 Chemical Compounds from the Genus Aspergillus

From static cultures of the fungus *Aspergillus sydowii* SP-1, collected from soil around the Chinese Antarctic station, a new alkaloid, named acremolin C, and four known compounds (*cyclo*-(*L*-Trp-*L*-Phe), 4-hydroxyphenylacetic acid, (7*S*)-(+)-hydroxysydonic acid, and (7*S*, 11*S*)- (+)-12- hydroxysydonic acid) were isolated (Li et al. 2018). Their antibacterial activities were evaluated. Acremolin C displayed weak inhibitory activity against methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE), as compared to tigecycline. On the other hand, known compounds *cyclo*-(*L*-Trp-*L*-Phe), (7*S*)-(+)-hydroxysydonic acid and (7*S*, 11*S*)- (+)-12- hydroxysydonic acid showed moderate inhibitory activity against the same bacteria. The antibacterial activity of *cyclo*-(*L*-Trp-*L*-Phe) and (7*S*)-(+)-hydroxysydonic acid had been already reported (Nishanth Kumar et al. 2014; Li et al. 2015).

Other species from the genus *Aspergillus*, *A. ochraceopetaliformis*, collected from soil around the Chinese Antarctic station, yielded five new highly oxygenated α-pyrone merosesquiterpenoids, named ochraceopones A–E, along with one new double bond isomer of asteltoxin, isoasteltoxin, and two known compounds, asteltoxin and asteltoxin B (Wang et al. 2015a). Ochraceopones A–D are the first examples of α-pyrone merosesquiterpenoids possessing a linear tetracyclic carbon

skeleton. All the compounds were tested for antiviral, cytotoxic, and antibacterial activities. Ochraceopone A, isoasteltoxin, and asteltoxin exhibited antiviral activities against the H1N1 and H3N2 influenza viruses. However, none of the compounds displayed cytotoxic or antibacterial activities.

Further research using this same strain yielded nine new humulane-derived sesquiterpenoids, named ochracenes A–I, along with previously reported compounds trans (3*R*,4*S*)-(-)-4-hydroxymellein, cis (3*R*,4*R*)-(-)-4-hydroxymellein, (3*R*,4*R*)-4,7-dihydroxymellein, 3,5-dimethylpyrone, stachyline B, and (*E*)-methyl-5-methylhexa-3,5-dienoate (Wang et al. 2017). Compared with previous humulane-type sesquiterpenoids, ochracenes A-I featured novel carbon skeletons with corresponding methyl migration, ring cleavage, and carbon loss. Notably, ochracenes B and C represent the first examples of 8,9-*seco*-humulane-type sesquiterpenoids. Ochracenes A–I were evaluated for their cytotoxic, antiviral, and NO inhibitory activities. Ochracenes B and C exhibited moderate inhibitory effects on lipopolysachharide-induced NO release in RAW 264.7 mouse macrophage cell lines. None of the compounds displayed cytotoxic and antiviral activities.

12.3.5 Chemical Compounds from Cadophora luteo-olivacea

The chromatographic separation of the methanol extracts from *Cadophora luteo-olivacea* (UMN PL12-3) collected at Port Lockroy (Antarctic Peninsula) yielded the isolation of nine new hexaketides, including spiciferone F, colomitides C and D, cadopheronenes A–D, similin C, and spicifernin B. In addition, the previously known polyketides spiciferone A, spiciferol A, dihydrospiciferone A, and dihydrospiciferol A were also isolated (Rusman et al. 2018). Isotope feeding experiments provided data supporting that colomitides and spiciferones are produced via a type III PKS pathway. A branched polyketide chain formed by incorporation of malonate and ethylmalonate is cyclized into one of two possible aromatic intermediates (Rusman et al. 2018). It should be noted that ethylmalonate-CoA is a rare extender unit in type III PKS pathways (Chan et al. 2009; Song et al. 2006).

Regarding the bioactivity of these compounds, they did not show inhibitory activity toward several bacterial or fungal pathogens or cancer cells (Rusman et al. 2018). Previous studies indicated that spiciferononones and spicifernin displayed phytotoxicity activity and plant growth-promoter activity, respectively (Nakajima et al. 1989; Nakajima et al. 1990).

12.3.6 Chemical Compounds from the Genera Geomyces *and* Pseudogymnoascus

Currently, the taxonomic position of *Pseudogymnoascus* species and its relative *Geomyces* is a matter of debate (Minnis and Lindner 2013). Taking into account that *Pseudogymnoascus* seems to be one of the fungal genera prevalent in Antarctica

(Ding et al. 2016), this controversy is important. However, the purpose of this chapter is not the re-evaluation of the taxonomy of fungal isolates, so they will be treated together.

A strain of *Geomyces* sp. isolated from a soil sample collected at the Fildes Peninsula, King George Island, Antarctica, was grown in solid-substrate fermentation culture (Li et al. 2008). The chromatographic separation of this extract yielded five new asterric acid derivatives, ethyl asterrate, n-butyl asterrate, and geomycins A–C. In addition, three previously known compounds (asterric acid, methyl asterrate, and bisechlorogeodin) were also isolated. Geomycins A and B have an unprecedent tetraphenyl core structure with two diphenyl ethers connected through an ester linkage. Geomycin C is also a structurally unique metabolite that is derived from a diphenyl ether and a spirocoumaranone through an ester linkage.

Antibacterial and antifungal activities of the new metabolites were tested. Geomycin B displayed significant antifungal activity against *A. fumigatus*, whereas geomycin C showed moderate antimicrobial activities against Gram-positive and Gram-negative bacteria (Li et al. 2008).

In another study (Figueroa et al. 2015), four new nitroasterric acid derivatives, pseudogymnoascins A–C and 3-nitroasterric acid, were identified from a *Pseudogymnoascus* strain isolated from an Antarctic marine sponge. These compounds are the first asterric acid derivatives bearing an unusual nitro group. In addition, the previously known compounds questin and pyriculamide were also isolated. Remarkably, the pyriculamide is a nitrated diketopiperazine, making this *Pseudogymnoascus* strain the first fungus capable of synthesizing two different families of metabolites carrying a nitro group. The new compounds were inactive against a panel of bacteria and fungi, suggesting that the presence of the nitro group inactivates these biological activities.

12.3.7 Chemical Compounds from the Genus Penicillium

Chemical investigation of the fungus *Penicillium crustosum* PRB-2, isolated from deep-sea sediment collected in Prydz Bay, led to the identification of two novel highly oxygenated polyketides, penilactones A and B (Wu et al. 2012). These compounds contain a new carbon skeleton which possesses a 3,3a,9,9a-tetrahydro-1*H*-furo[3,4-b] chromen-1-one core. This kind of core is rare in nature and has been previously reported only in one natural product, pallenic acid. Moreover, penilactone A and B are analogues with antipodal absolute stereochemistries. The isolation of two natural products analogues with this particularity is very unusual. The compounds did not show cytotoxicity against five human tumor cell lines, but penilactone A showed weak inhibitory activity of the nuclear factor NF-kB.

Kozlovskii et al. (2012) isolated several strains of the genus *Penicillium* from permafrosts and sediments at different geographical locations in Antarctica. Strains identified as *Penicillium commune* and *Penicillium solitum* produced α-cyclopiazonic acid and the benzodiazepine alkaloids cyclopenin and cyclopeptin, respectively.

Fractionation of the ethyl acetate extract of the fungus *Penicillium* sp. PR19N-1, obtained from deep-sea sediment collected in Prydz Bay, led to the identification of a new chloro-trinoreremophilane sesquiterpene, three new chlorinated eremophilane sesquiterpenes, along with a known sesquiterpenoid, eremofortine C (Wu et al. 2013). The new compounds were evaluated for cytotoxic activity. Only the chloro-trinoreremophilane sesquiterpene showed a moderate cytotoxic activity against HL-60 and A549 cancer cell lines (Wu et al. 2013). Further investigation on this strain yielded five new eremophilane-type sesquiterpenes, and a new rare lactam-type eremophilane, which is the first example of eremophilane-type lactam found in microorganisms (Lin et al. 2014). In addition, three known eremophylanes-type sesquiterpenes were also isolated. These compounds were evaluated for cytotoxicity on HL-60 and A-549 human cancer cell lines. Two of the new eremophilane-type sesquiterpenes, the 7 βH-eremophil-1(10), 11(12)-dien-2β, 8β-diol, and the compound analogue of tautomeric form of the eremofortine C, showed cytotoxicity.

From a soil sample collected from a penguin's nest on Paulete Island (Antarctica), a fungal strain with a strong antimicrobial activity, identified as *Penicillium nalgiovense*, was obtained (Svahn et al. 2015). The bio-guided fractionation of culture broths from this fungus led to the isolation of the antifungal secondary metabolite amphotericin B. This was the first isolation of amphotericin B in an organism other than the bacterium *Streptomyces nodosus*.

In another study, the bioassay-guided fractionation of extracts from the fungus *Penicillium* sp. SCIO 05705, obtained from a soil sample collected near to the Great Wall Station (Chinese Antarctic station), led to the isolation of three new indolyl diketopiperazine derivatives: penillines A and B and isopenilline A (Wang et al. 2015b). Also, seven known alkaloid compounds, (*E*)-3-(1*H*-imidazole-4-yimethylene)-6-(1*H*-indl-3-ylmethyl)-2,5-piperazinediol, penilloid, meleagrin, neoxaline, questiomycin A, N-(2-hydroxypehnyl)-acetamide, and 2-benzoxazolinone, were isolated. All the compounds were evaluated for antiviral (H1N1 and H3N2), antibacterial (*E. coli*, *S. aureus*, and *M. tuberculosis*), and cytotoxic (K562, MCF-7, A549, U937, Hela, DU145, HL60, and HT29 cell lines) activities. Meleagrin, neoxaline, and questiomycin A showed significant cytotoxicity against the human cell lines tested. In addition, questiomycin A showed potent anti-tuberculosis activity. However, none of the compounds exhibited antiviral or antibacterial activities.

The strain *Penicillium funiculosum* GWT2-24, isolated from moss collected near the China Great Wall Station, yielded two new meroterpenoids, named chrodrimanins I and J, along with five known biosynthetically related chrodrimanins (chrodrimanins A, B, E, F, and H) (Zhou et al. 2015). The chrodrimanins I and J have a unique cyclohexanone ring instead of the characteristic δ-lactone ring of the other reported chrodrimanins. Chrodrimanins A, E, and F displayed inhibitory activity against influenza virus H1N1. All compounds lacked cytotoxic and antibacterial activities. Further investigation on this same strain yielded six new pyridine alkaloids, named penipyridones A-F, and the known compound berkeleyamide C, all molecules with unusual phenyl-pyridone skeleton (Zhou et al. 2016). None of the compounds was cytotoxic. Penipyridones A, B, and E and the berkeleyamide C

elicited lipid-lowering activity in HepG2 hepatocytes. Interestingly, this is the first report of a lipid-lowering effect of this family of alkaloids.

In another study, a new furanone derivative, butanolide A, and a new sesquiterpene, guignarderemophilane F, along with six known compounds penicyclone A, xylarenone A, callyspongidipeptide A, *cyclo*-(*L*-Phe-4*R*-hydroxyl-*L*-Pro), *cyclo*-(*L*-Pro-*L*-Phe), and N-(2-hydroxypropanoyl)-2-aminobenzoic acid amide, were isolated from *Penicillium* sp. S-1-18 (Zhou et al. 2017). This fungus was isolated from Antarctic seabed sediments. The inhibitory effect against protein tyrosine phosphatase 1B was tested for all compounds. Only butanolide A showed moderate inhibitory activity against this enzyme.

Finally, a new polyene compound (8*E*,4*E*,6*E*,8*E*)-10-hydroxyundeca-2,4,6,8-tetraenoic acid and a new diketopiperazine, fusaperazine F, along with three previously known compounds (xylariolide D and two diketopiperazines) were isolated from the fungus *Penicillium crustosum* HDN153086 (Liu et al. 2019). This fungus was isolated from Antarctic sediment from Prydz Bay. The polyene compound showed a conjugated tetraene, an unusual structure in natural products. The compounds were evaluated for cytotoxic activities against K652 cell line. Only the new diketopiperazine exhibited moderate activity.

12.4 Conclusion

Fungi from extreme environments, including those living in Antarctica, may have developed specific metabolic pathways to produce singular natural products with bioactive properties. For this reason, these fungi represent potential sources of pharmaceutical molecules. Extracts obtained from fungi isolated in different Antarctic environments have shown promising antimicrobial, cytotoxic, antiparasitic, and antiviral activities. On the other hand, several pure compounds isolated from Antarctic fungal extracts show new carbon frameworks or unusual structural features, indicating that these fungi would be good sources of new chemical compounds. In this way, over the last years, the study of natural products from Antarctic filamentous fungi has attracted increasing interest from researchers. However, despite the recent advances described in this chapter, our current knowledge about the chemistry and bioactive properties of metabolites from Antarctic fungi barely represents the "tip of the iceberg". Therefore, further research about the chemistry of Antarctic fungi must be encouraged.

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Chapter 13
Antarctic Yeasts as a Source of Enzymes for Biotechnological Applications

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13.1 Introduction

Psychrophilic and psychrotrophic microorganisms present an enzymatic metabolism of outstanding characteristics arising from the extraordinary environmental challenges with which they must deal. This fact makes cold-adapted microorganisms a relevant target for scientific research and its possible use in several industrial processes (Cavicchioli et al. 2011). These enzymes present several advantages for use in industrial applications. Among these advantages is their thermolability, which makes them useful when a selective thermal inactivation step is necessary. In addition, as they are synthesized at low temperatures, there is a potential reduction in energy costs during their technological production and, additionally, their use in processes occurring at low temperatures greatly reduces the chances of contamination with mesophilic microorganisms (Javed and Qazi 2016).

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In the past 30 years, the interest in cold-adapted microorganisms, the main source for cold-active enzymes, has grown considerably. Nevertheless, most of the works report the prospecting and characteristics of enzymes obtained from prokaryotic microorganisms (De Maayer et al. 2014; Santiago et al. 2016), while enzymes from psychrophilic or psychrotolerant eukaryotes are reported less (Duarte et al. 2018). Even though yeasts are able to grow using several carbon sources and some groups are adapted to live in cold and extreme environments, the information on cold-active enzymes produced by yeasts is quite scarce. (Buzzini et al. 2012). As was suggested by Margesin et al. (2003), fungi, and yeasts among them, are better adapted than bacteria for living in cold environments.

13.2 Structural Features and Action Mechanism of Cold-Adapted Extremozymes

When mesophilic enzymes are exposed to low temperatures, the kinetic energy available for reaction is too low; it is insufficient to reach the activation energy for catalysis, and hence enzymatic activity is scarce. In addition, at low temperatures, proteins tend to denature because of the decrease in water molecules' availability, as these become more ordered and less associated with proteins (Karan et al. 2013).

Cold-active enzymes present a number of specific structural adaptations that result in a more flexible structure than their mesophilic and thermophilic counterparts. These adaptations allow higher catalytic activities at low temperatures and reduce their thermostability (Siddiqui and Cavicchioli 2006).

