Chapter 3
Antimicrobial Potential of Cold-Adapted Bacteria and Fungi from Polar Regions

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1 Introduction

The Earth's biosphere is predominantly cold in time (about 85 % of the year) being exposed to temperatures below 5 °C. Polar Regions constitute about 14 % of cold habitats on Earth. Such low-temperature environments often undergo a combination of environmental stresses including desiccation, nutrients limitation, high salinity, adverse solar radiation and low biochemical activity (Pearce 2012). Even though such harsh conditions preclude life in most of its forms, cold habitats have been successfully colonized by numerous organisms, especially by microorganisms that predominate over other organisms in terms of both biodiversity and biomass (Feller and Gerday 2003; Margesin 2007; Pearce 2012). On the basis of their cardinal temperatures, cold-adapted microorganisms are frequently distinguished in psychrophilic and psychrotolerant (or cold-loving and cold-tolerant, respectively) (Morita 1975). By definition, the optimal growth temperature of psychrophiles is ≤ 15 °C, whereas they are not able to grow above 20 °C. On the other side, psychrotolerants can grow over a wide temperature range with the fastest growth rates being above 20 °C. Accordingly, the heat-sensitive true psychrophilic microorganisms inhabit permanently cold habitats, whereas psychrotolerant are overrepresented in

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environments undergoing seasonal or diurnal thermal fluctuations (Margesin 2007). In global terms, psychrotolerants exhibit a much wider distribution than psychrophiles (Pearce 2012).

Since cold-adapted microorganisms have been subjected to a number of environmental stresses on a long timescale, they have evolved a variety of structural and physiological modifications to ensure survival in restrictive environmental conditions (Pearce 2012). These include the production of cold-active enzymes with a more flexible 3D structure at low temperature, cold-acclimation (CAPs) and cold-shock proteins (CSPs), the incorporation of high amounts of unsaturated fatty acids and carotenoids in cell membranes to maintain optimum fluidity and permeability, and the synthesis of cryoprotective substances (Margesin et al. 2007; Russell 2008). In addition to cellular modifications, the recently assessed cold-active antagonistic properties of cold-adapted microorganisms may reduce the presence of competitive microorganisms thus contributing to the microbial adaptation to permanently low temperatures (Lo Giudice et al. 2007a; Mangano et al. 2009; Prasad et al. 2011; Bell et al. 2013). On the other side, such capability has highlighted the possibility to use cold-adapted microorganisms as a novel source of industrial exploitable antimicrobial compounds. Thus, many different, complex and sophisticated survival strategies, which are quite relevant for the ecology of cold-adapted microorganisms, might render them valuable resources also for biotechnological purposes (Cavicchioli et al. 2002).

2 Antibiotic Potential of Cold-Adapted Microorganisms from Polar Regions

Several factors such as the increasing global resistance to existing antibiotics, the development of multiple-drug-resistant pathogens and the emergence of new infections constitute a pressing public health problem. Moreover, in recent decades there has been a dearth of new classes of discovered antibiotics. In attempts to overcome this emergency, the discovery of new and efficient antimicrobials has become of great interest for natural product chemistry. Historically, most bioactive microbial products have been obtained from actinomycetes and filamentous fungi and mainly from terrestrial habitats (Biondi et al. 2008). Recently, the exploration of unusual and underexplored sources of medically useful substances and the screening of less exploited microbial groups (e.g. cyanobacteria) have been recognized as promising tools for the discovery of new natural drugs. Particularly interesting from this viewpoint are microorganisms from extreme environments that produce biomolecules under unusual conditions, thus representing a valuable source of novel metabolites, including antimicrobial compounds with unique structures and specific biological activity (Hemala et al. 2014). For these reasons, cold-adapted microbial producers (heterotrophic and autotrophic bacteria and fungi) of antimicrobial compounds (mainly antibacterial and antifungal) have been isolated from various aquatic and

terrestrial environments in the Arctic and Antarctica. Several studies have been based on screening tests targeting human pathogens. In some cases, the microbial inhibitor has been extracted and (partially o fully) characterized. The versatile antimicrobial potential of microorganisms from Polar Regions will be discussed in the following sections.

2.1 Terrestrial Environments

Soil has been and is still the most exploited ecological niche for the discovery of useful natural bioproducts. Researches have been mainly addressed to the Actinobacteria and fungi, which are widespread in such habitat and able to produce several and different useful secondary metabolites and compounds exhibiting different biological properties. An overview of bacteria and fungi with antimicrobial potential from Polar soils is reported in this section.

2.1.1 Actinobacteria

Several bioactivity screenings on bacteria from Polar soils have been focused on the Actinobacteria (Moncheva et al. 2002; Nedialkova and Naidenova 2005; Gesheva 2010; Lee et al. 2012a; Pan et al. 2013). Such class of bacteria has represented the most fruitful source of antibiotics for decades; the largest fraction (about 80 %) of the new discovered antibiotics derived from *Streptomyces* species (order Actinomycetales), which possess a biosynthetic capacity that remains (at least so far) without rivals in the microbial world (Bérdy 2005; Bull and Stach 2007; Manivasagan et al. 2014).

However, nowadays, the discover of new commercially useful secondary metabolites from common streptomycetes is becoming increasingly difficult, thus emphasizing the need for isolating and testing novel members in this genus (Lyutskanova et al. 2009). Streptomycetes from underexplored habitats, including Polar soils, represent a rich source of novel bioactive compounds, as highlighted by results discussed below. A list of active actinobacterial isolates, including *Streptomyces* spp., is shown in Table 3.1.

Pan et al. (2013) selected 46 Antarctic actinobacterial isolates closely related to the psychrotolerant *Streptomyces beijiangensis* (Signy Island, South Orkney Islands) for their antibacterial activity against *Proteus vulgaris* and *Staphylococcus aureus*, whereas no activity was observed against *Escherichia coli* (only *Streptomyces* sp. PSY097 is reported in Table 3.1 as representative of the 46 *Streptomyces beijiangensis* related isolates). Interestingly, all strains were found to contain the nonribosomal peptide synthetase (NRPS) genes. As reported by Gesheva (2010), among actinomycetes that were able to synthesize extracellular biologically active products the methanol extract from *Streptomyces* sp. 5 biomass showed an antibiotic activity versus both Gram-positive bacteria and phytopathogenic fungi. Another psychrophilic

Table 3.1 Active actinobacterial isolates from Polar soils

	Isolate(s)	Location	Active against[a]	Main features of bioactive molecules	Reference
Antarctic	*Arthrobacter* sp. HPG8	East Antarctica	**7, 14, 15, 21, 25**	Proteinaceous nature	O'Brien et al. (2004)
	Arthrobacter sp. HPH17	East Antarctica	**7, 14, 25**	Proteinaceous nature	O'Brien et al. (2004)
	Arthrobacter sp. 1	East Antarctica	**5, 9, 11, 23, 25**		Gesheva (2010)
	Arthrobacter sp. 9	East Antarctica	**5, 23, 25**		Gesheva (2010)
	Brevibacterium spp. BV2, BV34 and BV35	Barrientos Island	**8**		Lee et al. (2012a)
	Brevibacterium sp. BV37	Barrientos Island	**17, 25**		Lee et al. (2012a)
	Demetria spp. DT40 and DT41	Barrientos Island	**25**		Lee et al. (2012a)
	Gordonia spp. G3 and G48	Barrientos Island	**8, 25**		Lee et al. (2012a)
	Janibacter sp. JB26	Barrientos Island	**17**		Lee et al. (2012a)
	Kocuria sp. KC21	Barrientos Island	**17, 25**		Lee et al. (2012a)
	Lapillicoccus sp. LC31	Barrientos Island	**8**		Lee et al. (2012a)
	Micromonospora sp. 18	East Antarctica	**5, 23, 25**		Gesheva (2010)
	Micromonospora sp. MM6	Barrientos Island	**17**		Lee et al. (2012a)
	Micromonospora sp. MM32	Barrientos Island	**20**		Lee et al. (2012a)
	Nocardioides sp. ND52	Barrientos Island	**8**		Lee et al. (2012a)
	Nocardioides sp. A-1	East Antarctica	**5, 16, 24, 27**	Glycolipidic and/or lipopeptidic nature	Gesheva and Vasileva-Tonkova (2012)

	Rhodococcus sp. 2	East Antarctica	**23**		Gesheva (2010)
	Rhodococcus sp. RC56	Barrientos Island	**25**		Lee et al. (2012a)
	Streptomyces sp. PSY097	Signy Island	**19, 25**		Pan et al. (2013)
	Streptomyces sp. NTK 97	Terra Nova Bay	**5, 25**	Frigocyclinone	Bruntner et al. (2005)
	Streptomyces flavovirens 6[7]	Livingston Island			Ivanova et al. (2002)
	Streptomyces sp. 5	East Antarctica	**5, 9, 11, 23, 25**	Actinomycin	Gesheva (2010)
	Streptomyces sp. 8	East Antarctica	**2, 5, 6, 9, 10, 11, 13, 18, 22, 23, 25**	Non-polyenic macrolide antibiotic, azalomycin B, and nigericin	Gesheva (2009)
Arctic	*Streptomyces* spp. SB9, SB72 and SB81	Svalbard Islands	**1, 3, 4, 5, 9, 12, 18, 22, 23, 25, 26**		Lyutskanova et al. (2009)
	Streptomyces spp. SB33 and SB47	Svalbard Islands	**1, 3, 4, 5, 9, 18, 22, 23, 25, 26**		Lyutskanova et al. (2009)

[a]Target strains: **1**, *Acinetobacter johnsonii*; **2**, *Aspergillus niger*; **3**, *Bacillus megatherium*; **4**, *B. mycoides*; **5**, *B. subtilis*; **6**, *Botrytis cinerea*; **7**, *Brochothrix thermosphacta*; **8**, *Candida albicans*; **9**, *C. tropicalis*; **10**, *C. utilis*; **11**, *Cladosporium cladosporioides*; **12**, *Escherichia coli*; **13**, *Fusarium oxysporum*; **14**, *Listeria innocua*; **15**, *L. monocytogenes*; **16**, *Micrococcus* sp.; **17**, MRSA; **18**, *Penicillium chrysogenum*; **19**, *Proteus vulgaris*; **20**, *Pseudomonas aeruginosa*; **21**, *P. fragi*; **22**, *Saccharomyces cerevisiae*; **23**, *Sarcina lutea*; **24**, *Staphylococcus* sp.; **25**, *S. aureus*; **26**, *Trichosporon cutaneum*; **27**, *Xanthomonas oryzae*

Streptomyces (strain 8) was able to inhibit the growth of Gram-positive bacteria, yeasts, and phytopathogenic fungi (Gesheva 2009). Antibacterial or/and antifungal activity (against *Bacillus subtilis*, *Candida tropicalis* and *Cladosporium cladosporioides*) of *Streptomyces* spp. were further reported by Gesheva and Negoita (2012) for *Streptomyces* spp. 10 and 21 isolated from soils of Haswell Island, Antarctica, but no details on their individual antimicrobial spectrum were reported.

From a panel of 91 psychrotolerant streptomycete strains isolated from the permafrost soils in Spitsbergen (Arctic Ocean) five ones exhibited a strong antimicrobial activity toward Gram-positive and Gram-negative bacteria, yeasts and fungi (Lyutskanova et al. 2009). The thin layer chromatography (TLC) profiles of their cell-free supernatants showed antibiotic complexes consisting of three major compounds, whose highest concentration was found in the supernatant obtained from cultures of *Streptomyces* sp. SB9. No biochemical characterization was reported.

Active actinobacterial isolates other than *Streptomyces* spp. have been also isolated from Polar soils. The methanol extract from the *Micromonospora* sp. 18 biomass showed a good antibacterial activity (Gesheva 2010). Actinobacterial strains from different locations of Barrientos Island, Antarctica, were selected by Lee and coworkers (2012) for their ability to produce secondary metabolites with antimicrobial and antifungal activities. Four screening models were used allowing identifying seven isolates that were active against *Candida albicans*, seven isolates versus *S. aureus*, four isolates toward methicillin-resistant *S. aureus* (MRSA) and one isolates against *Pseudomonas aeruginosa*. The most bioactive genus was *Brevibacterium* with four bioactive isolates. The relationship between taxonomic and metabolic diversity of bacteria was highlighted. Five isolates (within the genera *Demetria*, *Nocardioides*, *Lapillicoccus* and *Rhodococcus*) might be assigned to new genus or species, as they were separated from their respective type strains phylogenetic neighbors by sequence similarities of their 16S rRNA genes, strongly suggesting the possibility of discovering novel strains with antimicrobial activity.

