Psychrophilic microorganisms as important source for biotechnological processes

Sergiu Fendrihan[1] and Teodor G. Negoiță[2]

[1]Romanian Bioresource Centre and Advanced Research, Bucharest, Romania
[2]Romanian Institute of Polar Research, Bucharest, Romania

1 Introduction

The major parts of Earth's environments are cold and have temperatures below 5°C (Gounot 1999; Russell and Cowan 2005). About 70% of the freshwater is ice and about 14% from the Earth's biosphere is represented by terrestrial and aquatic polar areas (Priscu and Christner 2004). The depth of the oceans, the poles, and high mountains are the most important cold regions on Earth (Russell and Cowan 2005). Global ice, for example, covers 6.5 million km^2 which increases to 14.4 million km^2 in wintertime (Perovich et al. 2002). Here we can meet representatives from all domains of the living world. Two categories of microorganisms were discovered in such cold environments. First, the psychrophiles with an optimum growth temperature of about 15°C or even less, which cannot grow above 20°C (Moyer and Morita 2007); second, the psychrotolerants with an optimum growth temperature of 20–30°C, which are able to grow and exhibit activity at temperatures close to the freezing point of water (Madigan and Jung 2003). The lowest temperature for life's activities is −20°C under certain defined conditions (Rivkina et al. 2000; Gilichinsky 2002; D'Amico et al. 2006); others consider the temperature limits for reproduction as −12°C and for metabolism as −20°C (Bakermans 2008). *Colwellia psychrerythraea* strain 34H is motile at −10°C, as observed by transmitted light microscopy (Junge et al. 2003). Psychrophilic microorganisms are dominant in permanently cold environments such as Antarctic waters and have important roles in the biogeochemical cycles in the polar zones (Helmke and Weyland 2004). Not only are prokaryotes adapted to the cold, but also are many eukaryotes such as algae (Takeuchi and Kohshima 2004) and macroorganisms from crustaceans to fishes. The present work will focus on prokaryotes and some microscopic eukaryotes of biotechnological importance.

H. Stan-Lotter et al. (eds.), *Adaption of Microbial Life to Environmental Extremes*

2 Diversity of cold-adapted microorganisms

The psychrophilic and psychrotolerant microorganisms belong to all three of life's principal domains, Archaea, Bacteria and Eukarya. It is interesting to note that viruses are omnipresent and even so in those inhospitable places. Viruses from the families *Podoviridae, Siphoviridae,* and *Myoviridae* were identified in cold environments (Wells 2008). Bacteriophages were identified in inner polar waters and in ice (Säwström et al. 2007) infecting psychrophilic microorganisms; for example, phage 9A of *Colwellia psychrerythrea* strain 34 is capable of forming plaques at low temperatures, but not at 13°C (Wells 2008).

Archaea found in cold environments are methanogens for example, from genera *Methanogenium, Methanococcoides* and *Methanosarcina,* but halophilic (*Halorubrum*) and other strains can also occur (Cavicchioli 2006).

Bacteria. The majority of isolates from polar areas belong to the groups of *Beta-, Gamma-, Delta-Proteobacteria, Actinobacteria, Acidobacteria,* the *Cytophaga–Flexibacter–Bacteroides* group, and green nonsulfur bacteria. Many strains of Bacteria as well as Archaea and Eukarya were revealed by 16S rRNA and 18S rRNA gene clone libraries (Tian et al. 2009). Soils of the McMurdo Dry Valleys host species of *Pseudonocardia, Nocardioides, Geodermatophilus, Modestobacter, Sporichthya* and *Streptomyces* (Babalola et al. 2008). Cyanobacteria as photoautotrophs were retrieved from ice, soils, rocks, lakes, ponds, marine ecosystems, and alpine areas (Zakhia et al. 2008). *Chamaesiphon* sp., *Chroococcidiopsis* (from sandstone) and *Synechococcus* sp. (from lakes, marine water, and others) are examples of cyanobacterial genera with cold-adapted strains.

Algae. Species of *Chlamydomonas* were retrieved from water derived from melting glacier ice and from some layer species of *Rhodomonas* and *Chromulina*. Species of *Tribonemataceae* were found in Antarctic terrestrial environments (Rybalka et al. 2008). Several microalgae can be found in all known cold environments as in snow (*Chlamydomonas* and *Chloromonas*), seawater (diatoms), sea ice (diatoms and dinoflagellates), on rocks as endoliths (*Hemichloris antarctica*), ice-covered lakes (*Chloromonas* sp., *Chlamydomonas intermedia,* and *Chlamydomonas raudensis*) and at high altitudes (reviewed by Mock and Thomas 2008). Samples from the Tyndall Glacier in Patagonia, Chile contained algal species of the genera *Mesotaenium, Cylindrocystis, Ancylonema, Closterium, Chloromonas,* and some cyanobacteria (Takeuchi and Kohshima 2004).

Yeasts. Yeast strains such as *Sporobolomyces, Cryptococcus,* and *Rhodotorula* sp. were isolated from Lake Vanda (Goto et al. 1969) and from other Antarctic and alpine environments, including psychrophilic yeasts such as the novel species *Mrakia robertii, M. blollopis,* and *M. niccombsi* (Thomas-Hall et al. 2010). Several yeasts,

which are producers of lipases and proteases, were isolated from cold marine water and freshwater (Rashidah et al. 2007), such as *Cryptococcus antarcticus* and *Cryptococcus albidosimilis*, *Basidioblastomycetes* (Vishniac and Kurtzman 1992), *Cryptococcus nyarrowii* (Thomas-Hall and Watson 2002), *Cryptococcus watticus* (Guffogg et al. 2004), and *Leucosporidium antarcticum* – the latter from Antarctic waters (Turkiewicz et al. 2005) – and *Mrakia* strains (Thomas-Hall et al. 2010).