Based on previous reviews of cold-active enzymes, Sarmiento et al. (2015) listed the following features considered as important adaptations for maintaining high flexibility and high activity at low temperatures: decreased core hydrophobicity, increased surface hydrophobicity, changes in amino acid compositions (i.e., lower arginine/lysine ratio, more glycine residues for better conformational mobility, fewer proline residues in loops but more in α-helices, more non-polar residues on the protein surface), weaker protein interactions (such as interdomain and inter-subunit interactions, less disulphide bridges, fewer hydrogen bonds and other electrostatic interactions), decreased secondary structures and oligomerization (but an increase in the number and size of loops), and, finally, increased conformational entropy of the unfolded protein. Feller (2018) reported that one interesting consequence of these special characteristics is that the reaction rates of psychrophilic enzymes tend to decrease slowly when compared to similar enzymes from mesophilic or thermophilic microorganisms in response to a temperature decrease.

13.3 Biotechnological Potential of Cold-Active Enzymes

High activity at low temperatures and thermolability are valuable characteristics for different biotechnological applications in a wide variety of industries such as food, beverage, and household (Sarmiento et al. 2015). For this reason, psychrophilic enzymes are replacing mesophilic enzymes in several industrial processes (Fig. 13.1).

The food industry is one of the main potential users of cold-active enzymes, with special interest in dairy products, juice, meat, and baking industries. Intolerance to lactose, a disaccharide sugar naturally present in milk, represents a common health concern worldwide. Cold-active β-galactosidases are useful to reduce the lactose amount at low temperatures in the milk-processing industry, allowing intolerant people to consume milk and milk derivatives (Erich et al. 2015).

Throughout the world, pectinases are used in the fruit juice industry during the juice extraction process to reduce viscosity and refine the final product. Cold pectinases could be also used in wineries, where most of the fermentations are performed

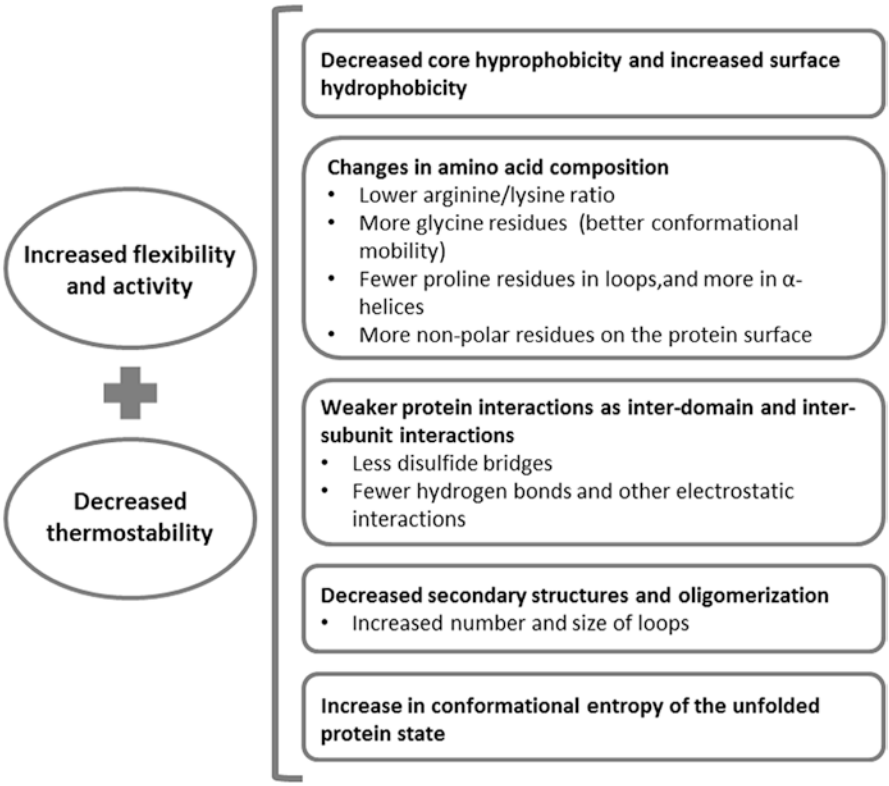

Fig. 13.1 Some relevant adaptations for keeping high flexibility and high activity at low temperatures in cold-active enzymes. (Adapted from Sarmiento et al. 2015)

at temperatures ranging from 15 to 20 °C to avoid evaporation of volatile molecules. Following this thinking, Merin et al. (2011) have proposed the use of psychrotolerant pectinase-producing yeast as adjuvant for wine fermentation. In the meat-processing industry, cold-active proteases are used during the tenderization process, which increases final product quality. Some enzymes, including proteases, amylases, and xylanases, are also helpful in baking processes by reducing the dough fermentation time along with retention of aromas and moisture levels (Kuddus 2018).

In the detergent industry, several enzymes are used for both household and industrial laundry and dishwashing processes. Lipases catalyze the hydrolysis of fats (lipids) and remove fatty stains (butter, oil, and sauces) from fabrics. The trend toward cold-washing to reduce energy consumption (and also to protect fabrics and extend their life) is one of the main reasons why search for the discovery and development of novel cold-adapted lipases from psychrophilic microorganisms has increased rapidly in the past years (Joseph et al. 2008). One example is Lipoclean®, which is a cold-adapted lipase produced by the world's leading producer of enzymes, Novozyme. This product targets stains from triglycerides at low temperatures (above 20 °C) and is very stable in multienzymatic solutions, being, therefore, suitable and effective as a cleaning mixture.

Proteases and celullases are also examples of enzymes used in detergent industries and their cold-active versions are as important as lipases in this industry. Proteases catalyze the hydrolysis of peptide bonds that link amino acids together, digesting proteins into smaller fragments. In the detergent industry, they help in breaking down protein stains, such as blood, egg, grass, cocoa, and human sweat (Joshi and Satyanarayana 2013). Commercial examples of these enzymes are Polarzyme® and (Novozyme), a serine protease for hand washing laundry able to maintain its high activity in a broad range of temperatures ranging from 5 to 60 °C. Purafect® Prime and Properase® are two cold-adapted proteases for laundry detergents with optimal activity for soil stains removal at temperatures ranging from 20 to 40 °C. On the other hand, Excellase® is a product for dishwashing at low or moderate temperatures. All these three enzymes are produced by Genencor.

Some of these biocatalysts may also provide a valuable tool for low-temperature biotransformations and bioremediation. Based on the high catalytic efficiency of cold-active enzymes and their unique specificity at low and moderate temperatures, psychrophilic and psychrotolerant microorganisms are powerful tools for bioremediation purposes. In most of these cases, purification of these enzymes for bioremediation is not necessary, and most of the reported protocols have been carried out using mixed cultures to provide a wide variety of enzymes able to degrade as many pollutants as possible (Welander 2005; Filler et al. 2008). Wastewater treatment and bioremediation of contaminated soils in cold environments also involve the potential application of cold-adapted microorganisms for reducing the amounts of toxic compounds, for example, nitrates, hydrocarbons, aromatic compounds, heavy metals, and biopolymers such as cellulase, chitin, lignin, proteins, and triacylglycerols (Gerday et al. 2000).

13.4 Antarctica as a Source of Yeast with Cold-Active Enzyme Production

Antarctica is one of the harshest environments on Earth, showing several extreme climatic factors including low temperature, low humidity, and high solar irradiation. Additionally, soil and water in Antarctic environment are exposed to successive freezing and thawing cycles, demanding specific adaptation responses to biological systems inhabiting them (Yergeau and Kowalchuk 2008). Considering both, continental and maritime regions, less than 2% of the Antarctic continental surface corresponds to ice-free land areas. Antarctic soils are mostly oligotrophic, except for areas where some macrorganisms, such as penguins or elephant seals settled down and breed, enriching soil with their excretions. Precipitations are scarce, and winds are both strong and variable. All these factors determine a wide range of extreme soil microhabitats that differ in temperature, moisture, organic carbon contents, and levels of nitrogen, phosphorous, and other macronutrients (Martorell et al. 2017). Figure 13.2 shows the scheme of bioprospection pathway for Antarctic yeasts with cold-active enzyme activities.

Such a profusion of different environmental conditions constitutes a huge challenge for the survival of microorganisms, which need to possess several physiological and metabolic adaptations to cope with these extreme conditions (Ruisi et al.

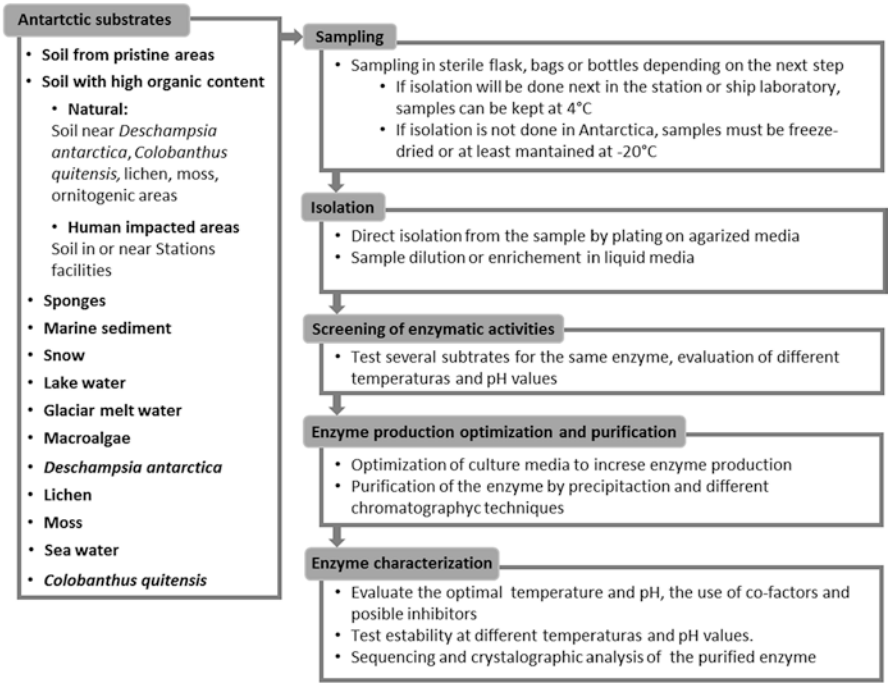

Fig. 13.2 Bioprospection pathway for Antarctic yeasts with cold-active enzyme activities

2007). Among these adaptations, the expression of a variety of cold-adapted enzymes able to work in a wide range of low temperatures is the main reason why in the recent decades Antarctic psychrophilic microorganisms (bacteria, archaea, algae, and, less frequently, fungi) and their enzyme production have been the focus of numerous investigations (Yárzabal 2016; Duarte et al. 2018). This chapter focuses on yeasts isolated from Antarctica and the cold-active enzymes produced by them. Table 13.1 lists, for the enzymes discussed in this chapter, the most promising yeasts isolated from Antarctica during the last 20 years and the main characteristics of the cold-active enzymes produced by them.

13.5 Lignocellulolytic Enzymes

Lignocellulose is the most abundant biomass on earth. Agricultural, forest, and agro-industrial activities generate tons of lignocellulosic waste annually, which present readily procurable, economically affordable, and renewable feedstock for various lignocellulose-based applications. Cellulose, hemicellulose, pectin (carbohydrate), and lignin (non-carbohydrate) polymers are the main substrates of lignocellulose-degrading enzymes (cellulases, hemicelullases, xylanases, pectinases, and laccases, among others) (Saini et al. 2015). The search of a replacement for fossil fuels reinforced the interest on processes that allow the use of renewable energy sources and there is another niche for cold-active lignocellulolytic enzymes (Budsberg et al. 2016).

13.5.1 Cellulases

Several substrates such as sponges, ornithogenic soils, soil near decaying wood and other organic materials have been reported as the source of fungi producing cold-active cellulases (Krishnan et al. 2011; Carrasco et al. 2012; Duncan et al. 2008; Vaca et al. 2013; Martorell et al. 2017, 2019). As a consequence of human activities, wood and other organic materials were introduced into Antarctica. Allochthonous fungi with the ability to degrade those organic compounds were introduced as well. These exogenous fungi faced the challenge of adapting to low temperature or dying.

A specific screening for cellulase-producing fungi can be done by adding CMC (carboxy methyl cellulose) (Bradner et al. 1999) to enrichment cultures. Another approach is to test already isolated fungi for cellulase activities in specific solid media supplemented with CMC and then measure the haloes of CMC consumption as a qualitative and preliminary estimation of the activity. Yeasts isolated from Antarctica with cellulase activity belong to several genera such as *Cryptococcus*, *Guehomyces*, *Leucosporidium*, *Mrakia*, *Leuconeurospora*, *Exophiala*, *Dioszegia*, *Leucosporidiella*, *Rhodotorula*, *Nadsonia*, *Filobasidium*, *Holtermanniella*, *Microglossum*, *Hiphozyma*, *Cystobasiduium*, *Fellozyma*, *Wickerhamomyces*,

Table 13.1 Some cold-active enzymes from yeasts isolated in Antarctica

Enzyme	Yeast	Substrate	Temperature of growth (°C)	Qualitative screening	Quantitative analysis	Comments	Reference
Xylanase	*Candida davisiana*	ND	15	ND	0.75 U mL^{-1} at 120 h	A	Duarte et al. (2013)
	Cryptococcus adeliensis	ND	15	ND	0.43 U mL^{-1} at 120 h	A	
	Guehomyces pullulans	ND	15	ND	0.43 U mL^{-1} at 120 h	A	
	Cryptococcus laurentii	ND	15	ND	1.09 U mL^{-1} at 120 h	B	
	Guehomyces pullulans	soil	15	6	ND	C	Martorell et al. (2017)
	Cryptococcus adeliae	ND	10	ND	385 nkat mL^{-1} at 111 h	D	Gomes et al. (2000)
Protease	*Rhodotorula mucilaginosa*	ND	25	ND	11.12 U mL^{-1} at 120 h	–	Duarte et al. (2013)
	Wickerhamomyces anomalus	Melt water	30	5	ND	C	Carrasco et al. (2012)
	Mrakia frigida	Human impacted lake	4	1.8	ND	E	Krishnan et al. (2011)
	Sporobolomyces roseus	Soil	22	8	ND	F	Troncoso et al. (2017)
	Leucosporidium creatinivorum		15	5	ND	C	Martorell et al. (2017)
	Mrakia frigida		15	5	ND	C	
	Rhodotorula muscorum		15	7	ND	C	
	Rhodotorula sp.		15	6	ND	C	

(continued)

Table 13.1 (continued)

Enzyme	Yeast	Substrate	Temperature of growth (°C)	Qualitative screening	Quantitative analysis	Comments	Reference
Lipase	*Cryptococcus laurentii*	ND	20	ND	0.143 U L^{-1} at 120 h	–	Duarte et al. (2013)
	Cryptococcus adeliensis	ND	20	ND	0.113 U mL^{-1} at 144 h	–	
	Leucosporidium scottii	ND	20	ND	0.230 U mL^{-1} at 120 h	–	
	Cystobasidium pallidum	Soil	30	8	ND	F	Troncoso et al. (2017)
Amylase	*Dioszegia fristingensis*	Soil	22	7	ND	C	Carrasco et al. (2012)
	Dioszegia sp.		15	7	ND	C	
	Cryptococcus victoriae		22	5	ND	F	Troncoso et al. (2017)
	Cryptococcus sp.		22	6	ND	F	
	Hyphozyma sp.		15	6	ND	F	
Cellulase	*Mrakia bollopsis*	Soil	15	8	ND	C	Carrasco et al. (2012)
	Mrakia psychrophila		10	10	ND	C	
	Mrakia frigida	Human impacted lake	4	1,7	ND	E	Krishnan et al. (2011)
	Cryptococcus victoriae	Soil	10	3	ND	F	Troncoso et al. (2017)
	Hyphozyma sp.		15	5	ND	F	
	Cryptococcus victoriae		15	6	ND	C	Martorell et al. (2017)
	Guehomyces pullulans		15	5	ND	C	