Gesheva and Vasileva-Tonkova (2012) tested the antimicrobial activity of cell-free supernatants of *Nocardioides* sp. A-1 cultures in mineral salts medium supplemented with different carbon sources. These latter favored the production of a broad spectrum compounds with antimicrobial activity against Gram-positive and Gram-negative bacteria, especially *S. aureus* and *Xanthomonas oryzae*. A preliminary analysis by TLC showed that compounds with antimicrobial activity were mainly glycolipids and/or lipopeptides depending on the used carbon source.

2.1.2 Other Bacteria

Reports on antimicrobial producing heterotrophic bacteria, other than the Actinobacteria, from Polar soils are reported in Table 3.2. A first study was carried out by O'Brien et al. (2004) by screening 4496 bacterial isolates from East Antarctica soils for the production of cold-active antimicrobials compounds potentially useful in chilled-food preservation. Four of the inhibitor–producers (0.29 % of total isolates) were affiliated to the genera *Arthrobacter*, *Planococcus* and *Pseudomonas*.

Table 3.2 Active cyanobacteria and heterotrophic bacteria (other than Actinobacteria) from Antarctic soils

Phylum or class	Isolate	Location	Active against[a]	Notes	Reference
Alphaproteobacteria	*Bradyrhizobium* sp. BR45	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR42	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR62	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR65	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR82	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR88	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR96	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR100	Barrientos Island	**5**		Lee et al. (2012b)
	Bradyrhizobium sp. BR105	Barrientos Island	**5**		Lee et al. (2012b)
	Methylobacterium sp. MB63	Barrientos Island	**18**		Lee et al. (2012b)
	Methylobacterium sp. MB104	Barrientos Island	**5**		Lee et al. (2012b)
	Methylobacterium sp. MB20	Barrientos Island	**5**		Lee et al. (2012b)
	Paracoccus sp. PC101	Barrientos Island	**5**		Lee et al. (2012b)
	Sphingomonas sp. SM14	Barrientos Island	**5**		Lee et al. (2012b)
Betaproteobacteria	*Janthinobacterium* sp. SMN33.6	Fildes Peninsula	**1, 10, 11, 14, 18**		Asencio et al. (2009)
Gammaproteobacteria	*Pseudomonas* sp. CrCD21	East Antarctica	**4, 12, 19**	Proteinaceous	O'Brien et al. (2004)
	Pseudomonas sp. CG21	King George Island	**9, 10**	Proteinaceous	Wong et al. (2011)
	Pseudomonas sp. MTC3	King George Island	**9, 10, 11, 16**	Proteinaceous	Wong et al. (2011)
	Pseudomonas sp. WEK1	King George Island	**20**		Wong et al. (2011)
	Pseudomonas sp. WEA1	King George Island	**20**		Wong et al. (2011)
	Pseudomonas sp. MA2	King George Island	**20**		Wong et al. (2011)
Bacteroidetes	*Pedobacter* sp. BG5	King George Island	**2, 9, 10, 11, 17**	Proteinaceous	Wong et al. (2011)
Firmicutes	*Planococcus* sp. CHF8	East Antarctica	**4, 12, 13**	Proteinaceous	O'Brien et al. (2004)
	Enterococcus sp. APR 210	Schirmacher Oasis	**5, 6, 11, 12, 13**	Bacteriocin-like	Shekh et al. (2011)
Cyanobacteria	*Fischerella* sp.	Haswell Island	**3, 7, 8**		Gesheva and Negoita (2012)

[a]Target strains: **1**, *Acinetobacter baumannii*; **2**, *Bacillus cereus*; **3**, *B. subtilis*; **4**, *Brochothrix thermosphacta*; **5**, *Candida albicans*; **6**, *C. krusei*; **7**, *C. tropicalis*; **8**, *Cladosporium cladosporioides*; **9**, *Enterobacter cloacae*; **10**, *Escherichia coli*; **11**, *Klebsiella pneumoniae*; **12**, *Listeria innocua*; **13**, *L. monocytogenes*; **14**, *Pseudomonas. aeruginosa*; **15**, *P. putida*; **16**, *Salmonella enterica*; **17**, *Salmonella* spp.; **18**, *Serratia marcescens*; **19**, *Staphylococcus aureus*; **20**, *Vibrio parahaemolyticus*

The proteinaceous nature of inhibitors synthesized by these strains was revealed by their sensitivity to protease. The same was true for antimicrobials from *Pedobacter* sp. BG5, and *Pseudomonas* spp. CG21 and MTC3 that were selected among 2465 bacterial isolates from Antarctic soils as they demonstrated inhibitory effects on the growth of one or more indicator foodborne pathogens (Wong et al. 2011). The activity of inhibitors from three additional *Pseudomonas* isolates (i.e. WEK1, WEA1 and MA2) was insensitive to catalase, lipase, α-amylase, and protease enzymes.

Shekh et al. (2011) selected *Enterococcus* sp. APR 210 from an Antarctic penguin rookery for its ability to inhibit the growth of the multidrug-resistant fungal pathogenic yeast strains *C. albicans* NCIM 3471 and *Candida krusei*, in addition to antibacterial activity. The effect of enzymes, heat and pH on the antifungal activity of cell-free supernatants of *Enterococcus* sp. APR 210 was investigated. The biological activity was completely lost at 100 and 121 °C, whereas it was maintained after repeated freezing and thawing or long-term storage at −20 and −80 °C. The activity was also completely lost at pH values 2, 4 and 10. However, no loss of activity at pH values 6, 6.9 and 8 was observed. The candidacidal principle was sensitive to proteinase K (which caused a complete loss of antimicrobial activity) and to pronase E (which led to partial loss of biological activity). Treatment with trypsin had no effect on the biological activity. . Results suggested that the antimicrobial compound(s) produced by *Enterococcus* sp. APR 210 belonged to a protein of class II bacteriocins or bacteriocin-like inhibitory substances.

Lee et al. (2012) tested the antibacterial and antifungal capability of 57 proteobacterium isolates from soils of Barrientos Island, Antarctica. After the screening, a total of 14 isolates producing bioactive metabolites were identified. Members of the genera *Bradyrhizobium*, *Paracoccus* and *Sphingomonas* were active against *C. albicans*, while three isolates from the genus *Methylobacterium* showed bioactivity against *S. aureus* and *C. albicans*. None of the isolates showed bioactivity against *P. aeruginosa* or MRSA.

The ethanolic extract of *Janthinobacterium* sp. SMN 33.6 from Antarctic soil possessed antibacterial activity against multi-resistant nosocomial isolates, such as *Serratia marcescens* (MIC = 0.5–2 μg/mL), *P. aeruginosa* (MIC = 1 μg/mL), *Klebsiella pneumoniae* (MIC = 16 μg/mL), *Escherichia coli* (MIC = 0.5–1 μg/mL) and *Acinetobacter baumannii* (MIC = 1 μg/mL) (Asencio et al. 2009).

Among Bacteria, cyanobacteria from Polar soils have been rarely reported as antimicrobial producers. Gesheva and Negoita (2012) described a *Fischerella* sp. isolate (Haswell Island, Antarctica) that inhibits the growth of *B. subtilis*, *C. tropicalis* and the ascomycete *C. cladosporioides*.

2.1.3 Fungi

Even though fungi may represent an important source of new natural bioactive molecules, few studies have been carried out on this issue. Among fungi, members of the genera *Penicillium* and *Aspergillus* generally prevail in soils and their antimicrobial activity has been often reported, including for Polar soils (Table 3.3).

Table 3.3 Ascomycetes with antimicrobial activity isolates from Polar soils

Isolate	Location	Mainly active against[a]	Notes	Reference
Aspergillus nidulans	Haswell Island, Antarctica	Not shown		Gesheva and Negoita (2012)
Aspergillus sydowii 9541	Ellsworth Mountains, Antarctica	**4, 7**		Godinho et al. (2015)
Geomyces sp. 2481	King George Island, Antarctica	**1, 5, 8**	Geomycin A-C	Li et al. (2008)
Penicillium allii-sativi 9451	Ellsworth Mountains, Antarctica	**8**		Godinho et al. (2015)
P. allii-sativi 9458	Ellsworth Mountains, Antarctica	**4**		Godinho et al. (2015)
P. allii-sativi 9508	Ellsworth Mountains, Antarctica	**8**		Godinho et al. (2015)
P. allii-sativi 9524	Ellsworth Mountains, Antarctica	**8**		Godinho et al. (2015)
P, brevicompactum 9446	Ellsworth Mountains, Antarctica	**8**		Godinho et al. (2015)
P, brevicompactum 9448	Ellsworth Mountains, Antarctica	**4, 8**		Godinho et al. (2015)
P. chrysogenum 9466	Ellsworth Mountains, Antarctica	**4**		Godinho et al. (2015)
P. chrysogenum 9534	Ellsworth Mountains, Antarctica	**8**		Godinho et al. (2015)
P. griseofulvum	Greenland		Griseofulvin Fulvic acid Mycelianamide Roquefortine C and D Chanoclavine I Elymoclavine	Frisvad et al. (2004)
P. griseofulvum strain VKM FW-2251	Kolyma Lowland, Russia	Not shown	Griseofulvin	Kozlovsky et al. (2012)
P. nalgiovense Laxa	Antarctica	**3, 5, 8**	Amphotericin B	Svahn et al. (2015)
P. rubens 9496	Ellsworth Mountains, Antarctica	**4**		Godinho et al. (2015)
P. verrucosum	Haswell Island, Antarctica	Not shown		Gesheva and Negoita (2012)
Phoma herbarum CCFEE 5015	Dry Valleys, Antarctica	**2, 5, 6, 7**		Onofri et al. (2000)
Phoma herbarum CFEE 5007	Dry Valleys, Antarctica	**2, 5, 6, 7**		Onofri et al. (2000)
Phoma herbarum CCFEE 5020	Dry Valleys, Antarctica	**2, 5, 6, 7**		Onofri et al. (2000)
Phoma herbarum CCFEE 459	Dry Valleys, Antarctica	**2, 5, 6, 7**		Onofri et al. (2000)

[a]Target strains: **1**, *Aspergillus fumigatus*; **2**, *Bacillus subtilis*; **3**, *Candida albicans*; **4**, *Cladosporium sphaerospermum* **5**, *Escherichia coli*; **6**, *Pseudomonas putida*; **7**, *Sarcina* sp.; **8**, *Staphylococcus aureus*

The profiles of secondary metabolites in fungal strains of the subgenus *Penicillium* (genus *Penicillium*) isolated from Arctic and Antarctic permafrost were studied to clarify their taxonomic positions (Kozlovsky et al. 2012). The strain *Penicillum griseofulvum* VKM FW-2251, from Kolyma Lowland (Russia), synthesizes a metabolite identical in terms of physicochemical properties to griseofulvin, a polyketide metabolite that is known as a fungicidal antibiotic but rarely used today because of its high toxicity. Gesheva and Negoita (2012) reported on the production of antibiotics by *P. verrucosum* and *A. nidulans* strains from Antarctic soil, but no details on their individual antimicrobial spectrum were reported. Additional 14 fungal strains isolated from Antarctic soils and rocks were screened for the production of antibiotic substances (Onofri et al. 2000). Among them *Phoma herbarum* CCFEE 5015 and CCFEE 5007 from soil, and strains CCFEE 5020 and CCFEE 459 from rocks showed inhibitory activity. The antibiotic activity of strain CCFEE 5020, showing the highest degree of bioactivity, was further experimentally characterized, revealing that it was still present in the temperature range 5°–25 °C, whereas at 30 °C it was reduced. Agitation and aeration strongly influenced the growth and antibiotic production by the same strain, suggesting that it required high levels of oxygen.