Fungi were isolated from many cold environments. For example, *Penicillium*, *Aspergillus*, *Paecilomyces*, *Cladosporium*, *Mortierella*, *Candida*, and *Rhodotorula* were isolated from soils of Terra Nova Bay and Edmonson Point, Antarctica (Gesheva 2009). Some authors described isolates from soils, such as *Chrysosporium* sp., *Phoma exigua*, *Heterocephalum aurantiacum*, *Aureobasidium pullulans*, *Fusarium oxysporum*, *Trichoderma viride*, and *Penicillium antarcticum* (Negoiță et al. 2001a). From the soils of Schirmacher Oasis, Antarctica, fungi such as *Acremonium*, *Aspergillus*, and *Penicillium* were isolated, the majority surviving as spores in those harsh environments, and some species possess unique features of their mycelia (Singh et al. 2006). Frisvad (2008b) reviewed the fungi from cold ecosystems and indicated their isolation from soils and permafrost, caves, rocks, mosses and lichens, glacier ice, freshwater, as well as from frozen foods. The fungi belong to the Ascomycetes (*Acremonium antarcticum*, *A. psychrophilum*, and *Penicillium antarcticum*), Zygomycetes (*Mortierella alpina* and *Absidia psychrophila*) and basidiomycetous yeasts, which are very rare in cold areas. Endolithic fungi resistant to low temperature and low water activity were isolated by Onofri et al. (2007).

3 Ecology and biology

Some of the microorganisms are polyextremophiles, for example halo-psychrophiles, or piezo-psychrophiles, which tolerate high pressure (Nogi 2008) and cannot grow at atmospheric pressure and at temperatures above 20°C, such as strains of *Shewanella*, *Colwellia*, *Moritella*, and *Psychromonas*. In these categories all the physiological and metabolic types can be found – anaerobes and aerobes, methanogens, methanotrophs, chemolithotrophs, sulfate reducers, and organotrophs. Anaerobic cold-adapted *Clostridium* sp. (e.g., *C. frigoris*, *C. bowmannii*, and *C. psychrophilum*) were isolated from Antarctic microbial mats (Spring et al. 2003) or some psychrotolerants, such as *C. frigidicarnis* and *C. algidixylanolyticum*, from frozen products (Finster 2008). Sulfate-reducing psychrophiles *Desulfotalea*, *Desulfofaba*, and *Desulfofrigus* (Knoblauch et al. 1999), sulfur-oxidizing bacteria (SOB), occurring in such organic carbon depleted environments as subglacial waters (Sattley and Madigan 2006), as well as denitrifying microorganisms in sea ice (Rysgaard et al. 2008) were found. Ammonia oxidizers were identified by genetic

methods in all of the samples taken from lakes Fryxell, Bonney, Hoare, Joyce, and Vanda in Antarctica, belonging to the *Proteobacteria* (Voytek et al. 1999). Acetogenic bacterial sequences originating from *Acetobacterium tundrae* and others related to *Acetobacterium bakii* (Sattley and Madigan 2007) were isolated from sediments of Lake Fryxell. From the same lake different phototrophic purple bacteria were identified with molecular methods (Karr et al. 2003) as well as methanogenic and other Archaea (Karr et al. 2006). Biological methane oxidation and sulfate reduction by Archaea occur in alpine lakes (such as Lake Lugano deeps) in anoxic zones (Blees et al. 2010). Methanogens were detected in soils, water sediments, sea and lake waters from cold environments (Cavicchioli 2006). Methanotrophy was detected indirectly in Lake Untersee (Antarctica) by identification of hopanoids and two steroids (4-methyl steroid and 4,4-dimethyl steroid), one hopanoid (diplopterol) having a specific low isotopic ^{13}C content, and originating from the aerobic methylotroph *Methylococcus* sp. (Niemann et al. 2010). Some *Shewanella* and *Pseudomonas* strains from Antarctic lakes are able to mediate redox reactions of manganese under stimulation by Co and Ni (Krishnan et al. 2009).

4 Cold environments

Cold deserts. There are cold deserts in Antarctica where the precipitation is very low, the temperatures range between −55°C and 10°C, UV radiation is high and water activity is low; these are some of the most extreme environments on Earth. Many microorganisms can be found in endolithic communities composed of cyanobacteria such as *Acaryochloris marina* and *Gloeocapsa* species (de los Ríos et al. 2007). Endolithic bacteria, fungi, archaea, green algae, yeasts, and lichens were found in McMurdo Dry Valley (Gounot 1999), analyzed by staining with the BacLight LIVE/DEAD kit and observed with confocal laser scanning microscopy to demonstrate their survival (Wierzchos et al. 2004).

Soils covered with snow. From Arctic wetland soil methanotrophic bacteria were retrieved such as *Methylocystis rosea* (Wartiainen et al. 2006). In soils of Lapland microbial communities were discovered, similarly as in soils from alpine zones, where the temperatures can reach −25°C in wintertime. In addition, soils from Spitsbergen contained many fungi such as *Mucor, Mortierella, Alternaria, Fusarium,* and *Zygorrhinchus* (Negoiță et al. 2001b), genera which are very probably psychrotolerants.