Enzyme	Yeast	Substrate	Temperature of growth (°C)	Qualitative screening	Quantitative analysis	Comments	Reference
	Mrakia frigida		15	5	ND	C	
Esterase	*Cryptococcus victoriae*	Soil	15	5	ND	C	Carrasco et al. (2012)
	Dioszegia fristingensis		22	6	ND	C	
	Dioszegia sp.		15	6	ND	C	
	Leuconeurospora sp.		15	6	ND	C	
	Mrakia psychrophila		10	7	ND	C	
	Cystobasidium pallidum		30	3	ND	F	Troncoso et al. (2017)
	Cryptococcus victoriae		22	10	ND	F	
	Cystobasidium laryngis		15	7	ND	F	
	Cryptococcus victoriae		15	7	ND	C	Martorell et al. (2017)
	Cystobasidium laryngis		15	5	ND	C	
	Guehomyces pullulans		15	6	ND	C	
	Holtermanniella sp.		15	6	ND	C	
	Mrakia frigida		15	7	ND	C	
	Pichia caribbica		15	6	ND	C	
Pectinase	*Dioszegia fristingensis*	Soil	22	7	ND	C	Carrasco et al. (2012)
	Dioszegia sp.		15	6	ND	C	
	Leuconeurospora sp.		15	6	ND	C	
	Sporobolomyces salmonicolor		22	6	ND	C	

(continued)

Table 13.1 (continued)

Enzyme	Yeast	Substrate	Temperature of growth (°C)	Qualitative screening	Quantitative analysis	Comments	Reference
	Cryptococcus sp.		10	20	ND	F	Troncoso et al. (2017)
	Cryptococcus adeliensis		15	6	ND	C	Martorell et al. (2017)

A = endoxylanase activity was measured, B = xylosidase was measured, C = corresponds to the halo of activity measured from the edge of the colony to the edge of the halo in mm, D = the enzyme was partially characterized, E = the numbers represent a relative activity (diam. of halo-diam. of colony/diam. of halo), F = corresponds to the halo (measured in mm) formed by the culture supernatant deposited into wells made in agarized media
ND not determined

Candida, and *Pichia* (Krishnan et al. 2011; Vaz et al. 2011; Carrasco et al. 2012; Vaca et al. 2013; Troncoso et al. 2017; Martorell et al. 2017).

13.5.2 Xylanases

Fungi able to produce xylanases have been isolated from several Antarctic samples, mainly from ornithogenic soils and marine invertebrates (Carrasco et al. 2012; Duarte et al. 2013; Martorell et al. 2017). For the screening of xylanase producers, xylan is added to solid media as a substitute for the original carbon source (glucose in most media). Martorell et al. (2017, 2019) reported the use of Yeast Morphology Media (YM) for the search of several enzyme activities, replacing the carbon source (glucose) for a particular substrate, in this case xylan. This strategy proved to be an efficient and simple way to analyze the presence of several enzymes using just one simple and standardized culture media. The most reported xylanase-producing yeasts genera isolated from Antarctica belong to *Candida*, *Cryptococcus*, *Mrakia*, *Wickerhamomyces*, *Dioszegia*, *Fellomyces*, *Guehomyces*, *Bullera*, *Leucosporidium*, *Phenoliferia*, and *Pichia* (Gomes et al. 2000; Carrasco et al. 2012; Duarte et al. 2013; Martorell et al. 2017).

13.5.3 Pectinases

Pectinases comprise a large group of enzymes that break down pectic polysaccharides of plant tissues into simpler molecules, such as galacturonic acid. Pectinases represent an important fraction of the food-processing market, where they are used for degradation of pectin and are involved in different processing steps such as liquefaction, clarification, and juice extraction. The industry currently uses pectinases from mesophilic or thermophilic microorganisms that are well established, but recently, there has been is a new trend in the food industry to adopt low-temperature processing. This trend is due to the potential economic and environmental advantages that the industry envisages for the near future. To achieve this change, cold-active pectinases and the microorganisms that produce them need to be studied more deeply (Adapa et al. 2014). In this sense, psychrophilic pectinases derived from cold-adapted yeasts are known to function at low to freezing temperatures and may be an alternative to address the problem. The isolation media for these enzymes generally contains a specific substrate for pectinase activity. Most researchers use pectin (Fenice et al. 1997; Vaz et al. 2011; Carrasco et al. 2012; Martorell et al. 2017); nevertheless, other compounds, such as polygalacturonic acid, have been used (Margesin 2000).

In Antarctica, yeasts able to produce these kinds of enzymes were mainly found in the marine environment or in soils surrounding *Deschampsia antarctica*, mosses, or lichens. Most of the isolated pectinase producer yeasts belong to *Cryptococcus*,

Wickerhamomyces, *Rhodotorula*, *Microglossum*, *Leuconeurospora*, *Leucosporidiella*, *Leucosporidium*, *Dioszegia*, and *Mrakia* (Vaz et al. 2011; Carrasco et al. 2012; Duarte et al. 2013; Martorell et al. 2017). All the isolates of *Mrakia frigida* reported by Martorell et al. (2017) presented pectinase activity, in accordance with two strains of *M. frigida* isolated from the European Alps and North Siberia by Margesin et al. (2005). These authors characterized the enzymes and concluded that both strains are capable of maximal enzyme production at temperatures below 5 °C, reflecting their adaptation to permanent cold natural place they inhabit. Maximal activity of the purified enzymes was observed at 30 °C, but the stability of both enzymes sharply decreased in the 10–40 °C range, classifying the enzymes as thermolabile. Similar characteristics are expected from the Antarctic strains of *M. frigida*.

13.5.4 Laccase, Lignin Peroxidase, and Decolorizing Enzymes

Rovati et al. (2013) evaluated the presence of these kinds of lignocellulolytic enzymes in yeasts isolated from King George Island in Antarctica. They used lignin, guaiacol, and a mixture of textile dyes as substrates to evaluate lignin peroxidase, laccase, and decolorizing activities, respectively. Lignin peroxidase activity was detected in yeasts belonging to *Leucosporidiella*, *Rhodotorula*, *Dioszegia*, *Debariomyces*, *Mrakia*, and *Cryptococcus*. Laccase activity was present in yeasts belonging to *Leucosporidiella*, *Rhodotorula*, *Dioszegia*, *Debariomyces*, *Bullera*, *Cylindrobasidium*, and *Cryptococcus*. Finally, yeasts with decolorizing activity belonged to *Leucosporidiella*, *Rhodotorula*, *Debariomyces*, *Mrakia*, *Bullera*, *Exophiala*, and *Cryptococcus*.

13.6 Lipolytic Enzymes

Microbial lipases are, besides proteases, the group of microbial enzymes showing the highest biotechnological potential. They catalyze hydrolytic reactions in media with low water activity. They show high regiostereo- and chemoselectivity. Many of them were obtained as crystals and, therefore, their structures have been deeply understood (Jaeger and Eggert 2002). These enzymes are used in pharmaceutical, food, and household chemicals industries and for the treatment of environmental pollution. Lipases production is increasing constantly, and it accounts for more than one-fifth of the global enzyme market nowadays (Szczęsna-Antczak et al. 2014).

It is important to highlight that special attention was paid to lipases and esterases producers, as these are enzymatic activities related to hydrocarbons degradation, a relevant catabolic ability with potential application in bioremediation processes of

cold polluted environments, as well as in biodiesel production. Although microorganisms able to degrade hydrocarbons efficiently have also been isolated from uncontaminated environments, their numbers, including fungi, significantly increase in oil-contaminated sites. In this sense, Aislabie et al. (2001) attributed the significant enhancement in numbers of cultivable yeasts and filamentous fungi in oil-contaminated cold soils to the important role of these microorganisms in the degradation of hydrocarbons or their metabolites (Fernandez et al. 2017). The production of esterases and lipases by Antarctic fungi may be associated with the mechanisms of cold tolerance, since the maintenance of the adequate cell membrane fluidity, essential to cellular survival, may be achieved by increasing the fraction of unsaturated fatty acids (Duarte et al. 2018).

To isolate or select yeasts with lipase activity, solid media is supplemented with olive oil and rhodamine B. After growth, a fluorescent halo under UV light is used to identify the colonies that exhibited lipase activity (Martorell et al. 2017). While this is a simple method used by most researchers, other substrates can be used for this purpose, such as vegetable and fish oils, animal fats, and synthetic triacylglycerides (Duarte et al. 2018).

13.6.1 Lipases

Reported Antarctic yeasts having lipase activity belong to the genera *Candida*, *Cryptococcus*, *Guehomyces*, *Hyphozyma*, *Hamamotoa*, *Kriegeria*, *Cystobasidium*, *Rhodotorula*, *Fellomyces*, *Sporidiobolus*, *Leucosporidium*, *Mrakia*, *Leuconeurospora*, *Wickerhamomyces*, *Exophiala*, *Phenoliferia*, and *Pichia* (Vaz et al. 2011; Carrasco et al. 2012; Duarte et al. 2013; Martorell et al. 2017; Troncoso et al. 2017). The most studied lipases from an Antarctic yeast are lipases A and B from *Pseudozyma* (*Candida*) *antarctica*, which have been thoroughly analyzed at molecular level and subjected to many genetic and chemical modifications, including immobilization (Szczęsna-Antczak et al. 2014). Lipase B is produced by Novozymes (Bagsvaerd, Denmark) and commercialized as Novozyme 435. This enzyme is involved in many organic synthesis applications related to food and feed processing, pharmaceuticals, and cosmetics (Yang et al. 2013; Duarte et al. 2018).

As was pointed out by Szczęsna-Antczak et al. (2014) in their extensive review on Lipases A and B from *Candida antarctica*, even though the number of reports and patents from Lipases A and B are impressive and increasing, the major portion have not been fully translated into industrial implementations, mainly because of the relatively high cost of the enzymes preparations, both in free or immobilized forms. The authors highlighted that the effort on the development of these enzymes must be focused on making them cheap, stable, and reusable.

13.6.2 Esterases

Unlike lipases, which prefer long-chain triglycerides, esterases hydrolyze short acyl-chain soluble esters (less than ten carbons) (Hashim et al. 2018). Esterase activity plays a major role in the degradation of natural materials and industrial pollutants, cereal wastes, plastics, and other toxic chemicals. They are useful for the synthesis of optically pure compounds, perfumes, and antioxidants (Panda and Gowrishankar, 2005).

For esterase activity screening from Antarctic yeasts, Tween 80 is the preferred substrate (Vaz et al. 2011; Carrasco et al. 2012; Martorell et al. 2017). Genera with esterase activity were reported by Vaz et al. (2011), Carrasco et al. (2012), Martorell et al. (2017), and Troncoso et al. (2017). It is a large group of microorganisms, comprising members of *Cryptococcus*, *Guehomyces*, *Leucosporidium*, *Mrakia*, *Leuconeurospora*, *Exophiala*, *Phenoliferia*, *Dioszegia*, *Glaciozyma*, *Leucosporidiella*, *Mrakia*, *Rhodotorula*, *Metchniskowia*, *Nadsonia*, *Cystobasidium*, *Holtermanniella*, *Protomyces*, *Hyphozyma*, *Kriegeria*, *Fellomyces*, and *Pichia.* Martorell et al. (2017) reported that esterase was the enzyme produced by most of the Antarctic isolated yeasts (72%). This result was in accordance with Carrasco et al. (2012), who reported the same situation for lipase and esterase. Interestingly, it was the only enzyme produced by all the *Cystobasidium larynges* isolates in the screening performed by Martorell et al. (2017).

Hashim et al. (2018) reported a new cold-active esterase-like protein with putative dienelactone hydrolase (GaDlh) activity from the psychrophilic yeast *Glaciozyma antarctica*. These authors characterized the purified recombinant GaDlh, which presented an optimal temperature of 10 °C and an optimum pH of 8.0. The behavior of the enzyme with its specific substrate, dienelactone, remains unknown due to the lack of the substrate commercially. Several cold-adapted features were observed in the predicted protein structure. Nevertheless, isolation, heterologous expression, and characterization of cold-active enzymes from Antarctic yeasts, as is the case of esterase from *G. antarctica*, are always appreciated and have become a key stone in the bioprospection of cold-active enzymes.

13.7 Amylases

Cold-active amylase activity was detected in members of several genera isolated in Antarctica including *Cryptococcus, Guehomyces*, *Leucosporidium*, *Mrakia*, *Leuconeurospora*, *Exophiala*, *Phenoliferia*, *Dioszegia, Glaciozyma, Hyphozyma, Hamamotoa, Kriegeria, Fellozyma, Leucosporidiella, Rhodotorula*, *Holtermanniella*, *Fellomyces*, and *Pichia* (Krishnan et al. 2011; Vaz et al. 2011; Carrasco et al. 2012; Vaca et al. 2013; Troncoso et al. 2017; Martorell et al. 2017). To evidence amylase activity, starch in agarized media is the main substrate used for screening. After incubation, revealing decolorization haloes with Lugol solution helps identify amylase-positive yeast.

De Mot and Verachtert (1987) purified α-amylase and a glucoamylase from *Candida antarctica* CBS. Both enzymes were monomeric glycoproteins with different amino acid composition. Kinetic analyses indicated that both enzymes preferentially hydrolyzed high-molecular-mass substrates, including some raw starches. α-Amylase was active on cyclodextrins, whereas debranching activity was demonstrated for glucoamylase.

Ramli et al. (2013) also purified and sequenced an *α-amylase from Glaciozyma antarctica* PI12. They developed and analyzed a 3D model for the α-amylase AmyPI12 structure and identified several novel characteristics of the newly isolated cold-adapted protein. They suggested that the AmyPI12 model developed for this enzyme could be used for further comparative structural analyses to understand the structure and activity relationships and may contribute to a better understanding of the structures and functions of other cold-adapted proteins in nature.

Cold-active amylases can be used in a wide range of processes, such as desizing operation (removal of starch) in textile industry, cold-washing with low-temperature detergents, direct fermentation of starch to ethanol, manufacture of maltotetraose syrup, manufacture of maltose, manufacture of high-molecular-weight branched dextrins, treatment of starch-containing wastewater, etc. (Kuddus et al. 2011). Also, cold-active but thermolabile amylases are important in the food industry, as their use can prevent substrates' and products' modification after enzymatic treatment (Kuddus et al. 2012).

13.8 Proteases

Antarctic yeasts showing cold-active protease activity belong to the genera *Candida*, *Cryptococcus*, *Exophiala*, *Guehomyces*, *Glaciozyma*, *Leuconeurospora*, *Leucosporidium*, *Mrakia*, *Rhodotorula*, *Leucosporidiella*, *Nadsonia*, *Pichia*, *Sporidiobolus*, and *Wickerhamomyces* (Krishnan et al. 2011; Vaz et al. 2011; Carrasco et al. 2012; Duarte et al. 2013; Vaca et al. 2013; Martorell et al. 2017; Troncoso et al. 2017). Ray et al. (1992) isolated a cold-active acidic protease from *Candida humicola*. These authors reported that the secretion of this protease was dependent on medium composition, being higher when the medium was supplemented with proteins. In addition, enzyme secretion during exponential growth was greater at low temperatures than at higher temperatures. Nevertheless, the extracellular protease isolated from *C. humicola* was active at temperatures ranging from 0 to 45 °C, with an optimum activity at 37 °C. Due to these particular features, this protease provides a powerful tool for processes where similar activity is required under temperature changes.