Very recently, Godinho et al. (2015) reported on 17 fungal isolates from cold-arid oligotrophic Antarctic soils (Ellsworth Mountains), mainly affiliated to the genera *Aspergillus* and *Penicillium*, with antibacterial and antifungal activities (the most active isolates are listed in Table 3.3). Bioactive extracts generally contained fatty acid functional groups and triglycerides. The presence of highly functionalized secondary metabolites due to the presence of protons in the aromatic and olefinic regions also occurred.

2.2 Polar Lakes and Ponds

Investigations on the antimicrobial activity of microorganisms inhabiting lakes in Polar Regions are very few and mainly addressed to Antarctic benthic mats, which have accumulated for thousands of years virtually undisturbed, due to the extreme climatic conditions and the absence of higher metazoans. The synthesis of antibiotics and/or toxins by microbes belonging to these dense communities may confer a survival advantage (Biondi et al. 2008).

2.2.1 Heterotrophic Bacteria

Rojas et al. (2009) screened bacterial strains isolated from microbial mats growing in the benthic environment of Antarctic lakes (Table 3.4). Among them, 122 isolates showed antibacterial activity against the Gram-positives *S. aureus* and to a lower extent *E. faecium*, and *vs* the Gram-negative *E. coli*. Few of these strains were also active against *Cryptococcus neoformans*, *A. fumigatus* and to a lower extent *C. albicans*. The active strains were affiliated to several lineages of the α-, β- and

Table 3.4 Active bacteria, cyanobacteria and fungi from Polar lakes and ponds

	Phylum or class	Isolate	Location	Active against[a]	Notes	Reference
Bacteria	Bacteroidetes	*Flavobacterium* sp. Ant342	Schirmacher Oasis	**7**	Flexirubin	Mojib et al. (2010)
	Betaproteobacteria	*Janthinobacterium* sp. Ant5-2	Schirmacher Oasis	**7**	Violacin	Mojib et al. (2010)
		Janthinobacterium sp. R-7687	Dry Valleys	**5, 6, 10**		Rojas et al. (2009)
	Gammaproteobacteria	*Shewanella* sp. R-8990	Vestfold Hills	**6, 10**		Rojas et al. (2009)
		Pseudomonas sp. R-12565	Larsemann Hills	**5, 6, 10**		Rojas et al. (2009)
		Pseudomonas sp. R-12533	Larsemann Hills	**5, 6, 9, 10**		Rojas et al. (2009)
		Pseudomonas sp. R-12535	Larsemann Hills	**6, 9, 10**		Rojas et al. (2009)
		Psychrobacter sp. R-12597	Larsemann Hills	**6, 10**		Rojas et al. (2009)
	Actinobacteria	*Arthrobacter* spp. R-7513 and R-7941	Dry Valleys	**5, 10**	Cyclic thiazolyl peptide	Rojas et al. (2009)
	Cyanobacteria	*Leptolyngbya antarctica* ANT.LG2.3	Not specified	**10**		Biondi et al. (2008)
		Leptolyngbya antarctica ANT.LG2.5	Not specified	**10**		Biondi et al. (2008)
		Leptolyngbya antarctica ANT.L18.2	Not specified	**10**		Biondi et al. (2008)
		Nostoc sp. ANT.L34.1	Not specified	**10**		Biondi et al. (2008)
		Nostoc sp. ANT.LPR.1	Not specified	**4**		Biondi et al. (2008)
		Nostoc ANT.L52B.1	Not specified	**4, 10**		Biondi et al. (2008)
		Nostoc ANT.L36.1	Not specified	**4, 10**		Biondi et al. (2008)
		Nostoc ANT.LG2.6	Not specified	**4, 10**		Biondi et al. (2008)
		Phormidium murray ANT.PE.1	Not specified	**10**		Biondi et al. (2008)
		Phormidium priesteyi ANT.LPR.6	Not specified	**10**		Biondi et al. (2008)
		Phormidium priesteyi ANT.L61.2	Not specified	**10**		Biondi et al. (2008)
		Phormidium priesteyi ANT.L52.4	Not specified	**1, 4**		Biondi et al. (2008)
		Phormidium priesteyi ANT.L52.6	Not specified	**1, 4, 10**		Biondi et al. (2008)
		Pseudophormidium ANT.LG2.1	Not specified	**4**		Biondi et al. (2008)
		Pseudophormidium ANT.LPR.2	Not specified	**10**		Biondi et al. (2008)
		Pseudophormidium ANT.LPR.3	Not specified	**4, 10**		Biondi et al. (2008)
		Pseudophormidium ANT.LG2.2	Not specified	**4, 10**		Biondi et al. (2008)

(continued)

Table 3.4 (continued)

	Phylum or class	Isolate	Location	Active against[a]	Notes	Reference
Fungi	Ascomycota	*Aspergillus clavatus* IWW 447	Lake Sarah Tarn	**2, 3, 5, 6, 10**		Brunati et al. (2009)
		Aspergillus niger IWW 1026	Lake Pendant	**10**		Brunati et al. (2009)
		Beauveria sp. IWW 1017	Lake Pendant	**3**		Brunati et al. (2009)
		Cladosporium sp. IWW 1019	Lake Pendant	**6**		Brunati et al. (2009)
		Penicillium sp. IWW 1054	Lake Pendant	**2, 3, 6**		Brunati et al. (2009)
		Penicillium sp. IWW 1059	Lake Ace	**2, 3, 6**		Brunati et al. (2009)
		P. chrysogenum IWW 1053	Lake Highway	**5, 6, 10**		Brunati et al. (2009)
		P. chrysogenum IWW 1055	Lake Pendant	**1, 6, 10**		Brunati et al. (2009)
		P. chrysogenum TF 3/3	Tarn Flat	**6, 8, 10**	beta-lactam	Montemartini Corte et al. (2000)
		P. chrysogenum G 3/2	Gondwana	**8,10**		Montemartini Corte et al. (2000)
		P. citrinum S1/4R	Skua Lake	**8,10**		Montemartini Corte et al. (2000)
		P. citrinum S1/4bis	Skua Lake	**8,10**		Montemartini Corte et al. (2000)
		P. crustosum IWW 1023	Lake Watts	**6**		Brunati et al. (2009)
		P. roseopurpureum S1/3 bis	Skua Lake	**8,10**		Montemartini Corte et al. (2000)
		P. waksmanii S1/5	Skua Lake	**6,10**		Montemartini Corte et al. (2000)
		P. waksmanii II/3	Inexepress. Island	**6, 8, 10**		Montemartini Corte et al. (2000)
		P. waksmanii G3/17	Gondwana	**6,10**		Montemartini Corte et al. (2000)
		P. waksmanii G3/18	Gondwana	**6,10**		Montemartini Corte et al. (2000)

[a]Target strains: **1**, *Aspergillus fumigatus*; **2**, *Candida albicans*; **3**, *C. neoformans*; **4**, *Cryptococcus neoformans*; **5**, *Enterococcus faecium*; **6**, *Escherichia coli*; **7**, *Mycobacterium tubercolosis*; **8**, *Micrococcus luteus*; **9**, *Pseudomonas aeruginosa*; **10**, *Staphylococcus aureus*

γ-proteobacteria, the Bacteroidetes branch, and the high and low G+C Gram-positives. LC–MS fractionation of extracts from a subset of strains that exhibited relatively strong antibacterial activity evidenced a chemical novelty that was further investigated. Two of these strains, R-7513 (from Lake Fryxell) and R-7941 (from Lake Hoare), both belonging to the genus *Arthrobacter*, produced potent antibacterial compounds active against Gram-positives. Since these drugs were related to siomycin, cyclothiazomycin or thiopeptin (with similar antibacterial spectrum against Gram-positive bacteria, including methicillin resistant bacteria) it is possible that they represent new cyclic thiazolyl peptide class of antibiotics.. In the case of the bioactive extracts obtained from *Janthinobacterium* sp. R-7687 and *Pseudomonas* sp. R-12535, the preliminary MS spectra were not associated with any known compound, suggesting the activity of these Antarctic bacteria might rely on production of novel chemicals..

2.2.2 Cyanobacteria

Members of the Cyanobacteria are over-represented in the microbial communities from benthic mats. Taton et al. (2006) and Biondi et al. (2008) first reported on mass cultivation and pharmaceutical screening of a number of Antarctic cyanobacteria for the production of new lead molecules for drug development (Table 3.4). Strains were isolated from 27 benthic microbial mat samples collected from 23 lakes and ponds in the Larsemann Hills, Bølingen Islands, Vestfold Hills, Rauer Islands and the McMurdo Dry Valleys. Seventeen cyanobacteria showed antimicrobial activity against *S. aureus*, the filamentous fungus *A. fumigatus* or the yeast *C. neoformans*. The highest frequency of strains exhibiting antimicrobial activity was detected for the genera *Pseudophormidium* and *Nostoc*. Seven isolates showed only antibacterial activity against *S. aureus*, three only antifungal activity and seven both antibacterial and antifungal activity. *P. priestleyi* strains ANT.L52.4 and ANT.L52.6, isolated from the same lake, showed potent antifungal and/or antibacterial activity and were further analyzed. The fractions active against *A. fumigatus* from the two strains showed very similar chromatographic profiles and eluted with the same retention time. In the same LC-MS system, *Pseudophormidium* sp. ANT.LPR.2, *L. antarctica* ANT.LG2.3 and *Nostoc* sp. L34.1 showed that the fractions active against *S. aureus* eluted with similar retention times, indicating that the three strains produced chemically similar antibacterial compounds and that bioactivity was strain-specific rather than species-specific. No known antimicrobial metabolite was identified thus suggesting that the activity of these Antarctic cyanobacteria was due to novel biochemical compounds.

2.2.3 Fungi

Brunati et al. (2009) isolated 47 filamentous fungi with antimicrobial activity from Antarctic lake microbial mats (Table 3.4). Most of them inhibited the growth of *S. aureus* (14 %), *E. coli* (10 %), and yeasts *C. albicans* (11 %) and *C. neoformans* (8 %).

Both enterobacteria and filamentous fungi were less sensitive. The most bioactive fungi were the cold-tolerant cosmopolitan hyphomycetes such as *Penicillium*, *Aspergillus*, *Beauveria* and *Cladosporium*. Cold-adapted Antarctic endemic *Thelebolus* was least productive in terms of antimicrobial activities than more opportunistic representatives of the genera *Penicillium*, *Aspergillus* and *Cladosporium*. LC–MS fractionation of extracts from *A. clavatus* IWW 447 and *A. niger* IWW 1026 that exhibited relatively potent antimicrobial activity, evidenced a chemical novelty of the antibiotic molecules. Indeed, two bioactive bis-anthraquinones, rugulosin and skyrin (having various medical and insecticidal applications) were identified by LC–MS as the main products in the *P. chrysogenum* strain IWW 1056 isolated from the saline Highway lake in the Vestfold Hills. They inhibit the growth of *S. aureus* (MIC, 8 and >128 μg/mL, respectively), *Moraxella catharrhalis* (MIC, 0.25 and 8 μg/mL, respectively), *E. coli* (MIC, 32 and >128 μg/mL, respectively), *P. aeruginosa* (MIC, >128 and >128 μg/mL, respectively), and *C. albicans* (MIC, >128 and >128 μg/mL, respectively),

Montemartini Corte et al. (2000) screened *Penicillium* spp. from the sediments of ponds in continental Antarctica for antibacterial activity. The MIC of liquid cultures of *Penicillium* strains was determined allowing the selection of nine isolates. Among them, *P. chrysogenum* TF 3/3 differed from the type strain in that it produced a red-brown pigmentation, and also in the presence of activity toward gram-negative bacteria. These preliminary data suggested the synthesis of beta-lactam antibiotics.

2.3 Marine Environments

More than 70 % of the Earth's surface is covered by water, mainly oceans; hence, the marine environment represents some of the world's most unexplored extreme environments. Today, the success rate of drug discovery from the marine world is 1 out of the 3140 known molecular entities, which is roughly twofold to threefold better than the industry average (i.e., 1 out of 5000–10,000 tested compounds) (Gerwick and Moore 2012; Giddings and Newman 2015). Hence, marine environments represent an untapped source to find microorganisms with the potential to produce new, invaluable pharmacophores (Giddings and Newman 2015). Microorganisms able to synthesize bioactive compounds with antimicrobial properties have been isolated also from Arctic and Antarctic marine environments, as described in the following sections.