Permafrost. Permafrost soils in the geological sense stay below 0°C for two consecutive years or more and are specific for arctic areas covering about 26% of the surface of the Northern Hemisphere. The average temperature is −16°C; in Siberia −11°C and in Antarctica −18°C to −27°C were measured (Vorobyova

et al. 1997). From those soils over 100 bacterial strains were isolated, also some methanogenic archaea from the families *Methanomicrobiaceae*, *Methanosarcinaceae*, and *Methanosetaceae* (Ganzert et al. 2007), methane oxydizing bacteria (Liebner and Wagner 2007), sulfate-reducing bacteria, aerobic and anaerobic heterotrophs (Gilichinsky 2002), denitrifiers, and iron and sulfate reducers (Rivkina et al. 1998). The majority of strains included species of *Micrococcus*, *Bacillus*, *Paenibacillus*, *Rhodococcus*, *Arthrobacter*, *Haloarcula*, and *Halobaculum* (Steven et al. 2007), which were isolated in quantities of 10^7–10^9 cells per gram of dry soil. From layers of permafrost which were demonstrated to be about 3–5 million years old, viable cells of bacteria were isolated (Rodrigues-Diaz et al. 2008), which have to face low temperatures and natural irradiation by radionuclides (Gilichinsky et al. 2008). From a layer of an arctic permafrost ice wedge from Canada (temperature −17.5°C, pH 6.5, salt concentration 14.6 g/l, age about 25,000 years) bacteria were isolated (Katayama et al. 2007) belonging to the classes *Actinobacteria* and *Gamma-Proteobacteria*.

Snow, ice, and glaciers. The ice glaciers in Antarctica contain approximately 90% of the ice of our planet according to the National Snow and Ice Data Centre of USA (http://nsidc.org/, cited by Christner et al. 2008). Some aspects of the soils covered partially with ice on the shore of the Antarctic sea are shown in Figs. 1 and 2. Sea and lake ice glaciers are hosting considerable quantities of biological material, consisting of microorganisms, bacteria, spores, and pollen grains, the majority being transported there by air. The microbiota can survive in crevices and capillary tunnels containing concentrated ionic solutions with a lower freezing point (Price 2006). The number of viable microorganisms decreases with the depth of the ice layers; there is a supraglacial community (bacteria, viruses, diatoms, tardigrades, and

Fig. 1. Larsemann Hills Coast, Law-Racovita Base area, East Antarctica, 69°23′0″S; 76°23′0″E (photo T.G. Negoita, 2007)

Fig. 2. Antarctic ice cap in the Schirmacher Oasis, 70°46′S; 11°50′E, 100 km inside the continent (photo T.G. Negoita, 2007)

rotifers), a subglacial community (aerobic and anaerobic) and an endoglacial community (Hodson et al. 2008). Hollibaugh et al. (2007) studied the Sea Ice Microbial Community (SIMCO) formed of bacteria, algae, and fungi of various metabolic types: sulfate reducers, chemolithotrophs, methanogens, anaerobic nitrate reducers (Skidmore et al. 2000), and viruses (Deming 2007). In ice there is an incredible diversity of *Proteobacteria*, of the phylum *Cytophaga–Flavobacterium–Bacteriodes*, high GC Gram positives and low GC Gram positives (Miteva 2008), and a large metabolic and physiological diversity. Some of them were entrapped for very long periods of time, such as the strain *Herminimonas glaciei*, a Gram-negative ultramicrobacterium, which was isolated from a 3042 m deep drilling core from a Greenland glacier of about 120,000-year-old ice (Loveland-Curtze et al. 2009), or *Chryseobacterium greenlandense* (Loveland-Curtze et al. 2010) and sequences from *Pseudomonas* and *Acinetobacter*, which stem from 750,000-year-old ice from the Qinghan-Tibetan plateau in Western China (Christner et al. 2003a). Many prokaryotes isolated from snow melt water belong to the *Beta-Proteobacteria* (21.3%), *Sphingobacteria* (16.4%), *Flavobacteria* (9.0%), *Acidobacteria* (7.7%), and *Alpha-Proteobacteria* (6.5%) and other groups (Larose et al. 2010). The cryoconite holes form another microhabitat containing various forms of life, such as diatoms, algae, prokaryotes, fungi, rotifers, and tardigrades (Wharton et al. 1985; Christner et al. 2003b).

Cold caves. Caves represent a constant temperature environment with low organic content, sometimes only 1 mg of organic matter per liter. Some strains are chemolithotrophs such as *Galionella*. In many cases there are more psychrotolerants than psychrotrophs. Some stenothermic bacterial strains were isolated which can grow at 10–20°C and only few which grow at 2°C or 28°C (Gounot 1999). The

strain *Arthrobacter psychrophenolicum* was isolated from an Austrian ice cave (Margesin et al. 2004).

Cold lakes. The cold lakes in the polar and alpine zones can be covered with an ice layer (Antarctic lakes), which practically isolates the lake from the rest of the environment, and their content of organic carbon and oxygen is rather low. Christner et al. (2008) pointed out in their comprehensive review that there are 141 subglacial Antarctic lakes having a total volume of about 10,000 km^3. One of the largest lakes is Lake Vostok of 14,000 km^2, covered by a 4000 m thick ice sheet and being 400–800 m deep. The bottom is covered with a thick sediment layer. The temperature of the ice layer is about −55°C, but the lake has a constant temperature of −2.65°C (Di Prisco 2007); the ice layer is about 15 million years old. Alpine lakes are only temporarily covered by ice and the microbiota there are subject to seasonal fluctuations (Pernthaler et al. 1998). The cold Antarctic lake environment is chemically driven, with reactions such as sulfide and iron oxidation (Christner et al. 2008), and contains methanogens such as *Methanosarcina*, *Methanoculleus*, and anoxic methanotrophs (Karr et al. 2006). The saline lakes host euryhalophiles related to *Halomonas* and *Marinobacter* (Naganuma et al. 2005).