In 2003, Turkiewicz et al. (2003) reported the purification to homogeneity and characterization of an extracellular serine proteinase, lap2, from Antarctic yeast *Leucosporidium antarcticum* 171. This proteinase showed to be halotolerant, whereas its activity and stability were dependent neither on Ca^{2+} nor on other metal ions. It showed an optimal temperature as low as 25 °C, poor thermal stability, rela-

tively small values of free energy, enthalpy and entropy of activation, and high catalytic efficiency in the range 0–25 °C. The 35 N-terminal amino acid residues of lap2 presented homology with subtilases of the proteinase K subfamily (clan SB, family S8, subfamily C). This proteinase lap2 was the first psychrophilic subtilase in this family.

The production, purification, and characterization of an extracellular protease released by *Rhodotorula mucilaginosa* L7 were reported by Lario et al. (2015). The enzyme production started at the beginning of the exponential growth phase and reached a maximum after 48 h. The purified protease presented optimal catalytic activity at pH 5.0 and 50 °C. In addition, the enzyme was stable in the presence of high concentrations of NaCl, in accordance with the marine environment from where isolation of the yeast was done.

Nowadays, around 60% of the total enzyme market is represented by proteases used in various industries. Proteases are applied in diverse fields such as detergent industry, leather processing, silk degumming, food and dairy, baking, pharmaceutical industries, silver recovery from x-ray films, waste management, and others (Joshi and Satyanarayana 2013).

13.9 Patents

Patenting is one of the ways for developments and discoveries to reach industrial use or market. As was described in this chapter, enzymes from cold-adapted yeast were reported as "powerful" tools for industry. Do they jump the hit from "potential biotechnological tools" to real applied products? The analysis of patents related to cold enzymes could be helpful to answer this question. Duarte et al. (2018) made and exhaustive research on patents (or patent requests) related to enzymes from Antarctic fungi from information deposited in 14 databases from around the world. Despite the high number of keywords used, their survey did not retrieve many relevant results, indicating that this environment has an enormous biotechnological potential, but it has not been reflected in patented products or process yet. They also reported that patents' records or requests are associated mostly with bacteria, such as the lipase from *Bacillus pumilus* from Antarctica (10–1596435-0000), the cold-adapted protease HSPA-2 from a marine bacterium (WO2013177834), and anti-tumor and anti-microbial compounds from Lakes Schirmacher Oasis' bacteria, in East Antarctica (US20110301216).

When it comes to patents of cold-adapted enzymes produced by Antarctic yeasts, they are all related to lipase produced by *Candida antarctica*. Several companies and organizations own patents and commercialize this enzyme as AstraZeneca (UK/Sweden), Du Pont (US), Novo Nordisk (Denmark), DSM NV (The Netherlands), Korea Ocean Research and Development Institute (Korea), Shin Dong Bang Corp. (Korea), and Nagata Sangyo (Japan) (Duarte et al. 2018).

13.10 Antarctic Regulation for Bioprospection

Bioprospecting in Antarctic environments is a relatively new phenomenon that generates both challenges and doubts, especially when the particular characteristics that rules Antarctica, as established by the Antarctic Treaty System (ATS 1959) and its Protocol, are taken into account. The guideline establishes that it is crucial to evaluate both all possible benefits and consequences from bioprospecting, looking for the protection of Antarctic ecosystems. The aim of the ATS is to keep the white continent as a territory used exclusively for peaceful purposes (Article I of the ATS). Commercial activities are not allowed, with the exception of tourism and fishery. Mining is forbidden, as well as the exploitation of other natural recourses. There have been multiple proposals by researchers and their countries, which have been presented in the ATS. However, the challenges are such that there is still a long way to go in the research field as well as in the ATS (Villamizar-Lamus 2015).

The first question to answer is: Does bioprospection endanger Antarctic ecosystems? The second one is: Does bioprospection represent a commercial exploitation of Antarctic life resources? To respond to these questions is not a simple task. Different approaches should be considered. So far, a regulated Antarctic bioprospect guideline has been gradually generated, with a starting point based on the requirement of channeling information about bioprospection activities to the Antarctic community to make proper decisions in the near future (ATCM 28, ATCM 36). As expressed in the documents signed during those meetings, the ATS is convinced of the benefits of scientific research in the field of biological prospecting for the progress of humankind and that scientific observations and results from Antarctica must be exchanged and made available for all countries.

13.11 Conclusions and Future Perspectives

Yeasts isolated from the Antarctic Continent provide a significant source for enzyme production. Enzymes from Antarctic yeast can be potentially applied in a myriad of industrial processes, with several examples of real and effective applications. In this chapter, we mainly reviewed hydrolases and oxidoreductases. Beyond bioprospection, it is important to consider the ecological role of Antarctic yeast and their battery of enzymes; through these enzymes Antarctic yeasts make essential contributions to nutrient cycling and organic matter mineralization in Antarctica, where nutrients are scarce.

Most of the cold-enzyme bioprospection in Antarctic yeasts has been done using cultivable, already isolated yeasts, with the consequent and beneficial creation of several culture collections in most of the countries with a research program focused on microorganisms.

As these studies started about 50 years ago and because it is difficult to carry out sampling in Antarctica, the knowledge about microorganisms and their metabolites

in general, and about yeasts in particular, is quite scarce. However, the use of metagenomics and culture-independent techniques is improving day by day, leading to an improvement in the understanding of the diversity and ecology of the Antarctic microbiota and an increase in the prospection of possible new metabolites, including enzymes.

Besides yeasts isolation and enzyme screening, more research on the purification and characterization of these enzymes needs to be done. Culture collections are rich and their hidden enzymatic potential is supposed to be abundant, but the number of cold enzymes that show effective application is not very large. Finally, as pointed out by the Antarctic Treaty, the white continent must remain a territory that is used exclusively for peace and science. The knowledge and benefits obtained from bioprospecting in Antarctica should be shared and made available for all countries.

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Chapter 14
Antarctic Fungi as Producers of Pigments

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14.1 Introduction

The Antarctic environment shows heterogeneous conditions and variations in different niches of the continent with respect to the type of soil, sediments, rocks, stones, and vegetation, which mainly comprises lichens, mosses, and only two flowering plants: *Colobanthus quitensis* and *Deschampsia antarctica* (Ruisi et al. 2007). All niches are characterized by extreme conditions of temperature and high levels of radiation, and most (about 98%) of the land surface is covered with desiccation, i.e. snow and ice (Jarvinen and Lepparanta 2013).

Therefore, organisms that inhabit this stressful environment must possess mechanisms to protect themselves from such conditions, otherwise several damages can be incurred, possibly resulting in the destruction of any life form (Convey 2005; Dieser et al. 2010). The restrictive environmental characteristics of the Antarctic continent have aroused great biotechnological interest, since the microorganisms adapted to these environments have developed a metabolic diversity that can be

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explored for biotechnological applications (Dieser et al. 2010). Among these biomolecules, we can highlight microbial pigments produced mainly in response to solar ultraviolet radiation (UVR 220–400 nm), especially ultraviolet B radiation (Wynn-Williams et al. 2002).

Polar natural compounds have become a promising source for extremophile compounds, from a pharmacological perspective, as well as for industrial and commercial applications (Coker 2016). Wynn-Williams et al. (2002) highlight pigmentation as an essential strategy in the survival of ancient and modern photosynthetic organisms and Archaea in extreme environments such as Antarctica (Wynn-Williams et al. 2002; Coker 2016). In this sense, there are studies in literature that indicate a high prevalence of pigments in yeasts (Vaz et al. 2011) and filamentous fungi (Rosa et al. 2009) from Antarctica. In this review, we have summarized the current understanding of Antarctic fungal pigments and their biological applications.

14.2 Microbial Pigments: General Features

Colour is an attribute related to molecules absorbing electromagnetic radiations associated with the so-called "visible spectrum" (wavelengths from *c.a.* 380 to 770 nm). Many natural substances present this property: carotenoids, the yellow-to-red pigments found in vegetables, microorganisms, and some animals; the red-to-purple/blue anthocyanins observed in fruits; chlorophyll, which gives leaves their green colour; the blue phycocyanins present in cyanobacteria; and melanin, the brownish pigment found in human skin and in fungi, are some of the main examples. Colour is a key feature of a product, in foodstuffs, as this is the first attribute to attract the consumer's attention and is therefore critical to determine acceptance or rejection. For this reason, colouring substances are usually used to confer, maintain, or intensify the colours of food products (Bicas et al. 2013).

Synthetic colourants have been widely used by the industry; however, only few options are commercially available, and their use is controversial mainly due to safety concerns (Kobylewski and Jacobson 2012). Therefore, biotechnology emerges as an important alternative to meet the increasing demand for natural colourants, by using microorganisms to produce such substances (Dufossé et al. 2005, 2014; de Carvalho et al. 2014). This strategy has attracted the interest of the scientific community due to some practical pros related to microbial pigments:

- Microorganisms may be regarded as a renewable and virtually inexhaustible source of pigments.
- Their production process, i.e. microbial fermentation, can occur uninterruptedly without seasonal/climatic interference, under controlled and optimizable conditions.
- Fermentations usually occur under milder conditions (temperature, pressure, pH, and others) when compared to conventional chemical syntheses, being more

environment friendly. Moreover, the residues from microbial fermentations are supposedly biodegradable.
- Fermentations can use underutilized and low-value raw materials such as agroindustrial wastes and by-products as substrates.
- Fermentation products may be labelled as "natural", which results in favourable marketing appeal and possibly higher commercial value.
- Microorganisms may be regarded as sources of colours that are scarcely available to the industry [e.g. blue pigments produced by *Antarctomyces pellizariae* isolated from Antarctic snow by de Menezes et al. (2017)].

Microbial-derived colours are frequently reported in literature, yellowish-orange pigments being the most commonly found (Aruldass et al. 2018). Beta-carotene, for instance, has been produced on a large scale using the fungi *Blakeslea trispora* or the microalgae *Dunaliella*. The microalgae *Haematococcus pluvialis* or the fungi *Xanthophyllomyces dendrorhous* have also been used to produce astaxanthin, another carotenoid, but these microbial processes are not commercially widespread (Berstenhorst et al. 2009). Moreover, yellow-to-red pigments from *Monascus* have been traditionally used in Asian countries for centuries (Feng et al. 2012).

Currently, the global trend for the use of natural substances instead of synthetic substances has been increasing (Akilandeswari and Pradeep 2016); particularly, the dye industry has been suffering from increased raw material and energy costs for the synthesis of pigments and is under constant pressure to minimize damage to the environment (Durán et al. 2002). Studies in pharmaceutical, food, and cosmetic industries due to the serious environmental and safety problems generated by many artificial synthetic pigments have been focused on producers of safer pigments obtained from natural sources (Lu et al. 2009). In addition, the search for more effective alternatives with sustainable and lower costs of production, which do not cause damage to the environment, is highly necessary. In this scenario, filamentous fungi might be an important source of pigments for food and industrial industries due their versatility in producing coloured pigments and the ease of cultivation on a large scale (Dufossé et al. 2014).

Although microbial-derived blue pigments such as Spirulina (from *Arthrospira platensis*) are commercially available, novel sources of this colour are highly desired. Moreover, functional properties of microbial colourants other than colouring power (e.g. antioxidant, antimicrobial, anticancer, anti-inflammatory, and immunomodulation) have also increased the scientific interest in such molecules (Tuli et al. 2015).

In this sense, most of these biological activities such as antimicrobial (Mojib et al. 2010; Asencio et al. 2014; Leiva et al. 2015; Vila et al. 2019), photoprotective and photosensitive (Mojib et al. 2013; Kimura et al. 2015; Órdenes-Aenishanslins et al. 2016), and antioxidant activity (Correa-Llantén et al. 2012; Kimura et al. 2015) have been widely reported in pigments produced by Antarctic bacteria. However, more recently, pigments produced by Antarctic fungi have also been considered promising with biological applications such as photoprotection (Moliné et al. 2009; Dimitrova et al. 2010), stress tolerance (Selbmann et al. 2011; Singh

et al. 2014), antioxidant activity (Dimitrova et al. 2013), and UV-C radiation tolerance (Villarreal et al. 2016).

Indeed, microbial pigments are commonly bioactive molecules produced in stress conditions (Gostincar et al. 2012), and a high frequency of pigment production is observed in isolates recovered from ice samples (Rodrigues and Tiedje 2008), marine surface waters (Stafsnes et al. 2010), and remote glacial areas from Antarctica (González-Toril et al. 2008), indicating the importance of pigmentation in adaptation to cold and high-UV environments.

In contrast to photosynthetic microorganisms, which produce pigments for harvesting light energy, these colourants are usually produced by microorganisms as a defence mechanism against UV radiation, oxidants, antimicrobials, and temperature extremes or as a competition strategy (antimicrobial activity against competitors) (Liu and Nizet 2009). In this context, extreme environments such Antarctica are good targets for bioprospecting microorganisms that produce pigments such as melanin (Chyizhanska and Beregova 2009; Gessler et al. 2014) or carotenoids (Villarreal et al. 2016).

14.3 Antarctic Fungal Pigments

Natural pigments are secondary metabolites produced by different organisms. Among these, Antarctic fungi are considered a significant source of pigments due to their great physiological plasticity (Fig. 14.1) and some species produce several kinds of pigments with diverse characteristics (Durán et al. 2002; Zhou and Liu 2010; Akilandeswari and Pradeep 2016). When the colonies are already well established or when the vital supplies become scarce, fungi produce pigments (Isaac 1994). Fungal pigments can demonstrate different functions such as protection against lethal photo-oxidation (like carotenoids) and protection to environmental stress (melanin) and can act as cofactors in enzymatic catalysis (flavins) (Mapari et al. 2005).

A summary of the diversity in pigmented yeast and filamentous fungi isolated from the Antarctic continent and seacoast is presented (Table 14.1). Moreover, it comprises an overview of reported pigmented, Antarctic substrates based on isolation, colour, and their functionality.

Pigmented yeasts have been isolated from Antarctic samples of zooplankton (Moliné et al. 2009), lichens (Dimitrova et al. 2010, 2013; Tashirev et al. 2010), sedimentary rocks (Barahona et al. 2016), soil and water (Villarreal et al. 2016), rhizosphere soil of *Deschampsia antarctica* (Trochine et al. 2017), and filamentous fungi from snow (de Menezes et al. 2017), soil (Arcangeli et al. 1997, 2000; Arcangeli and Cannistraro 2000), and moss (Singh et al. 2014).