2.3.1 Bacteria

Researches have been mainly addressed to the Actinobacteria and, at a lesser extent, to other heterotrophic bacteria, from Polar seawater and sediment (Table 3.5). Lo Giudice et al. (2007b) isolated from Antarctic seawater and sediments different

Table 3.5 Active bacteria from marine Polar environments

Phylum or class	Isolate	Origin	Active against[a]	Notes	Reference
Gammaproteobacteria	*Pseudoalteromonas* sp. F26	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Pseudoalteromonas sp. G24	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Pseudoalteromonas sp. 59	Antarctic seawater	**6**		Lo Giudice et al. (2007b)
	Pseudoalteromonas sp. 129	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Pseudoalteromonas sp. 131	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Pseudomonas sp. 65/3	Antarctic fish	**14**		Lo Giudice et al. (2007b)
	Pseudoalteromonas sp. MB33	Arctic copepods	**19**		Wietz et al. (2012)
	Pseudoalteromonas sp. MB205	Arctic surface water	**19**		Wietz et al. (2012)
	Pseudoalteromonas sp. MB220	Arctic sea-ice	**19**		Wietz et al. (2012)
	Pseudoalteromonas sp. MB240	Arctic sea-ice	**19**		Wietz et al. (2012)
	Psychrobacter sp. XX5	Arctic sea-ice	**19**		Wietz et al. (2012)
	Psychrobacter sp. ST4	Arctic sea-ice	**19**		Wietz et al. (2012)
	Vibrio sp. RR12	Arctic amphipods	**19**		Wietz et al. (2012)
	Vibrio sp. EF14	Arctic deep-sea	**17, 19**		Wietz et al. (2012)
	Vibrio sp. RS9	Arctic sea-ice	**17, 19**		Wietz et al. (2012)
Bacteroidetes	*Salegentibacter* sp. T436	Arctic sea-ice	**2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 15, 18**	Nitro compounds	Al-Zereini et al. (2007)

(continued)

Table 3.5 (continued)

Phylum or class	Isolate	Origin	Active against[a]	Notes	Reference
Actinobacteria	*Arthrobacter* sp. B20	Antarctic seawater	**9, 14**		Lo Giudice et al. (2007b)
	Arthrobacter sp. F40	Antarctic seawater	**4**		Lo Giudice et al. (2007b)
	Arthrobacter sp. G18	Antarctic seawater	**6, 9**		Lo Giudice et al. (2007b)
	Arthrobacter sp. G75	Antarctic seawater	**6, 9, 14**		Lo Giudice et al. (2007b)
	Arthrobacter sp. PP12	Arctic sea-ice	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. MB182	Arctic sea-ice	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. SS14	Arctic copepods	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. TT4	Arctic meltwater	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. ZZ3 18	Arctic sea-ice	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. LM7	Arctic surface water	**17, 19**		Wietz et al. (2012)
	Arthrobacter sp. WX11	Arctic deep-sea	**1, 3, 7, 16, 17, 19, 20, 21, 22, 23, 24**	Arthrobacilins	Wietz et al. (2012)
	Janibacter sp. B8	Antarctic seawater	**6, 9, 14**		Lo Giudice et al. (2007b)
	Janibacter sp. F21	Antarctic seawater	**14**		Lo Giudice et al. (2007b)
	Janibacter sp. F34	Antarctic seawater	**6, 9, 14**		Lo Giudice et al. (2007b)
	Janibacter sp. F39	Antarctic seawater	**14**		Lo Giudice et al. (2007b)
	Janibacter sp. G4	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Janibacter sp. G5	Antarctic seawater	**6, 14**		Lo Giudice et al. (2007b)
	Janibacter sp. I44	Antarctic seawater	**6, 9, 14**		Lo Giudice et al. (2007b)
	Microlunatus sp. y400	Antarctic sediment	**4**		Yuan et al. (2014)
	Nesterenkonia sp. S1-21	Antarctic sediment	**6**		Lo Giudice et al. (2007b)
	Nesterenkonia sp. S1-40	Antarctic sediment	**6**		Lo Giudice et al. (2007b)

	Nocardiopsis sp. y4	Antarctic sediment	**4**	Pradimicin, Tetarimycin A	Yuan et al. (2014)
	Nocardiopsis sp. y47	Antarctic sediment	**4**	Pradimicin, Tetarimycin A	Yuan et al. (2014)
	Nocardiopsis sp. y17	Antarctic sediment	**4, 5**	Pradimicin, Tetarimycin A	Yuan et al. (2014)
	Nocardiopsis sp. y18	Antarctic sediment	**4, 5**	Pradimicin, Tetarimycin A	Yuan et al. (2014)
	Nocardiopsis sp. y64	Antarctic sediment	**4, 5**	Pradimicin, Tetarimycin A	Yuan et al. (2014)
	Rhodococcus sp. B7	Antarctic seawater	**6, 9, 14**		Lo Giudice et al. (2007b)
	Rhodococcus sp. G77	Antarctic seawater	**6**		Lo Giudice et al. (2007b)
	Rhodococcus sp. W4-5	Antarctic seawater	**4, 6**		Lo Giudice et al. (2007b)
	Streptomyces sp. ART5	Arctic sediment	**5**	Articoside C-1027 chromophore V	Moon et al. (2014)
	Streptomyces sp. y146	Arctic sediment	**4, 5, 6**		Yuan et al. (2014)
	Streptomyces sp. y2	Arctic sediment	**4, 5, 17**		Yuan et al. (2014)
	Streptomyces sp. y23	Arctic sediment	**4, 5**		Yuan et al. (2014)
	Streptomyces sp. y222	Arctic sediment	**4,5**		Yuan et al. (2014)
	Streptomyces sp. y481	Arctic sediment	**4**		Yuan et al. (2014)

[a]Target strains: **1**, *Aeromonas salmonicida*; **2**, *Bacillus brevis*; **3**, *B. cereus*; **4**, *B. subtilis*; **5**, *Candida albicans*; **6**, *Escherichia coli*; **7**, *Listeria monocytogenes*; **8**, *Magnaporthe grisea*; **9**, *Micrococcus luteus*; **10**, *Mucor miehei*; **11**, *Nematospora coryli*; **12**, *Paecilomyces variotii*; **13**, *Penicillium notatum*; **14**, *Proteus mirabilis*; **15**, *P. vulgaris*; **16**, *Salmonella enterica*; **17**, *Staphylococcus aureus*; **18**, *Ustilago nuda*; **19**, *Vibrio anguillarum*; **20**, *V. parahaemolyticus*; **21**, *V. harveyi*; **22**, *V. vulnificus*; **23**, *Yersinia enterolitica*; **24**, *Y. ruckeri*

bacteria embedded with antimicrobial activity. Sixteen Actinobacteria phylogenetically affiliated to the genera *Janibacter*, *Nesterenkonia*, *Arthrobacter* and *Rhodococcus* were active toward *E. coli* and *P. mirabilis* strains, and at a lesser extent *vs M. luteus* and *B. subtilis*. Interestingly, the genera *Nesterenkonia* and *Rhodococcus* are not known as producers of bioactive molecules and they could represent a new source of antimicrobials. Among heterotrophic bacteria, other than Actinobacteria, isolates from seawater column (five *Pseudoalteromonas* isolates) and the intestinal content of the fish *Trematomus bernacchii* (one *Pseudomonas* isolate) from the Terra Nova Bay (Antarctica) exhibited a strong antibacterial activity against *E. coli* and *P. mirabilis* (Lo Giudice et al. 2007b). Moreover, the finding that different (even though closely related) isolates exhibit different inhibition pattern suggests that antibacterial activity was very likely strain-specific and that members of the same species might probably synthesize several compounds acting on multiple/different targets.

Of 511 randomly selected strains from different Arctic marine sources (e.g. sea-ice, surface seawater, zooplankton, deep sea, and meltwater), Wietz et al. (2012) selected 16 isolates showing considerable antibacterial activity against *Vibrio anguillarum* and *S. aureus* upon repeated testing. Antibiotic-producing isolates belonged to the genera *Arthrobacter* (7 strains), *Pseudoalteromonas* (4 strains), *Psychrobacter* (2 strains), and *Vibrio* (3 strains). Ethanolic extracts from *Arthrobacter* sp. WX11 inhibited the growth of *Aeromonas salmonicida*, *B. cereus*, *Listeria monocytogenes*, *S. aureus*, *Salmonella enterica*, *Vibrio vulnificus*, *V. parahaemolyticus*, *V. harveyi*, *V. anguillarum*, *Yersinia enterolitica*, and *Y. ruckeri*. Arthrobacilins A, B and C were detected in both ethanol and ethyl acetate extracts, but in this latter case it lacked antibacterial activity, indicating that antibiosis may rely on the synergistic action of different compounds.

Yuan et al. (2014) reported on 11 actinobacterial strains from Arctic deep-sea sediment samples showing antibacterial and/or antifungal activity. Among them, seven ones showed activity against both *B. subtilis* and *C. albicans*. However, *Streptomyces* sp. y146 and *Streptomyces* sp. y2 had activity against *E. coli* and *S. aureus*, respectively. Active strains belonged to the genera *Streptomyces*, *Nocardiopsis* and *Microlunatus*. The presence of genes coding for type I polyketide synthase (PKS I), PKS II, nonribosomal peptide synthase (NRPS), aminodeoxyisochorismate synthase (phzE), dTDP-glucose-4, 6-dehydratase (dTGD), halogenase (Halo) or cytochrome P450 hydroxylase (CYP) was checked through PCR amplification, revealing that the genome of all isolates harbored at least two gene clusters involved in the biosynthesis of secondary-metabolites. The PCR products of the ketosynthase (KS) domain of PKS II from five *Nocardiopsis* strain genomes were sequenced. The KS sequences from these strains displayed a 67 % and 69 % amino acid sequence identity with the KS domain of *fabF* encoded protein involved in the biosynthesis of pradimicin, an antifungal antibiotic from *Actinomadura hibisca*, and had also 68 % similarity with TamM, which is related to the biosynthesis of Tetarimycin A, a tetracyclic MRSA-active antibiotic (Yuan et al. 2014).

2.3.2 Fungi

Montemartini Corte et al. (2000) screened *Penicillium* spp. isolated from wooden baits sunk to a 50 m depth in the Ross Sea (Antarctica). The MIC of liquid cultures of *Penicillium* strains was determined allowing the selection of seven isolates, mainly *P. chrysogenum*, with activity against *S. aureus* and *M. luteus*. However, excellent activity was observed for *P. melinii* R55 might rely on the synthesis of patulin, as already demonstrated for the type strain (Table 3.6). Additional active fungi, listed in Table 3.6, were isolated also from Antarctic macroalgae and sponges (see Sects. 2.4.2 and 2.4.3).