Cold marine waters. From marine waters of Ushuaia, a sub-Antarctic town in Argentina, many sequences were identified belonging to the *Alpha-* and *Gamma-Proteobacteria*, *Cytophaga–Flavobacterium–Bacteroidetes* group, the genera *Marinomonas*, *Colwellia*, *Cytophaga*, *Glacieola*, *Cellulophaga*, *Roseobacter*, *Staleya*, *Sulfitobacter*, *Psychrobacter*, *Polaribacter*, *Ulvibacter*, *Tenacibacter*, *Arcobacter*, and *Formosa* (Prabagaran et al. 2007). In the depth of the ocean the temperature is about 3°C, and a considerable pressure exists (the pressure increases by 1 atm per each 10 m of depth). Here a very diverse bacterial community can be retrieved, for example, from the sediments of the Japanese Trench (Hamamoto 1993). From the deep sediments were, all the domains of life are represented, an important microbiota was identified by molecular methods (Tian et al. 2009). The archaeal sequences can reach 17% from total microbiota in marine coastal waters (Murray et al. 1998). Sulfate-reducing bacteria form a large community in sediments of the Arctic ocean, being active at about 2.6°C with a sulfate reduction rate similar to that under mesophilic conditions (Knoblauch et al. 1999).

Anthropic cold environments. Artificial cooling and freezing systems can be visualized as man-made environments. *Pseudomonas fluorescens* is one of the lipolytic food spoiling bacteria which is active in the cold, and its hydrolytic activity at low temperatures was studied as a function of water activity (Andersson et al. 1979). The lower temperatures and lower water activity did not affect the enzymatic activity since the substrates were hydrophobic. In

cooling devices the bacterium *Pseudomonas fragi* is frequently found, which is supported by temperatures between 2°C and 35°C; it possesses some cold shock proteins (Csps) and degrades frozen foods. Another bacterium from water-cooling systems is *Chryseobacterium aquifrigidense* (Park et al. 2008). The psychrophilic strain *Lactobacillus algidus* was isolated from refrigerated, packed beef meat (Kato et al. 2000).

Air. From the Antarctic continent air several psychrotolerant microorganisms were isolated, such as *Sphingomonas aurantiaca*, *Sphingomonas aerolata*, and *Sphingomonas faeni* sp. nov. (Busse et al. 2003).

5 Adaptation to cold environments

Growth and activity. The temperature has a direct influence on microbial growth and the relationship between growth and temperature generally conforms to the Arrhenius law (Gounot 1999). Christner (2002) reported the incorporation of DNA and protein precursors by *Arthrobacter* and *Psychrobacter* at −15°C. *Polaromonas hydrogenivorans* has a lower temperature limit of 0°C for growth (Sizova and Panikov 2007), and psychrophilic methanotrophs can grow at about 2°C (Liebner and Wagner 2007). The psychrophilic strain *Psychromonas ingrahami* showed growth at −12°C with a slow rate of 10 days of generation time (Breezee et al. 2004). The activity of microorganisms was proven by measurement of ATP as a result of biomass activity in soils and permafrost (Cowan and Casanueva 2007); truly psychrophilic microorganisms showed an increase of the ATP content at lower temperatures, which is the opposite reaction of mesophiles (Napolitano and Shain 2004). Other information can be obtained by determination of the Indicator of Enzymatic Soil Activity Potential, the Indicator of Vital Activity Potential, and Biologic Synthetic Indicator (Negoiță et al. 2001b). These indicators were introduced by Ştefanic (1994) in order to obtain comprehensive information about the biological activity of soils and to compare them for agricultural uses.

Membrane polar lipids. There are differences regarding the composition of membrane lipids and there are clear contributions to cold adaptation, depending also on bacterial taxonomy. The cytoplasmic membrane contains lipids with fatty acids of lengths ranging mainly between C_{14} and C_{18} Gram negatives possess in addition an outer membrane containing lipopolysaccharides, Archaea contain ether-linked glycerol alkyl lipids instead of fatty acids, and eukaryotes contain sterols (Russell 2008). Membrane fluidity depends on the degree of saturation of the polar lipids; the membranes from psychrophiles contain a higher amount of unsaturated and/or

polyunsaturated and branched fatty acids, with methyl groups and a larger percentage of double bonds of the *cis* type (Chintalapati et al. 2004). The changes in amount and type of methyl-branched fatty acids of Gram-positive bacteria are a possibility for increasing membrane fluidity at low temperatures. The amount of unsaturated fatty acids contributes to the flexibility of the membrane structure in cold-adapted microorganisms, including eukaryotic photobionts such as diatoms and algae (Morgan-Kiss et al. 2006). The presence of polyunsaturated fatty acids (PUFAs) does not completely explain the adaptation to cold environments, because there are many marine strains without them (Russell and Nichols 1999). Archaeal adaptation to the cold shows a similar increase in desaturation of their isoprenoids containing lipids; *Methanococcoides burtoni* for example generates unsaturated lipids during growth at low temperatures by selective saturation and not by using a desaturase such as bacteria (Cavicchioli 2006).

