Chapter 6
Fungi in Snow and Glacial Ice of Antarctica

Graciéle Cunha Alves de Menezes, Bárbara Alves Porto, Jefferson Cardia Simões, Carlos Augusto Rosa, and Luiz Henrique Rosa

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-

G. C. A. de Menezes (✉) · B. A. Porto · C. A. Rosa · L. H. Rosa
Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil

J. C. Simões
Centro Polar e Climático, Instituto de Geociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brasil

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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.

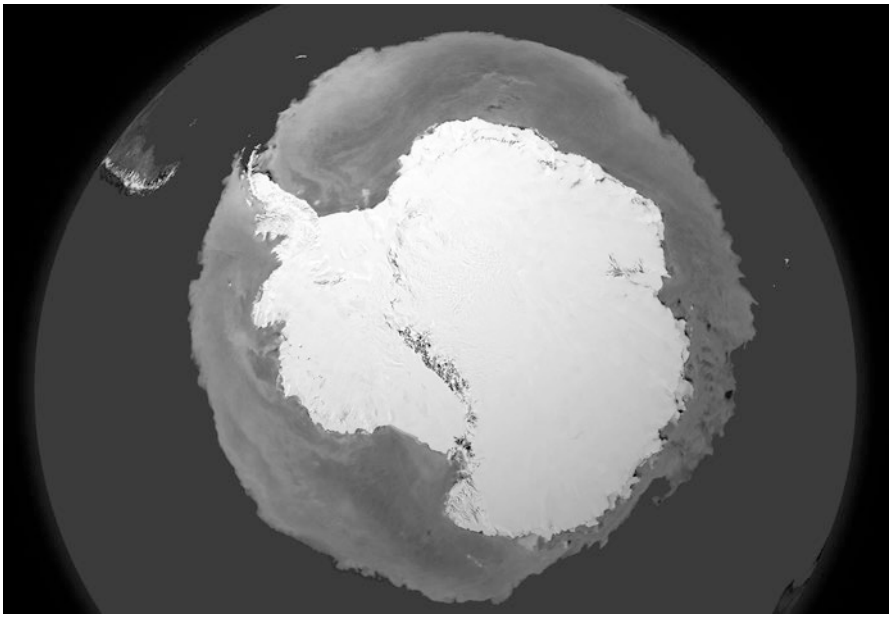

Fig. 6.2 Perspective view of the Antarctic cryosphere. The huge ice sheet (white) covers 99.7% of the continent (across 13.8 million km^2) and the Antarctic Peninsula. The dark spots represent the few mountainous regions with apparent rocks. Around the continent, grey represents sea ice at its maximum 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)

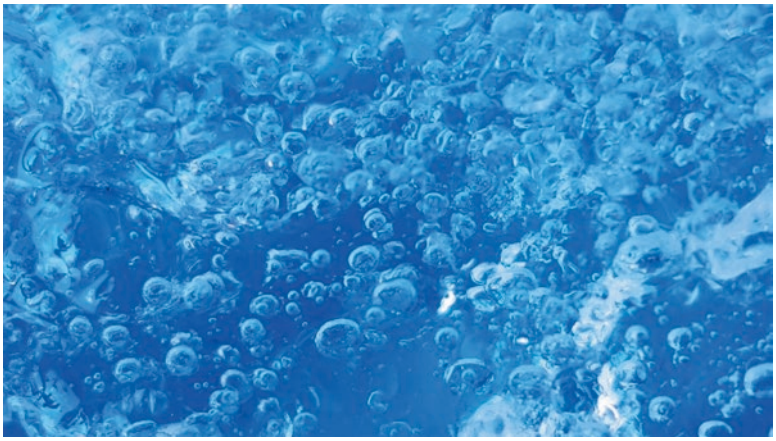

Fig. 6.7 Details of bubbles trapped in fragments of ice collected in Antarctica from glaciers containing atmospheric air of ancient Earth. (Photo Credits: LH Rosa)

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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