Table 3.6 Marine fungi showing antimicrobial activity

	Isolate	Origin	Target strains[a]	Notes	Reference
Ascomycota	*Dipodascus australiensis* 6031	Antarctic algae	**1, 2**		Furbino et al. (2014)
	Cladosporium sp. F09-T13-2	Antarctic sponge	**7**		Henríquez et al. (2014)
	Epicoccum sp. F09-T15-1	Antarctic sponge	**7, 8**		Henríquez et al. (2014)
	Epicoccum sp. F09-T15-4	Antarctic sponge	**8, 9**		Henríquez et al. (2014)
	Geomyces sp. F09-T1-8	Antarctic sponge	**4, 7, 8, 9**		Henríquez et al. (2014)
	Geomyces sp. F09-T3-19	Antarctic sponge	**4, 7, 8, 9**		Henríquez et al. (2014)
	Geomyces sp. F09-T3-5	Antarctic sponge	**7, 8, 9**		Henríquez et al. (2014)
	Metschnikowia australis MH47.1.2	Antarctic algae	**1, 2**		Furbino et al. (2014)
	Penicillium chrysogenum R9	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	Penicillium chrysogenum R28	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	Penicillium chrysogenum R31	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	Penicillium chrysogenum R34	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	Penicillium chrysogenum R36	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	Penicillium chrysogenum R38	Sunk wooden baits	**6, 8**		Montemartini Corte et al. (2000)
	P. commune F09-T8-1	Antarctic sponge	**8, 9**		Henríquez et al. (2014)

(continued)

Table 3.6 (continued)

	Isolate	Origin	Target strains[a]	Notes	Reference
	P. commune F09-T10-1	Antarctic sponge	**4, 8**		Henríquez et al. (2014)
	P. melinii R55	Sunk wooden baits	**5, 6, 8**	Patulin	Montemartini Corte et al. (2000)
	P. polonicum F09-T7-2	Antarctic sponge	**4, 8, 9**		Henríquez et al. (2014)
	Penicillium steckii 6012	Antarctic algae	**1, 2**		Furbino et al. (2014)
	Penicillium sp. 6034	Antarctic algae	**3**		Godinho et al. (2013)
	Penicillium sp. 6120	Antarctic algae	**3**		Godinho et al. (2013)
	Pseudogymnoascus sp. 1	Antarctic algae	**1, 2**		Furbino et al. (2014)
	Pseudogymnoascus sp. 2	Antarctic algae	**1, 2, 3**		Furbino et al. (2014)
	Pseudeurotium F09-T5-4	Antarctic sponge	**8**		Henríquez et al. (2014)
	Thelebolus F09-T14-3	Antarctic sponge	**8**		Henríquez et al. (2014)
	Trichocladium sp. F09-T24-1	Antarctic sponge	**4, 8, 9**		Henríquez et al. (2014)
Basidiomycota	*Guehomyes pullulans* MH33.1	Antarctic algae	**1, 2, 3**		Furbino et al. (2014)

[a]Target strains: **1**, *Candida albicans*; **2**; *C. krusei*; **3**, *Cladosporium sphaerospermum*; **4**, *Clavibacter michiganensis*; **5** , *Escherichia coli*; **6**, *Micrococcus luteus*; **7**, *Pseudomonas aeruginosa*; **8**, *Staphylococcus aureus*; **9**, *Xanthomonas campestris*

2.4 Microorganisms Living in Association with Other Organisms

The exceptional genetic and metabolic plasticity of microorganisms allow them to interact with many, if not all, eukaryotic (micro)organisms. Indeed, they commonly colonize either plants and/or animals with physiological and structural features enabling them to survive and synthesize a wide range of compounds that are able to protect their eukaryotic counterpart against pathogenic and fouling organisms. Several organisms (mainly invertebrates) have been attracting the attention of many researchers because of their diverse pharmaceutical potentials (antiviral, anti-proliferative, anti-inflammatory, anti-tumor, and antimycobacterial activities). However, some recent reports suggest that some metabolites obtained from algae, invertebrates and lichens might be synthesized by their fungal and bacterial symbionts. With regard to the Polar Regions, antimicrobial activity has been reported for bacteria and/or fungi associated with lichens and, as marine counterparts, algae and sponges.

2.4.1 Microorganisms Associated with Lichens and Mosses

The antibacterial potential of bacterial strains associated with Arctic and Antarctic lichens has been evaluated by Kim et al. (2012, 2013, 2014a, b). Active bacteria mainly belonged to the genera *Sphingomonas*, *Burkholderia* and *Rhodanobacter* within the Alpha-, Beta- and Gammaproteobacteria, respectively (Table 3.7). Bacterial extracts, obtained using different solvents (i.e. acetone, water, chloroform, diethyl ether, ethanol, methanol and petroleum ether), showed antibacterial activities against both Gram-positive (*S. aureus*, *B. subtilis*, and *M. luteus*) and Gram-negative (*E. cloacae*, *P. aeruginosa*, and *E. coli*) target pathogens. With respect to solvents, ethyl acetate extraction was more effective than solvents with high (e.g. ethanol) or very low polarity (e.g. petroleum ether). All the aqueous extracts do not exhibit any antibacterial activity against target bacteria, probably depending on the lack of water solubility of active compounds.

Melo et al. (2014) reported on the antibacterial activity of the Antarctic endophytic fungus *Mortierella alpina* strain ITA1-CCMA 952 isolated from the moss *Schistidium antarctici* (Admiralty Bay, King George Island). The strain inhibited the growth of *E. coli* (MIC of 26.9 μg/mL), *P. aeruginosa* and *E. faecalis* (both with a MIC of 107 μg/mL). Antifungal activity was absent. As it was revealed by the GC–MS analysis, antibacterial metabolites were identified as pyrrolopyrazine alkaloids.

To the best of our knowledge, there are no studies about the antimicrobial activity of fungi isolated from lichens or bacteria from mosses so far.

2.4.2 Microorganisms Associated with Sponges

Marine sponges are animals that harbour various organisms, and in some cases, up to 35 % of their wet weight is represented by microorganisms. Furthermore, high- and low-microbial abundance sponges can harbor microbial concentrations of 10^8–10^{10} cells/g and 10^5–10^6 cells/g, respectively, demonstrating the vast potential for the discovery of numerous new secondary metabolites isolated from microbes that live in environments with(in) other organisms (Hentschel et al. 2002). It is known that microbes associated to sponges possess tremendous biological activities covering a wide range of biological functions. The sponge-microbial association is a potential chemical and ecological phenomenon, which provides sustainable resource for developing new pharmaceutical leads. Keeping in view the importance of antimicrobial potential of microbes associated with marine sponge, targeting sponge microsymbionts is an essential focus nowadays. On this basis, since Antarctic sponges represent a potentially rich, untapped source of new antimicrobial compounds, the study by Papaleo et al. (2012) was aimed at characterizing the cultivable bacterial communities (a total of 132 strains) from three different Antarctic sponge species (*Lissodendoryx nobilis*, *Anoxycalyx joubini* and *Haliclonissa verrucosa*) from the Terra Nova Bay. The study was performed to

Table 3.7 Bacterial strains with antimicrobial activity living in association with lichens

Phylum or class	Isolate	Location	Active against[a]	Reference
Alphaproteobacteria	*Sphingomonas* sp. PAM26605	Svalbard Islands (Arctic)	**1, 3, 5, 6**	Kim et al. (2014a)
	Sphingomonas sp. PAM26625	Svalbard Islands (Arctic)	**3, 5, 6**	Kim et al. (2014a)
	Sphingomonas sp. PAMC26556	King George Island (Antarctica)	**5**	Kim et al. (2014b)
	Sphingomonas sp. PAMC26561	King George Island (Antarctica)	**3, 5**	Kim et al. (2014b)
	Sphingomonas sp. KOPRI26645	Svalbard Islands (Arctic)	**1, 2, 3, 4, 5**	Kim et al. (2012)
Betaproteobacteria	*Burkholderia* sp. PAM26606	Svalbard Islands (Arctic)	**1, 3, 4, 5**	Kim et al. (2014a)
	Burkholderia sp. PAM26607	Svalbard Islands (Arctic)	**3, 6**	Kim et al. (2014a)
	Burkholderia sp. PAM26608	Svalbard Islands (Arctic)	**1, 3, 5, 6**	Kim et al. (2014a)
	Burkholderia sp. PAMC26507	King George Island (Antarctica)	**4**	Kim et al. (2014b)
	Burkholderia sp. PAMC26537	King George Island (Antarctica)	**4**	Kim et al. (2014b)
	Burkholderia sp. PAMC26633	King George Island (Antarctica)	**1, 3, 5, 6**	Kim et al. (2014b)
	Burkholderia sp. KOPRI26643	Svalbard Islands (Arctic)	**1, 2, 3, 4, 5, 6**	Kim et al. (2012)
	Burkholderia sp. KOPRI26644	Svalbard Islands (Arctic)	**1, 2, 3, 4, 5, 6**	Kim et al. (2012)
	Burkholderia sp. KOPRI26646	Svalbard Islands (Arctic)	**1, 2, 3, 4, 5, 6**	Kim et al. (2012)
	Burkholderia sp. KOPRI26647	Svalbard Islands (Arctic)	**1, 2, 4**	Kim et al. (2012)
Gammaproteobacteria	*Rhodanobacter* sp. PAMC26515	King George Island (Antarctica)	**4**	Kim et al. (2014b)
	Rhodanobacter sp. PAMC26518	King George Island (Antarctica)	**4**	Kim et al. (2014b)
	Rhodanobacter sp. PAMC26538	King George Island (Antarctica)	**1, 2, 3, 5, 6**	Kim et al. (2014b)
	Rhodanobacter sp. PAMC26551	King George Island (Antarctica)	**1, 3, 5, 6**	Kim et al. (2014b)
	Rhodanobacter sp. PAMC26552	King George Island (Antarctica)	**4**	Kim et al. (2014b)
	Rhodanobacter sp. PAMC26557	King George Island (Antarctica)	**3, 4**	Kim et al. (2014b)
Bacteroidetes	*Hymenobacter* sp. PAMC26554	King George Island (Antarctica)	**1, 3, 6**	Kim et al. (2014b)
Actinobacteria	*Streptomyces* sp. PAMC26508	King George Island (Antarctica)	**1, 2, 3, 4, 5, 6**	Kim et al. (2014b)
	Frigoribacterium sp. PAMC26555	King George Island (Antarctica)	**1, 4, 6**	Kim et al. (2014b)
Firmicutes	*Paenibacillus* sp. PAMC26517	King George Island (Antarctica)	**1, 2, 6**	Kim et al. (2014b)

[a]Target strains: **1**, *Bacillus subtilis*; **2**, *Enterobacter cloacae*; **3**, *Escherichia coli*; **4**, *Micrococcus luteus*; **5**, *Pseudomonas aeruginosa*; **6**, *Staphylococcus aureus*

check whether some of these strains might inhibit the growth of a panel of more than 70 opportunistic pathogens, including those affecting cystic fibrosis (CF) patients. Data obtained revealed that most of these sponge-associated Antarctic bacteria were able to completely inhibit the growth of members of the *Burkholderia cepacia* complex (Bcc), representing one of the most important CF pathogens. On the other hand, the same strains did not interfere with the growth of other pathogenic bacteria, such as *P. aeruginosa* or *S. aureus*, suggesting a specific inhibition activity toward Bcc bacteria. Further experiments carried out on the most active isolates (i.e. *Pseudoalteromonas* spp. TB41 and AC163, *Shewanella* sp. TB4, *Psychrobacter* spp. TB47 and TB67) revealed that at least some of the antimicrobial compounds were very likely mVOCs, which constitute an important regulatory factor in the interrelationships among different organisms in microbial ecosystems. This finding was confirmed by solid phase micro extraction gas-chromatography mass-spectrometry (SPME-GC-MS) performed on, representative set of Antarctic bacteria, which revealed the production of a large set of mVOCs whose synthesis was very likely constitutive, in that it was not induced by the presence of target strains (Romoli et al. 2011). More interestingly, the activity of the VOCs seemed to be more effective than most of the commonly used antibiotics in inhibiting the growth of Bcc bacteria. A metabolomic approach applied to *Pseudoalteromonas* sp. TB41 allowed a selection of 30 compounds, some of which presumably responsible for the inhibition of *B. cenocepacia* (Bc) LMG16654 growth by the Antarctic isolate (Romoli et al. 2014). The mVOCs profiles obtained from strain TB41 when grown alone or in the presence of Bc-LMG16654 were not significantly different, suggesting that the production of such molecules may be constitutive and not modified by the presence of the target strain, as this latter neither interfered with the production nor induced the synthesis of different mVOCs. Recently, Papaleo et al. (2013) also reported on the volatile profiles of *Psychrobacter* spp. (isolated from *A. joubini*; Mangano et al. 2009) under aerobic conditions. Results suggested that Antarctic bacteria exhibited an antimicrobial ability that might rely on a complex mixture of mVOCs whose relative concentration was dependent on the growth conditions (presence/absence of oxygen and growth media used). Results also revealed that only *Pseudoalteromonas* sp. TB41 (Mangano et al. 2009) possessed some *nrps–pks* genes, similarly to *Arthrobacter* sp. TB23 (isolated from the Antarctic sponge *L. nobilis*; Mangano et al. 2009) whose genome sequence was recently reported (Fondi et al. 2012) (see Sect. 3).