The proteome. Cold-adapted bacterial proteins have a reduced amount of arginine, glutamic acids, and proline (salt bridge forming residues) and reduced amounts of hydrophobic clusters (Grzymski et al. 2006). A comparison of the contents of amino acids of psychrophilic enzymes was made by Gianese et al. (2001); they found that generally Arg and Glu residues in the exposed sites of alpha helices were replaced by Lys and Ala in psychrophiles. Studying the crystal structure of the β-lactamase from several psychrophilic strains (*P. fluorescens* and others) some authors found that the enzymes from psychrophiles have a lower content of arginine in comparison with lysine and a lower proline content than mesophilic enzymes (Michaux et al. 2008). The lysine residues are of great importance for the cold adaptation mechanism in enzymes, for example in α-amylase from *Pseudoalteromonas haloplanktis* (Siddiqui et al. 2006). A similar replacement is observed with Archaea having a higher content of noncharged amino acids (as glutamine and threonine) and lower contents of hydrophobic amino acids such as leucine (Cavicchioli 2006). At the same time the number of hydrogen bonds (Michaux et al. 2008) and the number of disulfide bridges are reduced (Sælensminde et al. 2009). The cellulase Cel5G from *P. haloplanktis* possesses a catalytic domain and a carbohydrate-binding domain which are joined by a long-linker region containing three loops closed by disulfide bridges. By experimental shortening of this linker region, the enzyme became less flexible approaching the activity of its mesphilic counterpart, which suggested that a long-linker region is an appropriate adaptation of this enzyme to low temperates (Sonan et al. 2007). Studying the thermal adaptations of psychrophilic, mesophilic and thermophilic DNA ligases, the conclusion was that "the active site of the cold-enzyme is destabilized by an excess of hydrophobic surfaces and contains a decreased number of charged residues compared with its thermophilic counterpart" (Georlette et al. 2003). The proteins must keep a balance between their stability and

flexibility, especially enzymes, which are to be active at lower temperatures than their mesophilic counterparts (Georlette et al. 2003). An intensive study of the proteomics of psychrophilic microorganisms (Kurihara and Esaki 2008) showed that there are various proteins involved in transcription, folding of RNA and proteins, modulation of gene expression, and others, which are inducibly produced at low temperatures.

The following three main types of proteins are of interest for mechanisms of adaptation:

Csps, which are induced by exposure to low temperatures, are involved in several cellular processes (fluidity of membranes, transcription, translation). Proteins from psychrophiles (Caps, cold acclimation proteins) are similar to the Csps. The regulation of the CspA protein takes place at the transcriptional level at the level of stabilization of mRNA (*cspA* mRNA) and at the level of translation (Phadtare and Inouye 2008). Numerous Csps and proteins helping in the adaptation to low temperatures were isolated and characterized (Russell 2008). They play a role in the cold adaptation during stress response and also act as RNA chaperones. Similar proteins can be found in Archaea, such as *Methanogenium frigidum*, which are bound to nucleic acids (Giaquinto et al. 2007).

Antifreeze proteins (AFPs). AFPs and antifreeze glycoproteins (AFGPs) can lower the temperature of the freezing point of water (D'Amico et al. 2006; Kawahara 2008). They can inhibit the formation of ice crystals and prevent the penetration of ice into cells (Zachariassen and Lundheim 1999). One of the examples is the protein Hsc25 produced by the bacterium *Pantoea ananatis* KUIN 3, which helps to refold denaturated proteins in the cold (Kawahara 2008).

Antinucleating proteins (ANPs). ANPs and other compounds inhibit ice nucleation and formation of intracellular ice crystals, avoiding thereby the damage of cells. *Acinetobacter aceticus* can release such antinucleating proteins with a mass of 550 kDa. The proteins can be used in the preservation of livers (in a concentration of 20 μg/ml) at subzero temperatures without freezing, with addition of an antioxidant such as ascorbic acid (Kawahara 2008). An ice-binding protein of a mass of 54 kDa, isolated from a bacterial strain from an ice core of over 3000 m depth, was able to inhibit the recrystallization of ice (Raymond et al. 2007).

Enzymes. The rate of a chemical reaction is temperature dependent, according to the Arrhenius equation $K = A \exp(-E_a/RT)$, where K is the reaction rate, E_a is the activation energy, R is the gas constant, T is absolute temperature in Kelvin, and A is a constant. It is well known that biological reactions showed a 16- to 80-fold reduction when the temperature is reduced from 37°C to 0°C (Collins et al. 2008).

While psychrophiles exhibit a high metabolic rate at cold temperatures, they are usually inactivated at mesophilic temperatures because of the flexibility and lower stability as a consequence of the plasticity of catalytic zones of their molecules, due to a reduction of hydrophobic and hydrogen bonds and a lower content of arginine and proline (D'Amico et al. 2006). Shifting to optimum activation energy allows them to keep normal reaction rates at low temperatures (Siddiqui and Cavicchioli 2006). At the same time the 3D structure is also important (Tkaczuk et al. 2005), such as intramolecular bond modifications (Feller and Gerday 1997; Bae and Phillips 2004), modification of amino acids in or near catalytic domains of enzymes (Papaleo et al. 2008), and a lower content of hydrogen bonds (Michaux et al. 2008). An intensive search for cold-adapted enzymes was performed by Morita et al. (1997) who isolated more than 130 bacterial strains and tested the properties of amylases, proteases and lipases, showing that they were easily inactivated at above optimum temperatures.

Other substances which can play a role in cold adaptation and cryoprotection are carotenoids, which contribute to the stability of cellular membranes (Russell 2008); extracellular polymeric substances (EPSs), some of them of high molecular weight or heteropolysaccharides (with additions of proteins), which are released by some microorganism into the neighboring environment and form a kind of gel with cryoprotective effects (Krembs and Deming 2008); polyhydroxyalkanoates (PHAs), which can reduce oxidative stress in the cold, maintaining the redox state (Ayub et al. 2009); trehalose, which is able to protect cells under conditions of shock exposure to high and low temperatures and osmotic stress (Phadtare and Inouye 2008) by stabilizing the cell membrane and removing free radicals, thus preventing denaturation of proteins. Generally speaking, when comparing with thermophiles and mesophiles, it appears that cold-adapted microorganisms adopted the strategy of more entropy by molecular mechanisms, which are allowing an enhanced flexibility for maintaining dynamics and functions of the molecules (Feller 2007).