The majority of pigmented yeasts mainly belong to the phylum *Basidiomycota*, the predominant genera being *Cystofilobasidium* (Moliné et al. 2009), *Dioszegia* (Trochine et al. 2017; Villarreal et al. 2016), *Sporobolomyces*, and *Collophora* (Dimitrova et al. 2013; Barahona et al. 2016), and the formerly polyphyletic genera

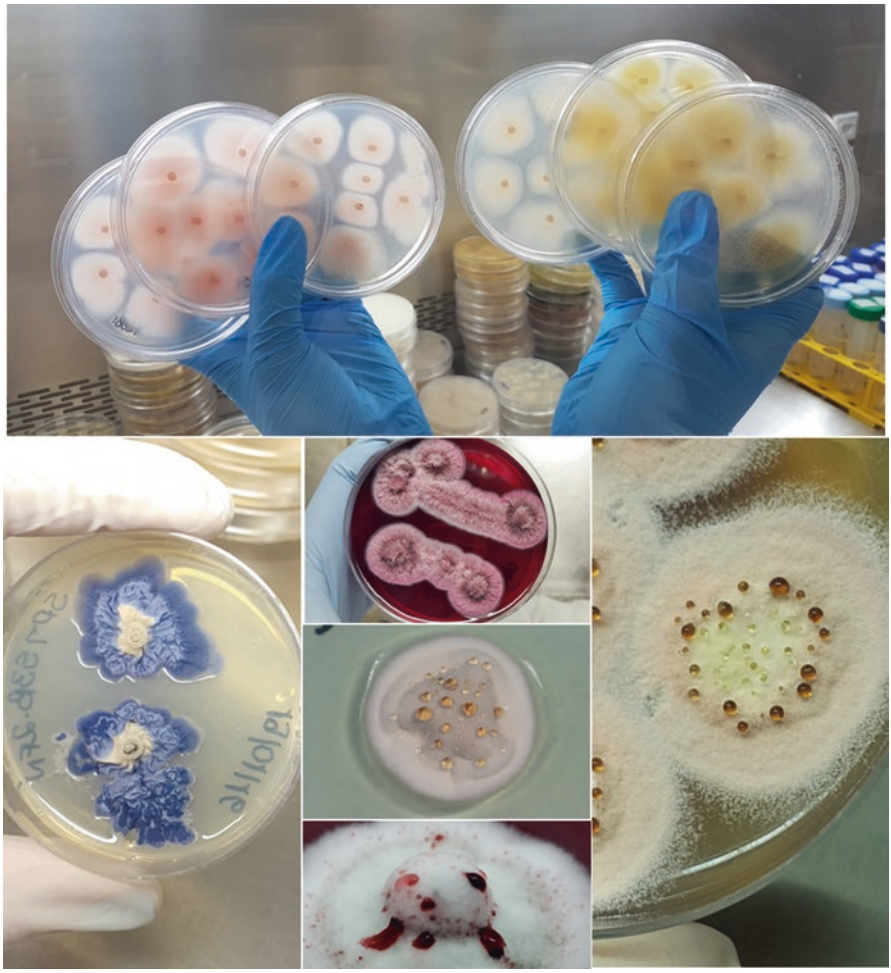

Fig. 14.1 Different kinds of pigments produced by Antarctic fungal colonies. (Photo credits, LH Rosa)

Cryptococcus and *Rhodotorula* (Dimitrova et al. 2010; Villarreal et al. 2016). In contrast, *Candida davisiana* U6 (pink) and black-pigmented *Exophiala xenobiotica* U7 isolated from green lichen on moss and green moss, respectively, have been reported from phylum *Ascomycota*, and these show high UV resistance (Vasileva-Tonkova et al. 2014).

The production of photoprotective compounds (melanin, carotenoids, and mycosporines) by yeasts and fungi could be a strategy to survive in extreme environments, and this behaviour can be evidenced by the high number of pigmented isolates recovered in Antarctica (Rosa et al. 2009; Vaz et al. 2011). Vaz et al. (2011) reported that 41.7% of Antarctic yeasts were producers of pigments and/or mycosporines. Among mycosporine-positive pigmented organisms, most belonged to

Table 14.1 Antarctic fungal producers of pigments: Characterization and bioactivities

Fungal species	Origin of sample	Pigment	Colour	Biological activities	References
Yeast					
Cystofilobasidium capitatum	Zooplankton (Antarctica)	β-Carotenoid	–	Resistance to ultraviolet B (UVB)	Moliné et al. (2009)
Sporobolomyces salmonicolor, *Cryptococcus albidus*, and *Cryptococcus laurentii*	Soil and lichens (Livingston Island)	β-Carotenoid	–	Resistance to ultraviolet A (UVA)	Dimitrova et al. (2010)
Sporobolomyces salmonicolor	Soil and lichens (Livingston Island)	β-Carotene, torularhodin, and torulene	–	Antioxidant activity	Dimitrova et al. (2013)
Sporobolomyces salmonicolor	Sedimentary rocks (Union Glacier)	2,3-Dihydroxy-γ-carotene, β-carotene, 4-ketotorulene, and torulene	Pink	–	Barahona et al. (2016)
Collophora sp.				–	
Sporobolomyces metaroseus		β-Carotene and 4-ketotorulene, furthermore, β-cryptoxanthin and spirilloxanthin	Red	–	
Dioszegia sp.	Soil and water samples (King George Island)	OHK torulene	Orange	UV-C radiation tolerance	Villarreal et al. (2016)
Rhodotorula larynges		Torulene and lycopene	Red		
Rhodotorula mucilaginosa		Torulene, γ-carotene, and lycopene	Pale red		
Cryptococcus gastricus		2-γ-Carotene	Pink		
Dioszegia patagonica	Rhizosphere of *Deschampsia antarctica* (King George Island)	Total carotenoid	Orange	–	Trochine et al. (2017)
Nadsoniella nigra	–	Melanine	Black	Increase resistance in piglets and reduction of morbidity and mortality	Chyizhanska and Beregova (2009)

(continued)

Table 14.1 (continued)

Fungal species	Origin of sample	Pigment	Colour	Biological activities	References
Candida davisiana	Green lichen on moss	Pink pigment	Pink	Protection against UV radiation	Vasileva-Tonkova et al. (2014).
Exophiala xenobiotica	Green moss	Melanine	Black		
Cryptococcus sp.	Sedimentary rocks (Union Glacier)	Mycosporine	Pink	–	Barahona et al. (2016)
Torrubiella sp.			Cream	–	
Filamentous fungi					
Thelebolus microsporus	Soil (Larsemann Hills)	β-Carotene	Bright orange to yellow-orange	Stress tolerance	Singh et al. (2014)
Arthrobotrys ferox	Moss samples (Wood Bay, Victoria Land)	Carotenoid and mycosporines	–	Resistance to ultraviolet-B (UVB)	Arcangeli et al. (1997, 2000), Arcangeli and Cannistraro (2000)

– not reported

Dioszegia, including the species *D. aurantiaca*, *D. crocea*, and *D. hungarica*, and the non-pigmented species *Naganishia antarctica* (formely *Cryptococcus antarcticus*); all of these isolates were recovered from the rhizosphere of *Deschampsia antarctica*. In another study, Rosa et al. (2009) reported that endophytic fungi recovered from the leaves of *D. antarctica* were mainly producers of melanin and included black moulds (about 80%).

Most studies report yeast as a good pigment producer, whereas few studies address pigment production by filamentous fungi. Among these, we can highlight the production of β-carotene by *Thelebolus microspores* (Singh et al. 2014) and carotenoid and mycosporine by *Arthrobotrys ferox* (Arcangeli et al. 1997, 2000; Arcangeli and Cannistraro 2000) as described in Table 14.1.

In general, the first screening for pigment production by Antarctic fungi is performed in a solid culture medium such as yeast malt agar and by direct visualization as described by Vaz et al. (2011). These pigments can be intracellular or extracellular (Fig. 14.2), the latter being more easily extracted as they do not require fungal lysis.

Some pigments are extracellular sunscreens and others have quenching properties to dissipate the excess energy from UV-B, which would alternatively generate single toxic oxygen; some absorb UV-B inside the cell before metabolically important molecules can be damaged (Wynn-Williams et al. 2001). In addition, there is often a combination of pigments that would not provide adequate protection individually, but together minimize the UVR damage to individual cells or a mutually terrestrial community (Wynn-Williams and Edwards 2002). Antarctic fungal pigments such as

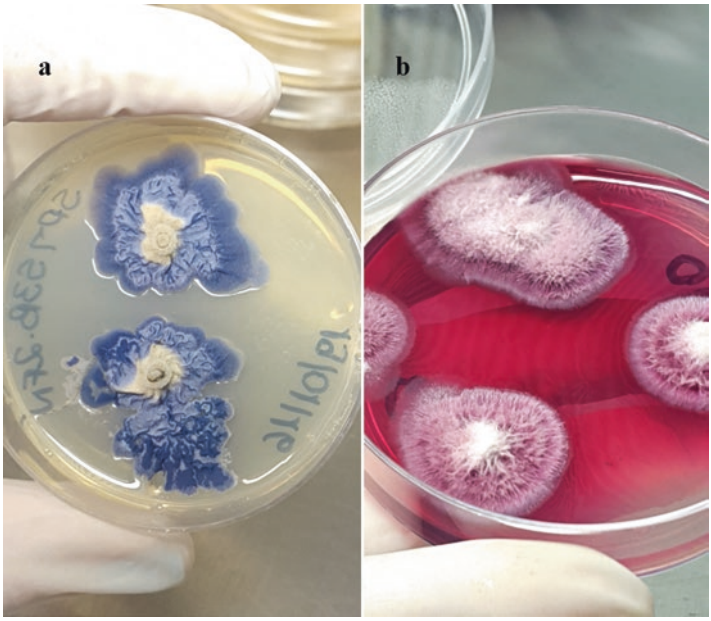

Fig. 14.2 Intracellular and extracellular pigments produced by Antarctic fungi. (**a**) *Antarctomyces pellizariae* isolated from snow, a producer of intracellular blue pigment. (**b**) *Pseudogymnoascus* sp. isolated from the lichen *Lecania brialmontii*, a producer of a reddish extracellular pigment. (Photo credits, LH Rosa)

carotenoids have already been identified with photoprotective functions as described by Moliné et al. (2009) and Dimitrova et al. (2010).

Important fungal pigments can be produced under controlled conditions through submerged, solid, or semi-solid fermentation (Vendruscolo et al. 2010; Akilandeswari and Pradeep 2016) using a wide variety of eco-friendly substrates (Gupta and Aggarwal 2014). Features such as temperature, aeration, agitation, pH, and culture medium directly affect the production and quantity of the pigments (Medentsev et al. 2005). Figure 14.3 shows the general steps for the production of a fungal pigment.

A variety of solvents such as acetone, acetonitrile, chloroform, cyclohexane, dichloromethane, ethanol, hexane, dimethylsulfoxide, pyridine, tetrahydrofuran, and water are used for the extraction of the produced fungal pigments (Fig. 14.4). The choice of solvent depends on the polarity of the molecule being studied. After obtaining the pigment, care must be taken with pH and temperature so that the pigment does not lose its original colorimetric characteristics.

Among the known pigments produced by fungi are melanin (black or dark brown), β-carotene (orange), γ-carotene and xanthophyll (orange-red), lycopene (dark red) (Vasileva-Tonkova et al. 2014; Barahona et al. 2016; Villarreal et al. 2016), catenarin (red), auroglaucin (orange), flavoglaucin (yellow), aurantin (yellow), aurofurasarin (orange yellow), and boletol (blue) (Durán et al. 2002) (Table 14.1 and Fig. 14.5).

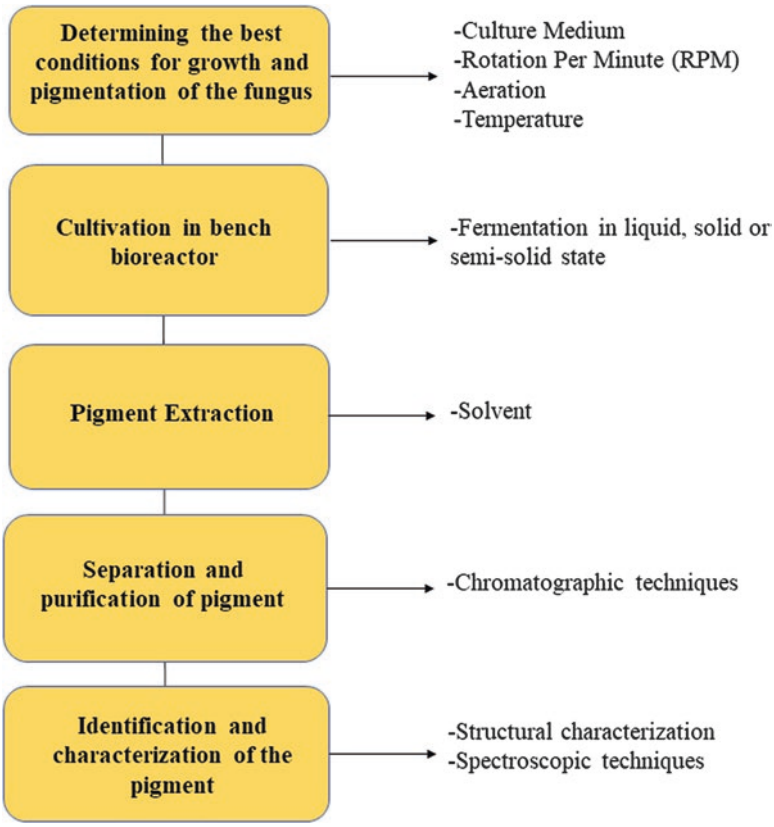

Fig. 14.3 General steps used for producing an Antarctic fungal pigment

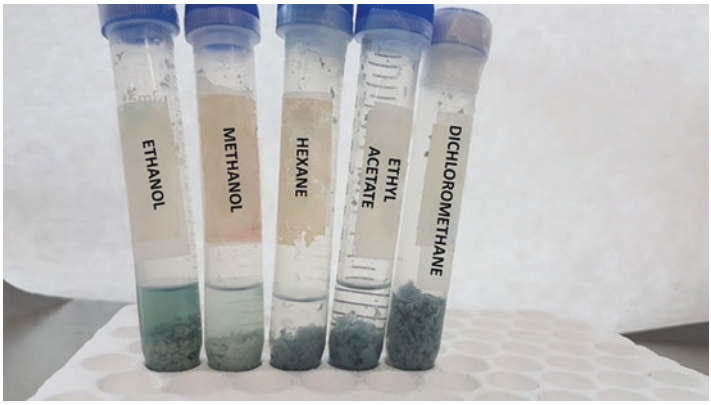

Fig. 14.4 Test using different solvents for the extraction of intracellular blue pigment from Antarctic fungus *Antarctomyces pellizariae*. (Photo credits, GCA de Menezes)