With regard to fungi, only recently Henríquez et al. (2014) described for the first time the biodiversity and the metabolic potential of fungi associated with Antarctic marine sponges. Fungal culture extracts were assayed for their possible antimicrobial activity (in addition to antitumoral and antioxidant ones, not reported). Among them, 52 extracts, mainly obtained from the genus *Geomyces* and unidentified relatives, showed antimicrobial activity against some of the bacteria tested, a selection of which is shown in Table 3.6. In general, fungal extracts were more active against Gram positive (particularly against *S. aureus*) than Gram negative bacteria. In particular, 5 isolates (belonging to the genera *Geomyces* and *Epicoccum*) exhibited

antibacterial activity against *P. aeruginosa*, 44 isolates showed inhibitory activity *versus S. aureus*, 11 isolates (belonging to the genera *Geomyces* and *Penicillium*, or not identified) exhibited antibacterial activity against *Clavibacter michiganensis*, and 22 isolates (belonging to the genera *Geomyces*, *Penicillium*, *Epicoccum* and *Cladosporium*, or not identified) exhibited antibacterial activity against *Xanthomonas campestris*. Several fungal isolates with the same ITS type showed different antimicrobial activity. *Geomyces* was the most prolific fungal genus, with 18 isolates (54.5 % of *Geomyces* sp.) showing antimicrobial activity. Among them, *Geomyces* spp. F09-T1-8, and F09-T3-19 resulted particularly active. The antimicrobial activity in the genus *Pseudeurotium* was first described by Henríquez et al. (2014). More interestingly, most of un-identified isolates (among the Leotiomycetes) also were prolific as producers of antimicrobial activities.

2.4.3 Fungi Associated with Macroalgae

Marine algae play a key role in organic matter mineral cycling, particularly in both littoral and infralittoral ecosystem in Antarctic shallow waters. The Antarctic macroalgae are highly endemic, play a fundamental role as primary producers, food for marine herbivores as well as in habitat structure. To date, only two reports exist on the antimicrobial activity of fungi associated with Antarctic algae (Table 3.6).

Godinho et al. (2013) analyzed the distribution and diversity of fungi associated with eight Antarctic macroalgae and their ability to synthesize bioactive compounds. *Penicillium* sp. strains 6034 and 6120, isolated from the endemic species *Palmaria decipiens* (Rhodophyta) and *Monostroma hariotii* (Chlorophyta), respectively, yielded extracts exhibiting high and selective antifungal (and/or trypanocidal) activity. Penicillium sp. 6120 displayed antifungal activity against the filamentous fungus *Cladosporium sphaerospermum*, producing 96 % inhibition and a MIC value of 250 mg/mL. The preliminary proton nuclear magnetic resonance spectroscopy analysis indicated the presence of highly functionalised aromatic compounds.

More recently, Furbino et al. (2014) characterized the fungal communities associated with the endemic Antarctic macroalgae *Pyropia endiviifolia* (Rhodophyta) and *Monostroma hariotii* (Chlorophyta), which are among the most abundant species across the Antarctic Peninsula. A total of six algicolous fungal taxa were able to produce compounds with biological activities. All extracts showed selective antifungal activities against *Candida albicans* and *C. krusei*, whereas those obtained by *Pseudogymnoascus* sp. 2 and *Guehomyes pullulans* MH33.1 also inhibited *Cladosporium sphaerosperum*. Additionally, the extract of *Penicillium steckii* 6012 inhibited 96 % of yellow fever virus. No extract displayed antibacterial or trypanocidal activities.

To the best of our knowledge, no study about the antimicrobial activity of fungi isolated from Arctic macroalgae has been carried out so far.

3 Genetic and Genomic Aspects of Sponge-Associated Antarctic Bacteria with Antibiotic Activity

Further insights in the metabolic potential of sponge-associated bacteria were obtained by the molecular and genomic analyses of Antarctic bacterial strains belonging to different genera whose draft genome sequence was recently obtained (Fondi et al. 2012; Papaleo et al. 2013; Maida et al. 2014; Orlandini et al. 2014; Bosi et al. 2015; Maida et al. 2015).

Among the isolates characterized by Papaleo et al. (2012), representatives of the genera *Gillisia* (i.e isolate CAL575), *Psychrobacter* (i.e. isolates TB2, TB15 and AC24) and *Arthrobacter* (i.e. isolates TB26, CAL618 and TB23) were further analyzed by a genomic approach in order to shed some light on their inhibitory activity against Bcc (Fondi et al. 2014; Maida et al. 2014; Orlandini et al. 2014). Maida et al. (2014) characterized *Gillisia* sp. CAL575 from the sponge *H. verrucosa* using a combination of different techniques, including genomics, phenotypic characterization and analysis of mVOCs. Fondi et al. (2014) reported the draft genomes of *Psychrobacter* spp. TB2, TB15 (both from the sponge *L. nobilis*) and AC24 (from the sponge *H. verrucosa*). In particular, *Psychrobacter* sp. AC24 efficiently inhibited the growth of almost all the Bcc strains tested, regardless of the growth medium. Conversely, TB2 and TB15 displayed a reduced inhibitory ability compared to AC24 and, in some cases, the effect on the growth of Bcc strains was influenced by the corresponding growth medium. Moreover, very similar inhibition pattern *vs* Bcc strains were exhibited by *Arthrobacter* spp. TB26 (from the sponge *L. nobilis*) and CAL 618 (from *H. verrucosa*), suggesting that the genetic determinants responsible for the biosynthesis of antimicrobial compounds belonged to the *core* genome (Orlandini et al. 2014). Additionally, the whole body of cross-streaking data suggested that also diffusible organic molecules (in combination with volatile compounds) might interfere with the growth of Bcc strains. This is also true for some *Pseudoalteromonas* strains that were shown to be able to interfere with the growth of Bcc strains (Maida et al. 2015).

The draft genome sequence of 38 Antarctic bacterial strains (including those mentioned above) were searched for genes involved in the biosynthesis of secondary metabolites, known to often possess antimicrobial activity. Data obtained revealed that, according to previous experimental data (Papaleo et al. 2012), no gene involved in secondary metabolite biosynthesis (*pks* or *nrps*), except for the presence of a *pks* type III gene and a terpene biosynthetic cluster, were identified in the *Gillisa* sp. CAL575 strain. Moreover, in the genome of the *Arthrobacter* sp. TB23 three gene clusters including a type III PKS, a NRPS gene, and terpene biosynthetic genes, respectively, responsible for the biosynthesis of antimicrobial compounds, which might be targeted towards Bcc bacteria were disclosed. The comparative analysis of the genome of these strains highlighted the presence of few genes belonging to the *core* genome involved in the secondary metabolites biosynthesis (Papaleo et al. 2013). Besides, it was also suggested that the biosynthesis of these compounds might be synthesized by still unknown metabolic routes.

Results suggested that the metabolic strategies exploited by the three *Psychrobacter* strains to inhibit the growth of *Burkholderia* representatives fall outside the range of already characterized biochemical systems.

4 Bioactive Metabolites with Antimicrobial Activities from Cold-Adapted Microorganisms

The number of new bioactive molecules from cold-adapted microorganisms has significantly increased in the last decade, with some of them that possess antimicrobial activity (for a recent review see: Bratchkova and Ivanova 2011; Liu et al. 2013). Several biomolecules have been cited in the Sect. 2. Additional information is reported below.

With regard to streptomycetes, Bruntner et al. (2005) isolated a new angucyclinone antibiotic, called frigocyclinone, from the Antarctic *Streptomyces griseus* strain NTK 97 (from soil), consisting of a tetrangomycin moiety attached through a C-glycosidic linkage with the aminodeoxysugar ossamine (Fig. 3.1). Frigocyclinone showed good inhibitory activity against Gram-positive bacteria (such as *B. subtilis*, MIC 4.6 μg/mL, and *S. aureus*, MIC 15 μg/mL), whereas Gram-negative bacteria (i.e. *E. coli*, *P. fluorescens* and *P. mirabilis*), filamentous fungi (i.e. *Botrytis cinerea*, *Aspergillus viridinutans*, *Penicillium notatum* and *Paecilomyces variotii*) and yeast (i.e. *S. cerevisiae* and *C. albicans*) were not sensitive.

Streptomyces flavovirens 6[7], isolated from soil samples in the region of Livingston Island, Antarctica, synthesized molecules embedded with antimicrobial and antitumour activity, which belonged to the actinomycins, within peptidic antibiotics (Ivanova et al. 2002) (Fig. 3.1). The new substance 2-amino-9,13-dimethyl heptadecanoic acid and phthalic acid diethyl ester, 1,3-bis(3-phenoxyphenoxy)benzene, hexanedioic acid dioctyl ester as natural products were found in the culture broth of the strain *Streptomyces* sp. 1010, isolated from water samples in the region Livingston Island, Antarctica. The phthalic acid diethyl ester possessed an antibacterial property against *M. luteus* (MIC, 3 μg/mL), *B. subtilis* (MIC, 12 μg/mL) and *S. aureus* (MIC, 25 μg/mL) (Ivanova et al. 2001).

Gesheva (2010), by comparison with different antibiotic standards, suggested that *Streptomyces* sp. eight produced three antibiotics: non-polyenic macrolide antibiotic (composed by two components), azalomycin B, and nigericin.

New benzoxazine secondary metabolites, articoside and C-1027 chromophore V, have also been obtained by Moon et al. (2014) from an Arctic marine *Streptomyces* strain ART5, isolated from surface sediment (East Siberian continental margin; depth of 354 m) (Fig. 3.1). Interestingly, articoside is a benzoxazine disaccharide, a structure type that has not been previously reported, whereas C-1027 chromophore-V possesses a chlorine atom, amino sugar, as well as cyclopenta[a]indene and 3′-chloro-5′-hydroxy-β-tyrosine moieties. Articoside and C-1027 chromophore-V inhibited *C. albicans* isocitrate lyase, an enzyme that plays a role in the pathogenicity of such yeast, with IC50 values of 30.4 and 37.9 μM, respectively.

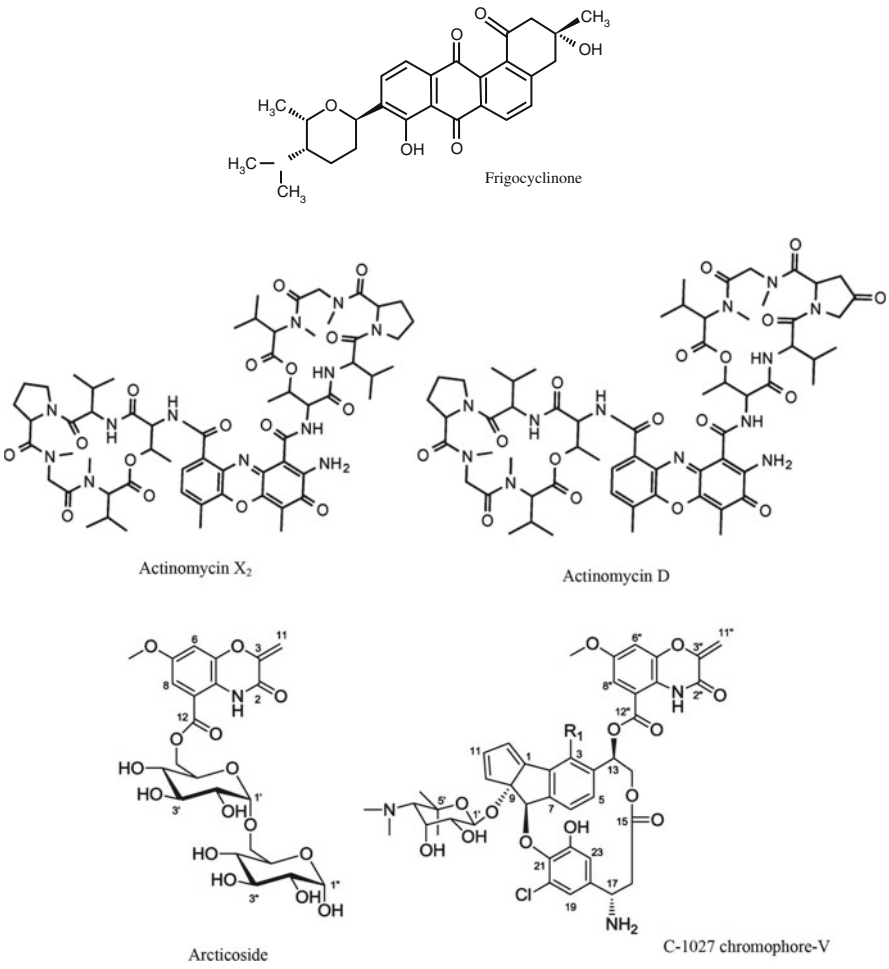

Fig. 3.1 Examples of bioactive compounds isolated from Actinobacteria from Polar sites. *Source*: Lo Giudice and Fani

Among bacteria, other than Actinobacteria, Mojib et al. (2010) tested for antimycobacterial activity two pigments, violacein, a purple violet pigment from *Janthinobacterium* sp. Ant5-2 (J-PVP), and flexirubin, a yellow-orange pigment from *Flavobacterium* sp. Ant342 (F-YOP). Both bacterial strains found were isolated from the land-locked freshwater lakes of Schirmacher Oasis, East Antarctica. Results indicated that the MICs of J-PVP and F-YOP were 8.6 and 3.6 μg/mL for avirulent *Mycobacterium smegmatis* mc^2155, 5 and 2.6 μg/mL for avirulent *Mycobacterium tuberculosis* mc^26230, and 34.4 and 10.8 μg/mL for virulent *M. tuberculosis* $H_{37}Rv$, respectively. The effectiveness of J-PVP on Multiple drug resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains was further investigated by Huang et al. (2012). The structures J-PVP and F-YOP have not been elucidated.