Genetic features as adaptation mechanisms. So far, the following genomes from psychrophiles and psychrotolerants have been sequenced: *Methanococcoides burtonii* (Allen et al. 2009); *Methanogenium frigidum* (Saunders et al. 2003); *Colwellia psychrerythrea* 34H (Methé et al. 2005); *Desulphotalea psychrophila* (Rabus et al. 2004); *Idiomarina loihiensis* L2TR (Hou et al. 2004), *Pseudoalteromonas haloplanktis* TAC125 (Médigue et al. 2005); *Shewanella frigidimarina* (Copeland et al. 2006); *Psychrobacter arcticus* 253-4 (Ayala-del-Río et al. 2010), *Psychromonas ingrahamii* (Riley et al. 2008); 14 *Shewanella* strains (Hau and Gralnick 2007); and several others, which are partially sequenced. The analysis of the genes showed some

principal features of the mechanisms for cold adaptation (Bowman 2008): a lower content in arginine and proline, which influences the flexibility of proteins, was observed, especially in sequences related to growth and development (Ayala-del-Río et al. 2010). Nucleic acids of psychrophiles showed a different proportion of uracil in 16S rRNA sequences, such as an inverse proportional relation to their optimum growth temperature (Khachane et al. 2005).

6 Applications of psychrophilic microorganisms

Bioprospecting and bioscreening of psychrophilic microbial resources (Nichols et al. 2002) have become real challenges and opportunities for biotechnology. Cold-adapted bioactive substances provide advantages in different areas, such as activity at low temperatures; the possibility of challenging reactions with a sufficiently high reaction rate; energy savings; efficient production with lower processing costs; thermal protection of the products; and better quality of products. Presently the market for bioactive products and industrial enzymes is growing. Archaea, Bacteria, and Eukarya can be sources of valuable products. Huston (2008) reviewed the enzymes from cold-adapted microorganisms, identifying compounds and enzymes for the food and cosmetic industry, pharmaceuticals, biofuels, substances for molecular biology studies, and even for nanobiotechnology. An extensive compilation of applications of psychrophilic and psychrotolerant microorganisms is presented in Table 1.

Bioscreening for valuable products is generally not made anymore in the classical way by isolation and cultivation of microorganisms. Now high-throughput culturing technologies enable the isolation of a major proportion of the microbiota in environmental samples; combined with metagenomics and gene expression studies, genome data mining permits an efficient search for bioproducts (see Huston 2008). Psychrophilic proteins, for example, can have some interesting applications, and their production can be achieved directly or expressed in an adequate host such as *Escherichia coli*, which was used for the α-amylase from *P. haloplanktis* (Feller et al. 1998). It can be difficult to obtain a stable production, due to autolytic deterioration. A possible solution is overexpression using a plasmid vector from *P. haloplanktis* pMTBL and the plasmid of *E. coli* pJB3 (Tutino et al. 2001). This type of expression technology promises a wide application for the problem of efficient gene expression systems and rapid purification steps. Recombinant proteins can be obtained by expressing them in prokaryotic cells of cold-adapted (*P. haloplanktis* TAC125) and eukaryotic cells (*Saccharomyces cerevisiae*; Parrilli et al. 2008).

Antibiotics. The isolates from the Antarctic Ocean, Ross Bay, were shown to have antibiotic activities which were tested with the terrestrial bacteria *E. coli*,

Table 1. Applications of psychrophilic and psychrotolerant microoganisms isolated from cold environments

Microorganism	Enzymes and other metabolites	Applications	References
Bacteria			
Acinetobacter sp.	Lipases	Lipid hydrolysis, detergent additives	Ramteke et al. (2005) and Joseph et al. (2007)
Achromobacter sp.	Lipases	Lipid hydrolysis	Ramteke et al. (2005) and Joseph et al. (2007)
Aeromonas sp.	Lipases	Lipid hydrolysis	Lee et al. (2003)
Arthrobacter strains	Antibiotics	Pharma industry	Lo Giudice et al. (2007) and Benešova et al. (2005)
Arthrobacter sp.	Alkaline phosphatases	Alkaline phosphatase (removal of 5′ phosphate groups from DNA and RNA)	De Prada et al. (1996)
Arthrobacter C2-2	α-Glucosidase, β-glucosidase	Cleavage of maltose at β-1,4 bonds; pharma industry, medicine	Benešova et al. (2005)
Arthrobacter strain 20B	β-Galactosidases	Lactose hydrolysis	Białkowska et al. (2009)
Arthrobacter psychrolactophilus strain F2	β-Galactosidase rBglAp	Produces trisaccharides from lactose; food industry	Nakagawa et al. (2007)
Arthrobacter psychrophenolicus		Degradation of phenol and phenolic compounds	Margesin et al. (2004)
Bacillus subtilis strain MIUG 6150	α-, β-Amylases	Starch hydrolysis; food industry	Bahrim and Negoiță (2004)
Bacillus subtilis strain MIUG 6150	Proteases	Protein hydrolysis	Bahrim and Negoiță (2004)
Brevibacterium antarcticum		Bioremediation; resistant to metals in soils (Cu, Cr, Hg, and others)	Tashyrev (2009)
Colwellia demingiae	Protease (azocasein)		Nichols et al. (1999)
Colwellia demingiae	Protease (azoalbumin)		Nichols et al. (1999)
Colwellia demingiae	Trypsin-like enzyme	Protein hydrolysis	Nichols et al. (1999)
Colwellia demingiae	Phosphatase		Nichols et al. (1999)
Colwellia-like strain	Trypsin-like enzyme		Nichols et al. (1999)
Colwellia-like strain	Phosphatase		Nichols et al. (1999)
Colwellia-like strain	β-Galactosidase	Lactose hydrolysis	Nichols et al. (1999)
Colwellia-like strain	Protease (azocasein)		Nichols et al. (1999)
Colwellia-like strain	Protease (azoalbumin)	Protein hydrolysis	Nichols et al. (1999)