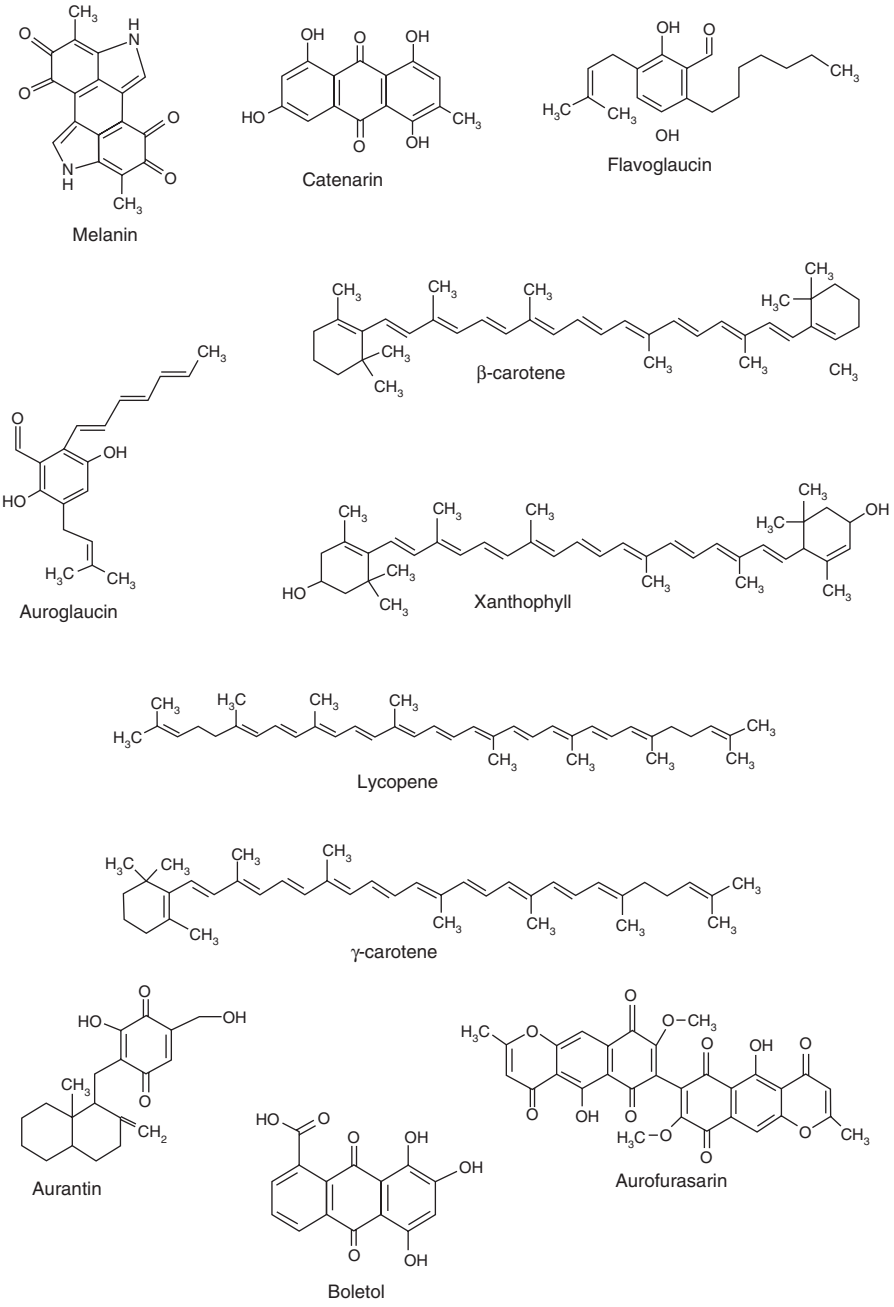

Fig. 14.5 General structure of pigments produced by Antarctic fungi

Mycosporine and mycosporine-like amino acids (MAAs) from fungi are capable of absorbing wavelengths between 310 nm and 360 nm, which may therefore confer photoprotective potential against UV as well as visible radiation. *Dioszegia*, a pigmented Antarctic yeast, and *Naganishia antarctica*, a non-pigmented Antarctic yeast (formerly *Cryptococcus antarcticus*), have been reported as mycosporine producers (Vaz et al. 2011; Barahona et al. 2016).

Melanin in fungi is an important protective factor against the adverse effects of environmental stresses such as UV radiation, drying, and high concentrations of salts and heavy metals (Jacobson 2000). Chyizhanska and Beregova (2009) suggested that melanin produced by the black yeast *Exophiala nigra* X-1 (formerly *Nadsoniella nigra*) can be used in livestock. These authors administered melanin to piglets (0.1 mg/kg) during weaning and showed that the weaning-associated morbidity and mortality was abrogated by 100%.

14.4 Conclusion and Perspective

The environmental impact of human activity has been promoting an intense debate involving the concept of sustainability. Simultaneously, there is a growing demand for natural ingredients and additives to be used in the formulation of industrial products. In this context, biotechnological processes have emerged as an attractive alternative to extraction and chemical synthesis processes, as they yield natural products, may use renewable and virtually inextinguishable sources, and do not generate toxic wastes. This fact has provoked an intense scientific race in recent years for bio-based materials.

The results indicate that the production and characterization of pigments produced by Antarctic fungi is a relatively recent and potentially promising option. Antarctic fungi have already been shown to be good producers of secondary metabolites including pigments, such as melanin and carotenoids, and may, in the future, be used in different biotechnological processes for applications, such as photoprotection or resistance to ultraviolet radiation.

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Chapter 15
Genomics of Antarctic Fungi: A New Frontier

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15.1 Introduction

Despite the extreme conditions of the Antarctic continent, life has managed to survive. Microorganisms are found in every Antarctic ecosystem (Godinho et al. 2013) from the ocean and ancient lakes to fresh snow, from lichens on rocks to the permafrost and ornithogenic soils (Duarte et al. 2016), and from prehistoric ice deposits to deep sea marine sediments (Vaz et al. 2011). The extreme Antarctic conditions – freezing cycles, low liquid water availability, and high UV incidence – have selected species with diverse unique features and adaptations in all cellular processes and structures, making the Antarctic biodiversity a reservoir of unique organisms and genes that could be exploited by the biotechnology industry (Godinho et al. 2013).

The microbiological, biochemical, and phylogenetic characterization of these Antarctic organisms and their features has been the main approach used to date (Wentzel et al. 2018; Yajima et al. 2017), followed by investigation of individual genes, in particular, those encoding antifreeze proteins and cold-adapted enzymes (Hashim et al. 2018). Further, bioprospection of extracts derived from cultivated fungi seems to be promising in the search for novel drugs with anti-pathogen and anticancer activities (Godinho et al. 2013).

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Recent advances in the techniques for DNA analysis, especially the advent of next-generation sequencing (NGS) (Goodwin et al. 2016) and improvements in bioinformatics tools, have enabled a new and complementary approach for the characterization of Antarctic organisms. The rationale is to investigate the genomes and transcriptomes of these organisms in the search for genes responsible for specific phenotypic traits (Firdaus-Raih et al. 2018) and also to peek into the molecular universe of the cell to determine unexpected features that are only accessible at the nucleic acid sequence level. This chapter is not intended as a detailed manual for genome analysis. Instead, its goal is to familiarize the reader with the concepts in the field and the basis for genome interrogation.

15.2 The Omics Era: Genomics and Other Omics

The term genome was coined in 1920, much before the recognition of DNA as the genetic information storage molecule, and was meant to designate the chromosomes and protoplasm (Goldman and Landweber 2016). With the rise of DNA sequencing in 1977 (Sanger et al. 1977), investigation of phenotypic traits at the molecular level was primarily performed on one or a few genes at a time. The last decades have witnessed rapid development of molecular biology and DNA sequencing platforms. Miniaturization allied to massive parallelization in high-throughput strategies have facilitated the extension of the context of sequencing from one gene to all genes (Heather and Chain 2016; Quail et al. 2012). Gathering of knowledge on biological gene structures and functions organized in large curated databases and the possibility to sequence the whole genome of an organism at once have given rise to the field of *genomics*.

More than all the genes of an organism put together, the genome is the set of all DNA molecules present in a cell (except for RNA viruses) organized as chromosomes. It is an ensemble of sequences comprising protein-coding genes and their regulatory sequences, non-coding RNA genes, pseudogenes, and protogenes (Dujon and Louis 2017). The genome also harbours repetitive sequences and many, only apparently, functionless stretches of DNA (Graur et al. 2015) along with informative regions, which carry the organism's evolutionary history. The genome is, in most cases, static during the cell lifetime. Some stresses or genotoxic agents can produce mutations that, if not corrected by the highly efficient DNA repair machinery, might be passed on through generations. Besides being considered as the molecule carrying the genetic information, DNA needs accessory proteins and RNAs to get its information interpreted in the context of an organism or cell.

The ability to study genomes is catalysing many research fields (Hittinger et al. 2015), virtually all fields related to biological sciences and, in particular, microbiology (Ziemert et al. 2016). The knowledge on all life domains is benefited by the capability of looking at any organism through the genomic lenses. This deeper vision has clarified and redefined taxonomic relationships and classifications (Choi and Kim 2017), elucidated ecological functions at the genetic

level (Chan et al. 2013), and made it possible to observe organisms that are invisible to classical approaches – the microbial dark matter – even in Antarctica (Bernard et al. 2018).

Derived from *genomics*, the field of *metagenomics* uses modern sequencing techniques to address the diversity of an ecosystem. The prefix "meta" refers to something beyond. Thus, metagenomics aims to analyse more than the genome of a given species, the complete set of genetic material present in a sample. By sequencing DNA from environmental or other complex samples and comparing it with known sequences from curated databases, it is possible to infer the composition and abundance of distinct taxa.

Composite samples can be assessed using two main strategies. The first is called *amplicon metagenomics*. In a similar manner as performed in *barcoding* analysis, PCR primers are designed to amplify the informative regions expected to be present in the investigated clades. For fungi, the most common regions are D1/D2 from the 18S ribosomal DNA (Baeza et al. 2017). Baeza et al. (2017) used these markers to perform amplicon metagenomic analysis in order to estimate the fungal diversity of Antarctica's terrestrial habitats, and identified 87 known genera and 123 species of 37 unknown genera. The most represented fungal classes were *Lecaronomycetes* and *Eurotiomycetes*.

The difference between the classical amplicon analysis with isolated organisms and *metagenomics* is the ability of the latter approach to process complex samples and infer the abundance of identified organisms from their proportion in the obtained sequencing reads. The drawback is that, for ecosystems where the biodiversity is little known, as for some Antarctic habitats, many sequences will not be identified, being only assigned to higher taxonomic ranks. Yet, with the constant advances in the gathering of biological information from Antarctica, this obstacle could be overcome in the near future.

The second strategy is called *shotgun metagenomics*. Its name is derived from the analogy to the genome sequencing strategy devised by Craig Venter in the race for the Human Genome Project (Venter et al. 2001), i.e. *shotgun sequencing*. Prior to its development, the current approach used to assemble genomes was the "contig strategy". To sequence long DNA stretches, one would work in an ordered manner by fragmenting the genome to different sizes and cloning its pieces into vectors with distinct insert size capabilities to produce libraries that were later organized by sequencing their insert ends. The strategy to reconstruct the genome was to use overlapping clones to produce a physical map, guiding the genome assembly, in a top-down approach.

The *shotgun* strategy, supported by Venter, was based on cutting the genome in a random manner using physical or enzymatic methods, as if shattering it with a shotgun shot, sequencing all the pieces and using computational power to determine overlapping parts in order to guide the contigs assembly, in a bottom-up approach. This strategy later proved to be the more efficient, especially when allied with modern sequencing capabilities. So, in *shotgun metagenomics*, all the DNA retrieved from a given sample is randomly sequenced, without any previous amplification step. The output data is a mixture of diverse fragments, corresponding to pieces of

the genomes present in the sample. More abundant organisms yield more DNA, generating more reads. For some organisms with small genomes, like viruses and some bacteria, it is sometimes possible to assemble entire genomes from these data. For others, with bigger genomes or that are proportionally less abundant in the sample, less data is retrieved, hindering full genome assembly. The advantage of *shotgun metagenomics* is that phylogenetically informative regions can be assessed along with virtually any part of the genome. From the pieces of the reconstituted genome, it is possible to assign taxonomic classification and also to infer an organism's role in the environment, as the presence of genes that perform specific molecular tasks denotes the ecological niche and functionality of the organism (Sharpton 2014; Calderoli et al. 2018).

The promising frontier of environmental DNA analysis has been largely explored recently, boosting the knowledge on Antarctic bacterial and viral diversity (Lloyd et al. 2018). As an example of the importance of such investigation, Koo et al. (2016) analysed metagenomic samples of six Antarctic microbial mats searching for the presence and distribution of bacterial cold adaptation proteins: antifreeze proteins (AFPs), ice nucleating proteins (INPs), cold shock proteins (CSPs), trehalose synthase (TA), and fatty acid desaturases (FADs). They found many CSPs, TAs, and FADs in all collected samples. However, the INPs and AFPs were less abundant, corroborating the fact that ice was not a constant selective pressure compared to low temperature, because the lakes from where the microbial mats were derived experience only mild freezing at localized sites.

Fungi, as organisms with genomes up to hundred times larger than those of bacteria, archaea, and virus, are harder to study and are usually not at the focus of most shotgun metagenomic studies conducted nowadays. However, an interesting feature of metagenomics is that as all the DNA of a sample is sequenced, information is gathered for all the constituent organisms, regardless of the groups that are the aim of the study. This facilitates investigation of sequences produced for a particular study with a different perspective compared to the original. As many metagenomic raw sequences are being deposited to public databases, a wealth of information is waiting to be mined with different optics. This richness was exploited by Donovan et al. (2018), who developed a pipeline to investigate public metagenomic data on fungal diversity. Thirteen metagenomic datasets of Antarctic soil, derived from studies on bacterial diesel degradation, were analysed. From those, 4.91% of the reads were assigned to the genus *Pseudogymnoascus*. They also postulated that some part of the oil-degrading capability was featured by these fungi, as one species found in two datasets, *P. pannorum*, had already been linked to diesel oil biodegradation in the Amazon.

Another facet of metagenomics is called *functional metagenomics*. In this approach, pieces of environmental DNA are randomly introduced into bacteria or yeast for heterologous expression. Colonies are later screened for a particular capability of interest, allowing identification of molecular activities without even knowing the donor organism. This strategy was used by Berlemont et al. (2013) to produce and screen a metagenomic library from Antarctic soil for lipase activity. A novel enzyme, Mhlip, was identified, characterized, and shown to be adapted to the

cold Antarctic conditions. Ferrés et al. (2015) also used this strategy to search metagenomic samples of glacial melt water for lipase/esterase, cellulase, and manganese oxidase activities. Additionally, an alkalophilic esterase was identified from Antarctic desert soil samples (Hu et al. 2012). These classes of enzymes are easily identified by functional metagenomic analyses because the activity assays are simple and low cost.

To be functional, any gene, protein-coding or not, must be transcribed from the genome to RNA. So, *transcriptomics* refers to the study of the transcriptome, the set of transcripts produced by a cell in a given moment. Its composition is directly influenced by the cell cycle, developmental stage, environmental conditions, and stresses, among other factors. It is extremely plastic and changes according to different circumstances. RNAs perform diverse roles in the cell: they are structural molecules, code for proteins, regulate chromatin modification, transcription, stability of transcripts, translation, and many other cellular processes. RNA molecules range from tens to thousands of bases, and are organized in classes related to their coding potential, size, and functionality (Wang et al. 2009). Investigation of an organism's transcriptome is paramount to identify the genetic agents underlying a response by comparing the gene expression profile before and after a specific stimulus.

Further, in an analogous way, *metatranscriptomics* refers to strategies where the RNA is assessed directly from environmental samples. It is similar to metagenomics, but this approach pictures the expressed genes involved in processes that are actually being carried out by the microbial community, whereas *metagenomics* pictures any parts of the composing genomes, regardless of their gene content or transcriptional state. From the assembled transcripts is also possible to identify the organisms of origin and reconstitute the microbial community diversity, based on sequence comparison (Bashiardes et al. 2016). This approach was used to compare three diatom communities of the Antarctic Peninsula for functional differences by Pearson et al. (2015), but its application in Antarctic fungi remains to be demonstrated.