Nineteen aromatic nitro compounds were isolated from *Salegentibacter* sp. strain T436 from Arctic pack ice, with four of them that were new and six that were never reported before from natural source (Al-Zereini et al. 2007; Schuhmann et al. 2009). The new natural products showed weak antifungal, antibacterial, and cytotoxic activities. The 2-nitro-4-(2′-nitroethenyl)-phenol was the most potent antimicrobial and cytotoxic substance, being active against all targets.

A bacterial strain of *Pseudomonas aeruginosa* from the Antarctic sponge *Isodictya setifera* was found to contain metabolites inhibiting the growth of *B. subtilis*, *S. aureus*, and *M. luteus* (Jayatilake et al. 1996). The culture broth of this bacterium contained a series of diketopiperazines, including a new natural product and two known phenazine alkaloid antibiotics.

Among cyanobacteria, a new lead antibacterial molecule with the proposed structure of 4-[(5-carboxy-2-hydroxy)-benzyl]-1,10-dihydroxy-3,4,7,11,11-pentamethyl-octahydrocyclopenta<a>naphthalene was reported by Asthana et al. (2009) to be produced by the Antarctic cyanobacterium *Nostoc* CCC 537. This intracellular biomolecule, structurally similar to noscomin, exhibited antibiotic activity against acid-fast *M. tuberculosis* H37Rv (MIC, 2.5 μg/mL), *S. aureus* (MIC, 0.5 μg/mL), *Enterobacter aerogenes* (MIC, 4.0 μg/mL), *Salmonella typhi* (MIC, 2.0 μg/mL), *P. aeruginosa* (MIC, 2.0 μg/mL), *E. coli* (MIC, 2.0 μg/mL), and multidrug-resistant strains of *E. coli* (MIC, 16 μg/mL).

Among fungi, the Antarctic ascomycete *Geomyces* sp. 2481, obtained from a soil sample (Fildes Peninsula, King George Island), was grown in solid-substrate fermentation culture (Li et al. 2008). Its organic solvent extract contained five new asterric acid derivatives, ethyl asterrate, n-butyl asterrate, and geomycins A-C. Geomycin B displayed significant antifungal activity against *A. fumigatus* with a MIC value of 20 μg/mL. Geomycin C exhibited antibacterial activities against *S. aureus* (MIC, 24 μg/mL) and *E. coli* (MIC, 20 μg/mL).

A number of metabolites with antimicrobial activity were isolated form *Penicillium griseofulvum* (Greenland) as follows: griseofulvin, fulvic acid, mycelianamide, roquefortine C and D, chanoclavine I and elymoclavine (Frisvad et al. 2004).

More recently, Svahn et al. (2015) isolated *Penicillium nalgiovense* Laxa from a soil sample of an abandoned penguin's nest. Amphotericin B, an antifungal agent used worldwide against fungal infections, was the only metabolite secreted from *P. nalgiovense* Laxa with noticeable inhibitory activity *versus C. albicans*, *S. aureus* and *E. coli*.

5 Conclusions

The review highlights the versatile antimicrobial potential of bacteria and fungi from Polar Regions, making such extreme and (often) underexplored habitats promising for the discovery of new natural compounds of pharmaceutical interest.

It is interesting to note that microorganisms belonging to the same species (isolated from the same or from different habitats) often show different antimicrobial spectra. This suggests that (1) inhibitory activity is more likely strain- rather than species-specific, (2) single species could probably synthesize a set compounds acting on multiple targets, and (3) the antimicrobial spectrum could depend on the habitat of origin. Thus, if secondary metabolic diversity is of interest, it becomes of great importance to keep different isolates of the same bacterial/fungal species in culture collections. To this purpose, our knowledge of the antimicrobial activity of microorganisms from Polar Regions derives from the screening of cultivable strains. This approach is the basis for most microbiological bioprospecting efforts as this both gives access to the totality of genomic information in microorganisms and makes the study of their phenotype possible in the laboratory (de Pascale et al. 2012). However, only a limited fraction of the total microbial community from a certain environment can be easily cultured under laboratory conditions, thus leaving the exceptional bioprospecting potential of the uncultured diversity unexplored (Vester et al. 2015). Recent developments in high-throughput molecular biology techniques have paved the way for employing cultivation-independent approaches (including metagenomics and single-cell genomic sequencing) for bioprospecting purposes. Coupling improved cultivation methods and metagenomics approaches for functional screening of cold-adapted microorganisms can lead to generate targeted information on communities enriched for antimicrobial activities. This emerging trend for microbial bioprospecting in Polar areas certainly merits to be taken into serious consideration in future research programs.

Conflict of Interest Angelina Lo Giudice and Renato Fani declare that they have no conflict of interest.

References

Al-Zereini W, Schuhmann I, Laatsch H, Helmke E, Anke H (2007) New aromatic nitro compounds from *Salegentibacter* sp. T436, an Arctic sea ice bacterium: taxonomy, fermentation, isolation and biological activities. J Antibiot 60:301–308

Asencio G, Lavina P, Alegría K, Domínguez M, Bello H, González-Rocha G, González-Aravena M (2009) Antibacterial activity of the Antarctic bacterium *Janthinobacterium* sp. SMN 33.6 against multi-resistant Gram-negative bacteria. Electron J Biotechnol 17:1–5

Asthana RK, Deepali TMK, Srivastava A, Singh AP, Singh SP, Nath G, Srivastava R, Srivastava BS (2009) Isolation and identification of a new antibacterial entity from the Antarctic cyanobacterium *Nostoc* CCC 537. J Phycol 21:81–88

Bell TH, Callender KL, Whyte LG, Greer CW (2013) Microbial competition in polar soils: a review of an understudied but potentially important control on productivity. Biology 2:533–554

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Biondi N, Tredici MR, Taton A, Wilmotte A, Hodgson DA, Losi D, Marinelli F (2008) Cyanobacteria from benthic mats of Antarctic lakes as a source of new bioactivities. J Appl Microbiol 105:105–115

Bosi E, Fondi M, Maida I, Perrin E, de Pascale D, Tutino ML, Parrilli E, Lo Giudice A, Filloux A, Fani R (2015) Genome-scale phylogenetic and DNA composition analyses of Antarctic

Pseudoalteromonas bacteria reveal inconsistencies in current taxonomic affiliation. Hydrobiologia 761:85–95.
Bratchkova A, Ivanova V (2011) Bioactive metabolites produced by microorganisms collected in Antarctica and the Arctic. Biotechnol Biotechnol Equip 25:1–7
Brunati M, Rojas JL, Sponga F, Ciciliato I, Losi D, Göttlich E, de Hoog S, Genilloud O, Marinelli F (2009) Diversity and pharmaceutical screening of fungi from benthic mats of Antarctic lakes. Mar Genomics 2:43–50
Bruntner C, Binder T, Pathom-aree W, Goodfellow M, Bull AT, Potterat O, Puder C, Hörer S, Schmid A, Bolek W, Wagner K, Mihm G, Fiedler H-P (2005) Frigocyclinone, a novel angucyclinone antibiotic by a *Streptomyces griseus* strain from Antarctica. J Antibiot 58:346–349
Bull AT, Stach JEM (2007) Marine actinobacteria: new opportunities for natural product search and discovery. Trends Microbiol 15:491–499
Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low-temperature extremophiles and their applications. Curr Opin Biotechnol 13:253–261
de Pascale D, de Santi C, Fu J, Landfald B (2012) The microbial diversity of Polar environments is a fertile ground for bioprospecting. Mar Genomics 8:15–22
Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1:200–208
Fondi M, Orlandini V, Maida I, Perrin E, Papaleo MC, Emiliani G, de Pascale D, Parrilli E, Tutino ML, Michaud L, Lo Giudice A, Fani R (2012) The draft genome of the VOCs-producing Antarctic bacterium *Arthrobacter* sp. TB23 able to inhibit Cystic Fibrosis pathogens belonging to the *Burkholderia cepacia* complex. J Bacteriol 194:6334–6335
Fondi M, Orlandini V, Perrin E, Maida I, Bosi E, Papaleo MC, Michaud L, Lo Giudice A, de Pascale D, Tutino ML, Liò P, Fani R (2014) Draft genomes of three Antarctic *Psychrobacter* strains known to have antimicrobial activity against *Burkholderia cepacia* complex opportunistic pathogens. Mar Genomics 13:37–38
Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus. Stud Mycol 49:201–241
Furbino LE, Godinho VM, Santiago IF, Pellizari FM, Alves TM, Zani CL, Junior PA, Romanha AJ, Carvalho AG, Gil LH, Rosa CA, Minnis AM, Rosa LH (2014) Diversity patterns, ecology and biological activities of fungal communities associated with the endemic macroalgae across the Antarctic peninsula. Microb Ecol 67:775–787
Gerwick WH, Moore BS (2012) Lessons from the past and charting the future of marine natural products drug discovery and chemical biology. Chem Biol 19:85–98
Gesheva V (2009) Distribution of psychrophilic microorganisms in soils of Terra Nova Bay and Edmonson Point, Victoria Land and their biosynthetic capabilities. Polar Biol 32:1287–1291
Gesheva V (2010) Production of antibiotics and enzymes by soil microorganisms from the Windmill Islands Region, Wilkes Land, East Antarctica. Polar Biol 33:1351–1357
Gesheva V, Negoita T (2012) Psychrotrophic microorganism communities in soils of Haswell Island, Antarctica, and their biosynthetic potential. Polar Biol 35:291–297
Gesheva V, Vasileva-Tonkova E (2012) Production of enzymes and antimicrobial compounds by halophilic Antarctic *Nocardioides* sp. grown on different carbon sources. World J Microbiol Biotechnol 28:2069–2076
Giddings L-A, Newman DJ (2015) Bioactive compounds from marine extremophiles. Springer Briefs Microbiol 1–124
Godinho VM, Furbino LE, Santiago IF, Pellizzari FM, Yokoya NS, Pupo D, Alves TM, Junior PA, Romanha AJ, Zani CL, Cantrell CL, Rosa CA, Rosa LH (2013) Diversity and bioprospecting of fungal communities associated with endemic and cold-adapted macroalgae in Antarctica. ISME J 7:1434–1451
Godinho VM, Gonçalves VN, Santiago IF, Figueredo HM, Vitoreli GA, Schaefer CE, Barbosa EC, Oliveira JG, Alves TM, Zani CL, Junior PA, Murta SM, Romanha AJ, Kroon EG, Cantrell CL, Wedge DE, Duke SO, Ali A, Rosa CA, Rosa LH (2015) Diversity and bioprospection of fungal community present in oligotrophic soil of continental Antarctica. Extremophiles 19:585–596