(*continued*)

Table 1 (*continued*)

Microorganism	Enzymes and other metabolites	Applications	References
Colwellia-like strain	Trypsin		Nichols et al. (1999)
Colwellia-like strain	β-Galactosidase	Removal of lactose	Nichols et al. (1999)
Colwellia-like strain	α-Amylase	Starch hydrolysis	Nichols et al. (1999)
Colwellia-like strain	Alkaline phosphatase		Nichols et al. (1999)
Colwellia demingiae	Synthesizes docosahexaenoic acid	PUFAs as precursors for prostaglandins, thromboxanes, leucotrienes; medicine, pharma industry	Bowman et al. (1998) and Lees (1990)
Colwellia hornerae	Synthesizes docosahexaenoic acid	Pharma industry	Bowman et al. (1998)
Colwellia maris	Malate synthase, *iso*-citrate lyase	Bioethanol and biomethane production; wastewater treatment, bioremediation	Brenchley (1996) and Cavicchioli et al. (2002)
Colwellia rossensis	synthesizes docosahexaenoic acid	Pharma industry	Bowman et al. (1998)
Colwellia psychrotropica	Synthesizes docosahexaenoic acid	Pharma industry	Bowman et al. (1998)
Dactylsporangium roseum	Antibiotics	Pharma industry, medicine	Nguyen et al. (2010)
Erythrobacter litoralis HTCC2594	Epoxide hydrolase	Epoxide hydrolase, for enantio-selective hydrolysis of styrene oxide	Woo et al. (2007)
Fibrobacter succinogenes S85	Cellulase	Animal food industry, detergents, textile industry	Cavicchioli et al. (2002)
Flavobacerium sp.	β-Mannanase	Decreases viscosity in food products	Zakaria et al. (1998)
Flavobacterium frigidarium	Xylanolytic and laminarinolytic	Xylane degradation	Humphry et al. (2001)
Flavobacterium frigidimaris	Malate dehydrogenase		Oikawa et al. (2005)
Flavobacterium hibernum sp. nov.	β-Galactosidase	Lactose degradation	McCammon et al. (1998)
Flavobacterium limicola		Organic polymer degradation	Tamaki et al. (2003)
Glaciecola chathamensis	Exopolysaccharides	Food processing industry; medical and industrial uses	Matsuyama et al. (2006)
Glaciecola chathamensis	Polysaccharide-producing strain	Exopolysaccharides, industrial applications	Matsuyama et al. (2006)
Instrasporangium sp.	Antibiotics	Pharma industry	Nguyen et al. (2010)

(*continued*)

Table 1 (*continued*)

Microorganism	Enzymes and other metabolites	Applications	References
Janibacter sp.	Antibiotics	Pharma industry, medicine	Lo Giudice et al. (2007)
Kordiimonas gwangyangensis	Cold-adapted enzymes	Capable of degrading polycyclic aromatic hydrocarbons (PAHs)	Kwon et al. (2005)
Micromonospora sp.	Antibiotics	Pharma industry	Nguyen et al. (2010)
Moraxella sp.	Lipases	Pharma industry, medicine, food additives	Ramteke et al. (2005) and Joseph et al. (2007)
Oceanibulbus indolifex	Indole and several indole derivatives	Cosmetics industry, pharma industry, cancer prevention	Wagner-Döbler et al. (2004) and Auborn et al. (2003)
Oceanibulbus indolifex	Cyclic dipeptides cyclo-(Leu,Pro), cyclo-(Phe,Pro), and cyclo-(Tyr,Pro)	Compounds with antiviral, antibiotic, and antitumor activity	Wagner-Döbler et al. (2004) and Milne et al. (1998)
Oceanibulbus indolifex	Tryptanthrin	Activity against some Gram-positive bacteria and fungi	Wagner-Döbler et al. (2004)
Oleispira antarctica	Cold-adapted enzymes	Hydrocarbonoclastic; for bioremediation	Yakimov et al. (2003)
Photobacterium frigidiphilum	Lipases	Lipid hydrolysis	Seo et al. (2005)
Planococcus sp.	β-Galactosidase	Lactose hydrolysis	Sheridan and Brenchley (2000)
Polaromonas naphthalenivorans	Enzymes	Degrades naphthalene	Jeon et al. (2004)
Polaromonas sp. strain JS666	Enzymes	*cis*-1,2-Dichloroethene as carbon source; for bioremediation	Mattes et al. (2008)
Pseudoalteromonas sp.	Protease (azocasein)		Nichols et al. (1999)
Pseudoalteromonas sp.	Trypsin-like enzyme		Nichols et al. (1999)
Pseudoalteromonas	Antibiotics	Pharma industry, medicine	Lo Giudice et al. (2007)
Pseudoalteromonas sp.	Lipases		Ramteke et al. (2005)
Pseudoalteromonas haloplanktis TAE 47	β-Galactosidase	Lactose hydrolysis	Hoyoux et al. (2001)
Pseudomonas sp. strain B11-1	Lipases, esterases		Suzuki et al. (2001)
Pseudoalteromonas sp. SM9913	Subtilase		Yan et al. (2009)
Psychrobacter okhotskensis	Lipase-producing strain		Yumoto et al. (2003)

(*continued*)

Table 1 (*continued*)