15.3 Sequencing: From Molecules to Data

To investigate and study the genome or transcriptome of any given organism, it is necessary to sequence its DNA or RNA. Sequencing is the process by which DNA/RNA molecules have their information decoded and digitalized, allowing their computational manipulation. The last decades have witnessed the rise and improvement of many different sequencing strategies, each with its pros and cons, but all participating in the field's revolution.

The first sequencing platforms were based on the chain termination method, also called dideoxy method, developed by Frederick Sanger in 1977 (Sanger et al. 1977). Dideoxynucleotide triphosphate molecules (ddNTPs) are nucleotides lacking the hydroxyl group to which the next base to be incorporated in the elongating strand

would attach. In this method, the DNA to be sequenced is amplified in four separate PCR reactions, each one with conventional nucleotides (dNTPs) plus a small fraction of one of the distinct ddNTPs – ddATP, ddCTP, ddTTP, or ddGTP – which, when added, terminate the elongation of the newly synthesized strand. As each ddNTP incorporation happens only once on each newly produced molecule, at the end of the reaction, many DNA strands with different lengths are generated, each corresponding to every possible strand size. The amplification products are then resolved by electrophoresis in a denaturing acrylamide gel where the material of each of the four reaction tubes is run separately. The nucleotide sequence is inferred from the position of the bands in each of the four lanes. The platforms based on this method were automated to detect the DNA fragments with lasers, as long as the products composed of one fluorescent nucleotide migrated through the gel. Moreover, the labelling of each ddNTP with a distinct fluorescence tag made it possible to amplify and run the four reactions together in a single lane (Smith et al. 1986). These automated sequencers were capable of generating intermediate size reads from 300 to 1000 bases long, with relatively low error rates and are still used nowadays in cases where few sequences are to be investigated, or to aid in short reads assembly.

The Sanger sequencing method opened the doors for sequence-level genetic analysis but was not powerful enough for applications such as eukaryotic genome sequencing, making it extremely labour-intensive and expensive. The Human Genome Project triggered the race for the development of other derivate techniques devised to overcome sensitivity, throughput, and cost limits, aiming at lowering the price to sequence a full human genome at USD 1000 or less. The new techniques and platforms are together referred to as *next-generation sequencing* or, shortly, *NGS* (Heather and Chain 2016).

In NGS technologies, more powerful sensors allowed working with much lower sample input, but great improvement was obtained with highly massive parallelization. The sequencing process is now based on nucleotide incorporation detection rather than on chain termination. At each new incorporation cycle, the added nucleotides are detected directly, by fluorescence labelling (Illumina platforms), detection of pyrophosphates released on base incorporation (Pyrosequencing, Roche), or by proton release and pH variation sensors (Ion platforms, Thermo Fisher Scientific). Moreover, by the molecular addition of index sequences in the sample preparation process, many samples can be multiplexed to be analysed in a single run. However, most NGS platforms still output short reads when compared to Sanger sequencing, ranging from 35 (the first ones) to a little more than 1000 bases long, with the current technologies. This size limitation is bypassed with computational and bioinformatics tools, which have evolved side by side with the sequencing platforms. Yet, computational power sets the limits nowadays. As a wide variety of sequencers have become popular equipment in every field of life sciences and adjoining areas, the amount of generated raw sequencing data greatly surpasses the ability to process and interpret it. Currently, the most used NGS platforms are produced by Thermo Fisher, with Ion PGM, Ion Proton, and S5 sequencers, and by Illumina, with the

benchtop sequencers iSeq, MiniSeq, and MiSeq, and the production-scale sequencers HiSeq, NextSeq, and NovaSeq.

In the sequencing process, the same fragment can be sequenced in different manners. It is possible to sequence one end, which generates single end reads (SE), or both ends, which generates paired end reads (PE). In the latter case, if shorter than the sum of two reads size, the sequenced ends overlap in the middle of the fragment sequence, representing it all. The PE reads bear information of synteny, even when they do not overlap. The reads might belong to the ends of a fragment of up to 800 bases. Sequence assembler softwares use this valuable information to connect regions and extend the assembly. A derivation of PE sequencing is mate pairs (MP). In this technique, longer fragments from 2 kb up to 5 kb are circularized by biotinylation, fragmented, and sequenced to produce associate reads belonging to the ends of the original fragment. They are also used to infer contiguity, but at greater distances, and are fundamental to assemble DNA species like chromosomes, from short reads data.

The more recent achievements of the sequencing revolution are altogether called *third-generation sequencing* (*TGS*) techniques (van Dijk et al. 2018). These techniques have in common the ability to sequence very long fragments of single molecules, reaching read lengths longer than 2,000,000 base pairs (Payne et al. 2018), with no need of amplification (Heather and Chain 2016). Some, like SMRT (single-molecule real-time) sequencing developed by PacBio (Pacific Biosciences) are based on incorporation detection with high sensitivity for a fluorescent nucleotide in a very small volume – zeptoliters ($10e^{-21}$) – in the bottom of a well where a DNA polymerase is attached. Others, like the MinIon, GridION, and PromethION (Oxford Nanopore), work on direct detection of sequence composition by reading electric variations in a membrane pore when each base of the sequence fragment passes through, also allowing detection of base chemical modifications, such as methylation. At first these platforms had high error rates up to 13%, but with the advances in sequence chemistry and the ability to re-sequence the same molecule, this drawback is being put aside. In particular, the Oxford Nanopore platforms are already able to sequence RNA directly (Garalde et al. 2018).

NGS strategies can be used alone or in combination, complementing each other in a hybrid approach. Inaccurate long reads can aid the scaffolding of accurate contigs generated from short reads (Sohn and Nam 2016) or can aid in determining the length of repetitive regions. Repetitive regions were regarded for a long time as useless products of expansion of repetitive elements, transposons, and viral remains and remained ignored on genetic analyses. Although some of these elements have no function at all, others are essential. They have been domesticated to play important roles in cellular processes such as formation of the kinetochore and other nucleoprotein complexes (Shapiro and von Sternberg 2005), and being part of the bacterial and archaeal adaptative immune system (Kunin et al. 2007). Concerning Antarctic biotechnological prospection, repetitive sequences should not be ignored. Many AFPs have active sites composed of repetitive blocks of amino acids, which can be encoded by repetitive nucleotides (Davies 2014; Baalsrud et al. 2017).

15.4 Assembling the Genome

Any reads generated on sequencing, big or small, are usually fragments of a given genome (or transcriptome). To reconstitute the original full genome sequence, it is necessary to use computational strategies in a process analogous to assemble a jigsaw puzzle but with millions of pieces. The information needed to accomplish this task is contained in the reads to be assembled, as it is in the pieces of the puzzle. In the latter, adjacent pieces are connected based on their complementary shapes and patterns. For a genome or transcriptome, the assembly is done by finding overlapping parts in read pairs. When a pair of reads shares a common sequence, they are connected, generating a continuous sequence called contig (Fig. 15.1). This process is repeated progressively until no more shared sequences are found in the reads universe.

There are two main assembling strategies, de novo assembly and referenced assembly. The de novo assembly (also called ab initio assembly) uses only the information contained in the reads to reconstitute the original sequence. It is the preferred strategy and usually the first to be attempted. The outcome depends on the read quality, coverage, and the complexity of the sequence to be assembled.

The sequence quality refers to the probability of each position of a read to actually represent the original nucleotide from which the sequence is derived. It is measured at the sequencing process and relates to the intensity of the signal for each base sequenced. The quality can vary drastically along the read, and checking it is the first step before the start of any assembly. Full reads or read pieces of low quality must be removed from the set to assure that only quality reads are used in the assembly, in a process known as trimming.

The coverage refers to the percent of the sequenced fragment that is covered by the generated reads. The coverage is complemented by another concept, sequencing depth, which refers to the number of times each position of the sequence is represented in the reads set. Due to biases inherent to the sample nucleotide composition and size, and to the library construction and sequencing process, some parts of the genome will be better represented than others. Despite being fully covered in the sequencing output, a sequenced fragment might have inner regions that vary in depth. Prior to assembly, the average expected depth can be estimated by multiplying the number of output reads by the read size and dividing by the approximate size of the genome or the sequence to be assembled. Both concepts are also used at the end of the assembly process as quality metrics. Higher depth values for a given position mean that there is more evidence in the reads set to guarantee that this position truly represents the actual base in the original sequence. In this manner, coverage and depth are measures of assembly reliability.

The sequence complexity corresponds to the content of the original sequence. Sequences of high complexity are easier to assemble as there is low probability that a read aligns to more than one position. Low-complexity sequences such as repetitive regions are harder to assemble, especially if they are longer than the read size. They make it difficult for the assembly software to precisely define the connection

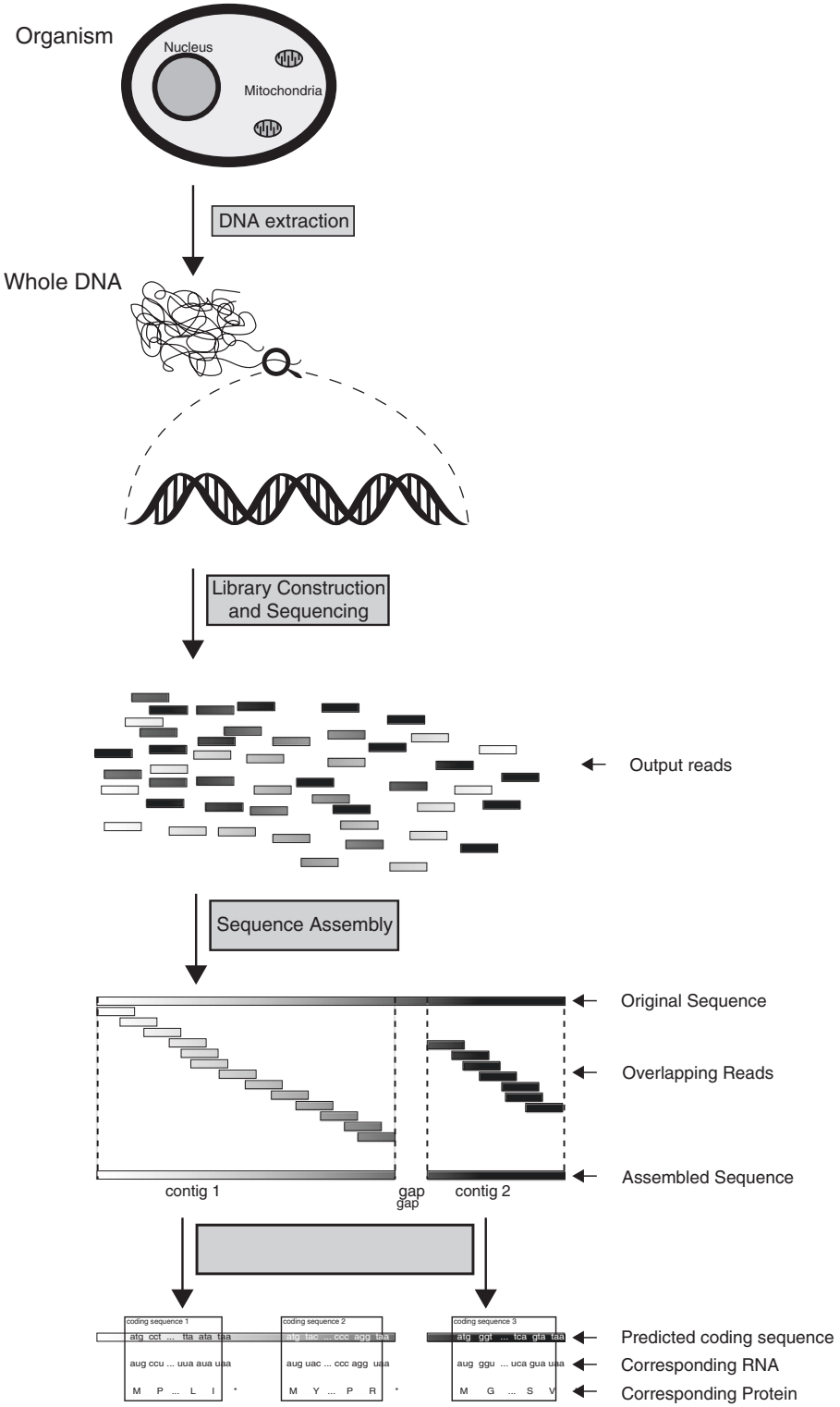

Fig. 15.1 Summarized workflow of the genome sequencing, assembly, and prediction process

between the reads in the read set, as there are greater possibilities of read pairs to concatenate.

With appropriate coverage and high-quality reads, it possible to assemble an entire genome or, more commonly, its regions of high complexity. As low complexity regions do not contain genes at most times, it is not unusual to proceed to the next analytic steps, namely gene prediction, annotation, and genetic comparison, before fully assembling the genome. Moreover, some genomic analyses require full genome reconstruction, which makes it necessary to connect the contigs to structures that are closer to the genomic structures of chromosomes. This process is called scaffolding. It can be approached by sequencing the ends of long fragments that will be later used to connect and position the contigs relatively to each other, the formerly referred *mate pairs*. As these fragments are much longer than the reads and as it is not possible most of the times to precisely determine their length, the resulting scaffolds will be contigs connected by stretches of a sequence of arbitrary size and unknown composition. These connecting regions are represented by the letter N, meaning any nucleotide. Despite being a very elegant strategy, mate pair reads may become obsolete with the popularization of long read platforms.

An alternative way to perform scaffolding is to use another assembled genome of the same species, when available, or a genome of a close species as a reference. This process is called *referenced assembly*. This would be the least desired alternative as even close species have their own genomic particularities that may introduce biases. Further, for using genomes of the same species, it is necessary to be sure of the reference genome assembly quality and reliability, as errors in the reference genome will be propagated to the new assembly.

With improvements in sequencing technologies yielding longer and more accurate reads, the *referenced assembly* strategy may become obsolete. Despite applying the referenced, de novo, or mixed strategies, many softwares are available to perform the assembling task, each with its own particularities, most appropriated for different types of organisms and their genomic structures. Each assembler also presents many possibilities of configuration, which drastically affects the outcome. It is usual to try different softwares and configurations and compare the output assemblies based on their quality metrics to determine the best suited assembly to each case. Assemblies with a longer span, structured in fewer contigs are preferred. Two metrics to be considered are the *L50* and *N50*. The first refers to the number of the largest contigs that are, together, equivalent to half of the assembly size. The lower this value, the less fragmented is the assembly. From the L50 contigs, the N50 corresponds to the size (in base pairs) of the smallest contig, thus allowing comparison of two assemblies with the same L50 value.

In addition to numerical metrics, the quality of an assembly can be evaluated in terms of its completeness. It refers to the percentage of expected genes for that species, which are present in the assembly. As genomes vary in content even between close species, this measure is an estimate and depends on the previous knowledge on the close species genomes. The key genes used to calculate the completeness are single-copies orthologous genes, which are a special type of homologous genes.