Hemala L, Zhanga D, Margesin R (2014) Cold-active antibacterial and antifungal activities and antibiotic resistance of bacteria isolated from an alpine hydrocarbon-contaminated industrial site. Res Microbiol 165:447–456

Henríquez M, Vergara K, Norambuena J, Beiza A, Maza F, Ubilla P, Araya I, Chávez R, San-Martín A, Darias J, Darias MJ, Vaca I (2014) Diversity of cultivable fungi associated with Antarctic marine sponges and screening for their antimicrobial, antitumoral and antioxidant potential. World J Microbiol Biotechnol 30:65–76

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440

Huang JP, Mojib N, Rr G, Watkins S, Waites KB, Ravindra R, Andersen DT, Bej AK (2012) Antimicrobial activity of PVP from an Antarctic bacterium, *Janthinobacterium* sp. Ant5-2, on multi-drug and methicillin resistant *Staphylococcus aureus*. Nat Prod Bioprospect 2:104–110

Ivanova V, Oriol M, Montes M-J, García A, Guinea J (2001) Secondary metabolites from a *Streptomyces* strain isolated from Livingston Island. Antarctica Z Naturforsch C 56:1–5

Ivanova V, Yocheva L, Schlegel R, Graefe U, Kolarova M, Aleksieva K, Naidenova M (2002) Antibiotic complex from *Streptomyces flavovirens* 6^7, isolated from Livingston Island, Antarctica. Bulgarian Antarct Res Life Sci 3:35–42

Jayatilake GS, Thornton MP, Leonard AC, Grimwade JE, Baker BJ (1996) Metabolites from an Antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*. J Nat Prod 59:293–296

Kim M-K, Park H, Oh T-J (2012) Antibacterial properties associated with microorganisms isolated from Arctic lichens. Korean J Microbiol Biotechnol 40:380–388

Kim M-K, Park H, Oh T-J (2013) Antibacterial properties of the bacterial associates of the Arctic lichen *Stereocaulon* sp. Afr J Microbiol Res 7:3651–3657

Kim M-K, Park H, Oh T-J (2014a) Antibacterial and antioxidant capacity of polar microorganisms isolated from Arctic lichen *Ochrolechia* sp. Pol J Microbiol 63:317–322

Kim M-K, Park H, Oh T-J (2014b) Antibacterial and antioxidant potential of polar microorganisms isolated from Antarctic lichen *Psoroma* sp. Afr J Microbiol Res 8:3529–3535

Kozlovsky AG, Zhelifonova VP, Antipova TV, Baskunov BP, Kochkina GA, Ozerskaya SM (2012) Secondary metabolite profiles of the *Penicillium* fungi isolated from the Arctic and Antarctic permafrost as elements of polyphase taxonomy. Microbiology 81:308–313

Lee L-H, Cheah Y-K, Sidik SM, Ab Mutalib N-S, Tang Y-L, Lin H-P, Hong K (2012a) Molecular characterization of Antarctic actinobacteria and screening for antimicrobial metabolite production. World J Microbiol Biotechnol 28:2125–2137

Lee L-H, Cheah Y-K, Syakima AMN, Shiran MS, Tang Y-L, Lin H-P, Hong K (2012b) Analysis of Antarctic proteobacteria by PCR fingerprinting and screening for antimicrobial secondary metabolites. Genet Mol Res 11:1627–1641

Li Y, Sun B, Liu S, Jiang L, Liu X, Zhang H, Che Y (2008) Bioactive asterric acid derivatives from the Antarctic ascomycete fungus *Geomyces* sp. J Nat Prod 1:1643–1646

Liu J-T, Lu X-L, Liu X-Y, Yun G, Bo H, Jiao B-H, Zheng H (2013) Bioactive natural products from the Antarctic and Arctic organisms source. Mini Rev Med Chem 13:617–626

Lo Giudice A, Brilli M, Bruni V, De Domenico M, Fani R, Michaud L (2007a) Bacterium-bacterium inhibitory interactions among psychrotrophic bacteria isolated from Antarctic seawaters (Terra Nova Bay, Ross Sea). FEMS Microbiol Ecol 60:383–396

Lo Giudice A, Bruni V, Michaud L (2007b) Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. J Basic Microbiol 47:496–505

Lyutskanova D, Ivanova V, Stoilova-Disheva M, Kolarova M, Aleksieva K, Raykovska V, Peltekovavska V, Peltekova V (2009) Isolation, characterization and screening for antimicrobial activities of psychrotolerant Streptomycetes isolated from polar permafrost soil. Biotechnol Biotechnol Eq 23.

Maida I, Fondi M, Papaleo MC, Perrin E, Orlandini V, Emiliani G, de Pascale D, Parrilli E, Tutino ML, Michaud L, Lo Giudice A, Romoli R, Bartolucci G, Fani R (2014) Phenotypic and genomic characterization of the Antarctic bacterium *Gillisia* sp. CAL575, a producer of antimicrobial compounds. Extremophiles 18:35–49

Maida I, Bosi E, Fondi M, Perrin E, Orlandini V, Papaleo MC, Mengoni A, de Pascale D, Tutino ML, Michaud L, Lo Giudice A, Fani R (2015. Antimicrobial activity of Pseudoalteromonas strains isolated from the Ross Sea (Antarctica) vs Cystic Fibrosis opportunistic pathogens. Hydrobiologia 761:443–457

Mangano S, Michaud L, Caruso C, Brilli M, Bruni V, Fani R, Lo Giudice A (2009) Antagonistic interactions among psychrotrophic cultivable bacteria isolated from Antarctic sponges: a preliminary analysis. Res Microbiol 160:27–37

Manivasagan P, Venkatesan J, Sivakumar K, Kim S-K (2014) Pharmaceutically active secondary metabolites of marine actinobacteria. Microbiol Res 169:262–278

Margesin R (2007) Alpine microorganism: useful tools for low-temperature bioremediation. J Microbiol 45:281–285

Melo IS, Santos SN, Rosa LH, Parma MM, Silva LJ, Queiroz SC, Pellizari VH (2014) Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. Extremophiles 18:15–23

Mojib N, Philpott R, Huang JP, Niederweis M, Bej AK (2010) Antimycobacterial activity in vitro of pigments isolated from Antarctic bacteria. Antonie Van Leeuwenhoek 98:531–540

Moncheva P, Tishkov S, Dimitrova N, Chipeva V, Antonova-Nikolova S, Bogatzevska N (2002) Characteristics of soil Actinomycetes from Antarctica. J Cult Collect 3:3–14

Montemartini Corte A, Liotta M, Venturi CB, Calegari L (2000) Antibacterial activity of *Penicillium* spp. strains isolated in extreme environments. Polar Biol 23:294–297

Moon K, Ahn C-H, Shin Y, Won TH, Ko K, Lee SK, Oh K-B, Shin J, Nam SI, Oh DC (2014) New benzoxazine secondary metabolites from an Arctic actinomycetes. Mar Drugs 12:2526–2538

Morita RY (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167

Nedialkova D, Naidenova M (2005) Screening the antimicrobial activity of Actinomycetes strains isolated from Antarctica. J Cult Collect 4:29–35

O'Brien A, Sharp R, Russell NJ, Roller S (2004) Antarctic bacteria inhibit growth of food-borne microorganisms at low temperatures. FEMS Microbiol Ecol 48:157–167

Onofri S, Fenice M, Cicalini AR, Tosi S, Magrino A, Pagano S, Selbmann L, Zucconi L, Vishniac HS, Ocampo-Friedmann R, Friedmann EI (2000) Ecology and biology of microfungi from Antarctic rocks and soils. Ital J Zool 67:163–167

Orlandini V, Maida I, Fondi M, Perrin E, Papaleo MC, Bosi E, de Pascale D, Tutino ML, Michaud L, Lo Giudice A, Fani R (2014) Genomic analysis of three sponge-associated *Arthrobacter* Antarctic strains, inhibiting the growth of *Burkholderia cepacia* complex bacteria by synthesizing volatile organic compounds. Microbiol Res 169:593–601

Pan SY, Tan GYA, Convey P, Pearce DA, Tan IKP (2013) Diversity and bioactivity of actinomycetes from Signy Island terrestrial soils, maritime Antarctic. Adv Polar Sci 24:208–212

Papaleo MC, Fondi M, Maida I, Perrin E, Lo Giudice A, Michaud L, Mangano S, Bartolucci G, Romoli R, Fani R (2012) Sponge-associated microbial Antarctic communities exhibiting antimicrobial activity *against Burkholderia cepacia* complex bacteria. Biotechnol Adv 30:272–293

Papaleo MC, Romoli R, Bartolucci G, Maida I, Perrin E, Fondi M, Orlandini V, Mengoni A, Emiliani G, Tutino ML, Parrilli E, de Pascale D, Michaud L, Lo Giudice A, Fani R (2013) Bioactive volatile organic compounds from Antarctic (sponges) bacteria. New Biotechnol 30:824–838

Pearce DA (2012) Extremophiles in Antarctica: life at low temperatures. In: Stan-Lotter H, Fendrihan S (eds) Adaption of microbial life to environmental extremes. Springer, Vienna, pp 87–118

Prasad S, Manasa P, Buddhi S, Singh SM, Shivaji S (2011) Antagonistic interaction networks among bacteria from a cold soil environment. FEMS Microbiol Ecol 78:376–385

Rojas JL, Martín J, Tormo JR, Vicente F, Brunati M, Ciciliato I, Losi D, Van Trappen S, Mergaert J, Swings J, Marinelli F, Genilloud O (2009) Bacterial diversity from benthic mats of Antarctic lakes as a source of new bioactive metabolites. Mar Genomics 2:33–41

Romoli R, Papaleo MC, de Pascale D, Tutino ML, Michaud L, Lo Giudice A, Fani R, Bartolucci G (2011) Characterization of the volatile profile of Antarctic bacteria by using solid-phase microextraction – gas chromatography mass spectrometry. J Mass Spectrom 46:1051–1059

Romoli R, Papaleo MC, de Pascale D, Tutino ML, Michaud L, Lo Giudice A, Fani R, Bartolucci G (2014) GC-MS volatolomic approach to study the antimicrobial activity of the Antarctic bacterium *Pseudoalteromonas* sp. TB41. Metabolomics 10:42–51

Russell NJ (2008) Membrane components and cold sensing. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 177–190

Schuhmann I, Yao CB, Al-Zereini W, Anke H, Helmke E, Laatsch H (2009) Nitro derivatives from the Arctic ice bacterium *Salegentibacter* sp. isolate T436. J Antibiot 62:453–460

Shekh RM, Singh P, Singh SM, Roy U (2011) Antifungal activity of Arctic and Antarctic bacteria isolates. Polar Biol 34:139–143

Svahn KS, Chryssanthou E, Olsen B, Bohlin L, Göransson U (2015) *Penicillium nalgiovense* Laxa isolated from Antarctica is a new source of the antifungal metabolite amphotericin B. Fungal Biol Biotechnol 2:1. doi:10.1186/s40694-014-0011-x

Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, Biondi N, Tredici MR, Mainini M, Losi D, Marinelli F, Wilmotte A (2006) Polyphasic study of antarctic cyanobacterial strains. J Phycol 42:1257–1270

Vester JK, Glaring MA, Stougaard P (2015) Improved cultivation and metagenomics as new tools for bioprospecting in cold environments. Extremophiles 19:17–29

Wietz M, Månsson M, Bowman JS, Blom N, Ng Y, Gram L (2012) Wide distribution of closely related, antibiotic-producing *Arthrobacter* strains throughout the Arctic Ocean. Appl Environ Microbiol 78:2039–2042

Wong CMV, Tam HK, Alias SA, González M, González–Rocha G, Domínguez–Yévenes M (2011) *Pseudomonas* and *Pedobacter* isolates from King George Island inhibited the growth of foodborne pathogens. Pol Polar Res 32:3–14

Yuan M, Yu Y, Li H-R, Dong N, Zhang X-H (2014) Phylogenetic diversity and biological activity of Actinobacteria isolated from the Chukchi Shelf marine sediments in the Arctic Ocean. Mar Drugs 12:1281–1297