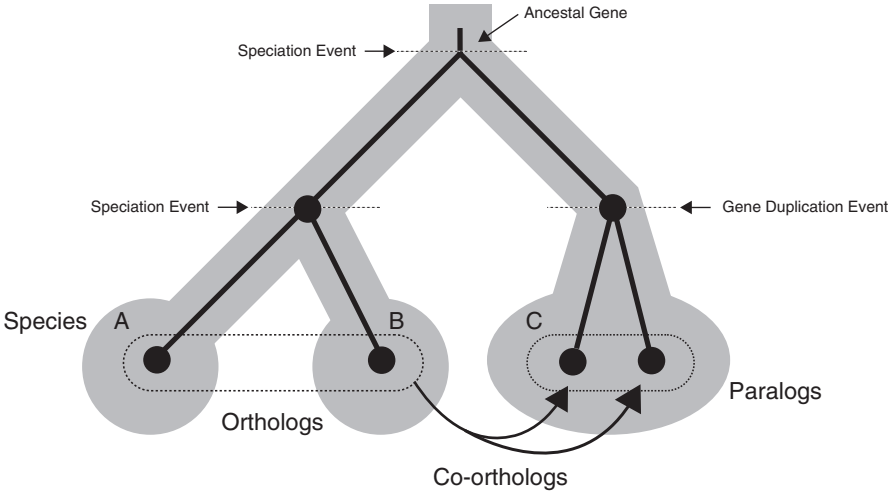

Fig. 15.2 Summary of homology relationships: genes related in terms of ancestry (homologous) that retained the same function in distinct species are called orthologous genes (orthologs). Genes duplicated along the evolutionary history of a species, whether they have retained the same function or not, are coined as paralogous genes (paralogs)

Homology is the existence of shared ancestry between a pair of structures or genes (Fig. 15.2). A gene that is common to two (or more taxa) is said to be homologous. Eventually, through the course of generations, a gene might be duplicated. The organism's genome will now carry two or more copies of this sequence. As the original molecular function of the gene product is maintained by one of the copies, the others are free to mutate. These new copies might assume other functions that, if beneficial to the organism, will therefore be maintained. This is one of the ways in which new genes arise (Wu and Knudsona 2018). Homology relations are subdivided into two main types. If two genes (or DNA sequences) share ancestry because of a speciation event, they are said to be *orthologous*. If they share ancestry because of a duplication event in a species, they are said to be *paralogous*.

By analysing high-quality genome assemblies of related species, it is possible to identify orthologous genes that are always present on the genomes of clade members. These are called the *core orthologous genes* (*COGs*) for that clade. Some of these genes are virtually vital for the organisms, and thus always tend to be present in any related genome. The closer the species are, the greater will be the number of COGs. So, a new genome assembly of another member of the clade is expected to contain almost all of these genes. The absence of expected COGs might indicate that this assembly is not truly representing the actual genome, as some parts – *contigs* containing these genes – might be missing. With a good assembly in hand, one can proceed to a deeper investigation for understanding the organisms' biology from a genetic and molecular perspective.

15.5 Investigation: From Bases to Genes, from Genes to Function

The choice to bioprospect an organism usually comes from an interest to understand a remarkable capability, but any organism has more to tell than the phenotypic traits that catch one's attention at first. Identifying genomic traits related to the particular phenotype of interest is not a simple task, and resembles the search for a needle in a haystack. However, it can be immensely facilitated depending on the availability of close species genomes, transcriptomes, and genetic data or previous knowledge on similar gene functionalities. In the *comparative genomics* field, the genomic information of different species is compared to highlight the differences or similarities that can be accounted for particular traits. For example, AFPs are a class of proteins that has evolved independently many times in different taxa. Some AFP classes are well understood and characterized, but some are yet unknown, with evidence of their existence coming only from the observation that the organism can survive freezing (Bar Dolev et al. 2016). If one aims to identify AFPs in novel organisms, comparison with sequences of other AFPs from closely related organisms might indicate the most probable candidates. Conversely, if one is working with lineages from which AFPs have not been identified yet, a subtractive approach can be applied. By comparing the genome of an AFP-producing organism to a close one that does not have this ability, the exclusive regions might contain the AFP-coding genes that are sought for. This approach greatly reduces the regions of the genome to be mined.

Once assembled, the genome is purely a set of strings carrying letters with no evident significance. To describe it, it is necessary to identify where all the genetic features are and to assign their functions. This can be done by two main strategies. The first one is by searching for similarities with sequences from previously studied organisms. Genetic information is available in many specialized curated sequence databases, where sequences are stored together with their attributes, obtained by experimental, or theoretical approaches. The second is to work with information contained in the genome itself. This strategy is called ab initio gene prediction and involves identifying the regions of interest in the genome with the aid of computational tools trained to identify specific features. Locating these regions and assigning their composing elements, such as protein-coding genes, regulatory sequences, intron-exon boundaries, repetitive elements, as well as inserting additional information and comments, is a process called *genome annotation*.

Despite differences, all known life forms work on an almost identical coding system (Koonin and Novozhilov 2017). Their protein-coding genes are all virtually structured as *open reading frames* (*ORFs*): a start codon in frame with amino acid-coding codons, ending in a stop codon. Gene prediction softwares and algorithms use this principle to identify every piece of the genome that matches these characteristics. This does not imply that the region is actually a gene, but it is an indication. Since the probability of a stop codon occurrence is approximately 1 to every 20 codons, ORFs with 60 nt or smaller tend to be disregarded. Classical ORF predictors

usually consider only ORFs longer than 150 nt (50 amino acids) by default (Rombel et al. 2002), but there is growing evidence that small ORFs also code active genes (Couso and Patraquim 2017). The longer the ORF, the lower is its probability to originate and be maintained in the genome if not under selective pressure. Further, similar to eukaryotes, fungi have some of their coding sequences (CDSs) interrupted by introns, that must be spliced out after transcription to generate a mature RNA. In the annotation process, the intronic sequences must be considered, and this is achieved by using an intron-aware annotation software.

Further, identification of sequences with self-complementarity and palindromic sequences indicate possible ncRNAs. There are many gene predictors and pipelines available to accomplish the annotation task, most in constant improvement. All follow the same logic but are better suited to specific situations, such as organisms that have an exon/intron structure, gene editing processes, or those that are derived from metagenomic samples, among other particularities.

Proof of a gene's existence, protein coding or not, is the presence of its corresponding RNA in the cell. However, as many genes are expressed only in particular situations, it is not always easy to provide the right stimuli, especially when the cultivating conditions are so extreme and hard to replicate, as for most Antarctic microorganisms.

Once we know the possible genes, the next step is to assign their function. The best way to do so is to compare each gene's sequence to the characterized sequences deposited in databases. The UniProt/Swiss-Prot is the most suited database for this purpose, as it is composed of curated information, which is sometimes experimentally validated. Many other databases are available, dedicated to specific taxa and organisms, with or without experimental evidences.

Pairwise comparison between sequences is performed with alignment tools like *BLAST* (Basic Local Alignment Search Tool) (Altschul et al. 1990) or *DIAMOND* (Buchfink et al. 2015). These tools look for sequence similarity that ultimately suggests a taxonomic relationship and make it possible to extend information from known sequences to the query sequence. The search must be performed by choosing extremely low e-value cutoffs and selecting only highly similar alignments. These characteristics might indicate same ancestor origin and homology relations. It is also common to look for characterized protein domains/signatures using protein domain databases as a reference. *InterProScan* is the most commonly used software for this, and Pfam is the most used reference database.

These strategies are efficient at finding the core metabolism and structural genes, as most of them are extremely conserved throughout life domains. As the Antarctic biodiversity is yet poorly characterized and has thousands of years of divergence, many of their organisms' new genes do not share nucleotide or amino acid sequence similarities with any known sequences. This is the case for most antifreeze proteins, as most of them are unique to each organism; having evolved independently many times (Davies 2014).

15.6 Omics Studies in Antarctica

The omics strategies have been largely used in the study of bacteria and archaea, as their genomes are usually much smaller than those of eukaryotes, making their investigation considerably cheaper and more feasible. Some studies have characterized archaea (Anderson et al. 2016) and bacteria (Dsouza et al. 2015; Han et al. 2016) endemic to the Antarctic region. The advantages of using omics to investigate Antarctic fungi can be exemplified in a recent study on a psychrophilic basidiomycete yeast isolated in the Antarctic region, *Glaciozyma antarctica*. Firdaus-Raih et al. (2018) have characterized this yeast. It has a 20 Mb genome, composed of 7857 predicted CDSs, of which 67% were confirmed by EST (Expressed Sequence Tag) sequencing. Nine genes were found to have similarity with known AFP-coding genes. These genes had their expression patterns characterized after sub-zero temperature exposure and were also heterologously expressed in bacteria to characterize their antifreeze properties. Other genes related to organism freezing tolerance were investigated. FADs are enzymes responsible for the introduction of double bonds in fatty acids that compose the lipid membrane. The double bond content is directly related to membrane fluidity, and the expression of these FADs was found to increase after sub-zero temperature exposure. The purified proteins were also submitted to thermal hysteresis assays to determine their influence on the freezing/melting point of water, and were found capable of lowering the water freezing point by 0.04–0.08 °C.

Another example of Antarctic fungi genomic investigation is the study on the black cryptoendolithic *Cryomyces antarcticus* (Sterflinger et al. 2014). The genome of 24.3 Mb was assembled in 12,492 contigs larger than 300 bp, with an N50 of 4.72 Mb. Gene prediction identified 10,731 putative protein-coding genes and the genome was compared to those of five other close species of melanized fungi from non-extreme habitats that were available, but no gene related to freezing tolerance was found.

Genomic analyses are being carried out by our group on the genomes of Antarctic fungi. *Metschnikowia australis* is a marine yeast that lives in close association with diverse Antarctic marine algae. It was selected for genomic analysis due to its capability to withstand freezing down to −80 °C, without the addition of any cryoprotectant (i.e. glycerol). The yeast's genome was sequenced, assembled, and annotated (Batista et al. 2017), totalling 4442 protein-coding genes along its 14.3 Mb genome. Since no similarity was found with any known antifreeze protein gene, a different approach to investigate this phenotypic trait is necessary. One possible strategy is to identify genes directly associated with this feature using comparative genomics. The genome of the organism in study is compared to other genomes from close species that lack the freeze tolerance capability. Exclusive genes present only in the interrogated species can be identified, thus reducing the candidates to be screened for the antifreeze property.

15.7 Ontology and Metabolic Analyses

In some cases, the same molecular activity can be performed by different and unrelated genes. These situations require a classification system not based on homology. Further, the grouping of an organism's genes into functional classes allows rapid, albeit superficial, assessment of the processes that are enriched in a genome or transcriptome. Similar and related functions are organized in hierarchies that ultimately encompass all cellular processes, with specific associations to a unified, computable terminology, in what is called *ontology*. One widely used ontology classifier is *gene ontology* (Carbon et al. 2017), which describes genes by three major classifications: cellular component, biological process, and molecular function. Another one is the KEGG BRITE, a highly organized classification of hierarchical functions and networks of molecular interaction for genes constituting the KEGG Orthology database (Kanehisa and Goto 2000). These initiatives are fundamental to unify biological knowledge under a common vocabulary and provide important resources to investigate any genome functionality.

Secondary metabolites (*SMs*) are molecules produced by an organism that are not necessary for normal development or growth (Fox and Howlett 2008). Fungi are molecular factories capable of producing many SM compounds with various functions. These compounds act as antibiotics, virulence factors or pigments, and are related to processes like signalling, among other functions (Macheleidt et al. 2016). These molecules are incredibly diverse and have immense biotechnological applications, and fungal extracts are commonly prospected for activities against pathogens and cancer (Godinho et al. 2013; Spiteller 2015).

Genomic investigation of fungi shows that there is still a lot to comprehend. It is now easier to identify many gene clusters related to SM production by sequence similarity, than it is to identify their final products. For example, sixty-eight gene clusters are known in the model ascomycete *Aspergillus nidulans*, but the products for only 20 of those are known (Macheleidt et al. 2016). There are databases dedicated to storing information on secondary metabolites and their related genes. These databases also provide computational tools to assess genomes in pursuit of SM producing genes. Once a specific activity of interest is detected by extract screening, one important starting point for the identification of the responsible pathway is to seek genes possibly involved in secondary metabolite production. By revealing these genes, one can devise strategies to boost pathway activity or even proceed to cloning its component genes for heterologous expression (Macheleidt et al. 2016). One example is the work performed by Hossain et al. (2016), in which the three genes of the itaconic acid (IA) production pathway from *Aspergillus terreus* were cloned into *A. niger* and overexpressed to increase the IA production fivefold.

It is documented that many Antarctic yeasts are capable of producing compounds like pigments, microsporines, and other UV-protective compounds, as screened by biochemical analysis (Vaz et al. 2011). Yet, most of these molecules and their biosynthetic pathways remain to be characterized. This reinforces the potential of

Antarctic microorganisms in the production of new biomolecules. As the genetic knowledge on Antarctic organisms increases, bioinformatic tools will aid in comprehending and exploiting this potential.

15.8 Phylogenomic Analysis

An organism's capabilities are as important as its relations to other known species. Genetic marker-based phylogeny is widely employed, especially in microbiology. Fungi, as aforementioned, are commonly classified based on the sequence of ITS regions in the ribosomal RNA gene (Schoch et al. 2012). Despite being powerful, easy to scale up, and widely used, ITS phylogeny is based on one or a few markers that might not be robust enough in some cases. The possibility to use hundreds or thousands of genes in this analysis has brought phylogeny to the omics era, as *phylogenomics*.

With many accessible genomes, there is no need to limit their comparison to short stretches of sequences. These comparisons can be performed using all correspondent sequences among the analysed organisms. As genetic similarity weakens in distantly related individuals, more informative shared regions exist in closer individuals to support their phylogenetical relationship. In phylogenomics there are no preferred genes. All that is common might be compared. Yet, some organisms have gene duplications and gene deletion events that would skew the comparison. A good strategy to overcome this problem is to use only common single-copy orthologous genes.

For phylogenomic analysis, despite having a complete or partial genome, it is important to compare only sequences that have equivalents in all the genomes to be analysed, the aforementioned orthologous genes. It is also important that these sequences have only one copy in each genome – paralogs must be avoided. This guarantees that the information used is unique (Simão et al. 2015). Genes to be compared must be aligned and have the same size. In the alignment process, inner regions that are not shared on the sequences are extended as gaps. After that, the unshared outer regions are trimmed off, leaving all sequences with the same number of positions.

You can either use amino acid or nucleotide sequences for phylogenomic analysis. As many of the investigated sequences correspond to proteins that are under strong selective pressure to maintain their functions and, as amino acids can be coded by more than one codon, close species may bear features almost identical at the protein level, but with DNA sequences being less conserved. In this sense, phylogenomic trees made from nucleotides tend to be better suited to resolve close species relations. Conversely, when studying distantly related species, phylogenomic trees generated from proteins should be used. There is no ultimate rule and both strategies must be tested and compared to choose the most robust and adequate solution. The reliability of output trees can be evaluated by analysing the terminal branches bootstrap – a measure of how frequent a branch configuration is maintained

after a random subsampling of the data. The higher the bootstrap values, the more reliable is the tree.

15.9 Conclusions and Perspectives

The advances in nucleic acid sequencing and interpretation are revolutionizing many fields of biological sciences. These powerful omics tools are available to every microbiologist and give uncountable perspectives on the investigation of microbial diversity, genetic capabilities, and environment deciphering. To use these tools, it is crucial for a microbiologist to be familiar with the fast-evolving approaches, strategies, logic, and vocabulary of the omics field. Nonetheless, their use has unprecedented potential to help understand the immense microbiological diversity that remains to be investigated, especially in the Antarctic continent.

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