Microorganism	Enzymes and other metabolites	Applications	References
Psychrobacter sp.			Ramteke et al. (2005)
Rhodococcus	Antibiotics	Pharma industry, medicine	Lo Giudice et al. (2007)
Rhodococcus sp.		Biodegradation of phenol and phenolic compounds	Margesin and Schinner (1999)
Rhodococcus sp. strain N774	Nitrile hydratase	Acrylamide production	Kobayashi et al. (1992)
Rhodococus sp. Q15 i		Degrades short- and long-chain aliphatic alkanes from diesel fuel	Whyte et al. (1998)
Rhodococcus ruber			Murygina et al. (2000)
Rhodococcus erythrococcus		Product "Rhoder" for bioremediation of oil polluted environments	Murygina et al. (2000)
Serratia proteamaculans	Trypsin-like protease		Mikhailova et al. (2006)
Shewanella sp.	Produces omega 3 fatty acids	Essential fatty acid for humans	Hau and Gralnick (2007)
Shewanella sp.		Waste removal of radionuclides (uranium, technetium)	Hau and Gralnick (2007)
Shewanella sp.		Reduction of organic chlorine compounds	Hau and Gralnick (2007)
Shewanella donghaensis	High levels of polyunsaturated fatty acid	Medicine, food supplements	Yang et al. (2007)
Shewanella gelidimarina	β-Galactosidase	Lactose hydrolysis	Nichols et al. (1999)
Shewanella frigidimarina	Eicosapentaenoic acid (20:w503)	Food additives	Bowman et al. (1997) and Bozal et al. (2002)
Shewanella pacifica	Produces polyunsaturated fatty acids	Food additives, pharma industry	Ivanova et al. (2004)
Serratia proteamaculans	Trypsin-like protease	Protein hydrolysis	Mikhailova et al. (2006)
Serratia sp.	Lipases	Lipid hydrolysis	Ramteke et al. (2005)
Sphingmonas paucimobilis	Proteases	Meat industry, detergent industry, molecular biology	Cavicchioli et al. (2002)
Streptomyces sp.	Amylases, proteases, cellulases, lipases, antibiotics, other bioactive compounds	Detergent additives, starch industry, bread industry, antibiotics, immuno-suppressants, anticancer agents, extracellular hydrolytic enzymes, degradation of ligno-cellulosic materials	Cavicchioli et al. (2002), Galante and Formantici (2003), and Morita et al. (1997)

(*continued*)

Table 1 (*continued*)

Microorganism	Enzymes and other metabolites	Applications	References
Streptomyces fradiae	Antibiotics, amylase, protease, cellullase, lipases	Pharma industry, medicine, food industry, detergent additives	Nguyen et al. (2010)
Streptomyces anulatus	Dextranase	Dextrane hydrolysis; sugar industry	Doaa Mahmoud and Wafaa Helmy (2009)
Streptoverticillium	Antibiotics	Pharma industry	Nguyen et al. (2010)
Fungi			
Candida antarctica	Lipases		Joseph et al. (2007)
Candida antarctica		Conversion of *n*-alkanes into glycolipid; biosurfactants	Kitamoto et al. (2001)
Cryptococcus albidus	Xylanase	Hydrolyzing xylane for improvement of food, waste treatment, food industry	Amoresano et al. (2000)
Cryptococcus laurentii	Phytase	animal feeding	Pavlova et al. (2008)
Cryptococcus laurentii	β-Galactosidases	Dairy industry	Law and Goodenough (1995)
Cryptococcus cylindricus	Pectinases	Clarification of fruit juices; improving filterability, and extractability of juices	Nakagawa et al. (2004)
Cystofilobasidium capitatum	Pectinases	Clarification of fruit juices; improving filterability, and extractability of juices	Nakagawa et al. (2004)
Mrakia frigida	Pectinases	Clarification of fruit juices; improving filterability, and extractability of juices	Nakagawa et al. (2004)
Pichia lynferdii strain Y-7723	Lipase		Kim et al. (2010)
Rhodotorula psychrophenolica		degradation of phenolic compounds	Margesin et al. (2007)
Algae			
Porphyridium cruentum	Eicosapentaenoic acid, arachidonic acid	Pharma industry	Cohen (1990)

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Proteus mirabilis*, *Salmonella enterica*, and the yeast *Candida albicans*, following incubation at 37°C on nutrient agar (Lo Giudice et al. 2007). The isolates were identified by 16S rRNA and characterized by biochemical tests. From 580 isolated strains belonging to *Arthrobacter*, *Rhodococcus*, *Pseudoalteromonas*, and *Janibacter*, some were able to inhibit the test strains. The actinomycetes *Intrasporangium* sp., *Micromonospora* sp., *Streptoverticillium* sp., *Streptomyces* sp., and *Dactylsporangium roseum*, isolated from the soils from India at over 4000 m altitude, showed different

antibiotic activities against strains of *Streptococcus* isolated from dental plaque (Raja et al. 2010). From cold environments, strains close to *Serratia* and *Pseudomonas* were isolated; both are producers of antimicrobials, probably class II microcins acting in the cold (Sanchez et al. 2009).

Enzymes. Many psychrophilic and psychrotolerant bacteria possess the capacity to produce extracellular enzymes such as lipases, proteases, amylases, cellulases, chitinases, and β-galactosidase, when induced by the presence of specific substrates. Producers were strains of sea ice microorganisms, for example, for lipases *Colwellia psychroerythrea*, *Shewanella livingstonensis*, and *Marinomonas prymoriensis*; for hydrolysis of polysaccharides *Colwellia*, *Marinomonas*, *Pseudoalteromonas*, *Pseudomonas*, and *Shewanella*; for hydrolysis of chitin *Pseudoalteromonas tetraodonis*, *Pseudoalteromonas elyakovii*, *Bacillus firmus*, and *Janibacter melonis*, which degrade the organic matter from phyto- and zooplankton (Yu et al. 2009). A simple screening of 137 cold water isolates belonging to *Moraxella*, *Pseudomonas*, *Aeromonas*, *Chromobacterium*, *Vibrio*, and others showed that about 62% can produce gelatinase, 71% proteases, 31% lipases, 47% amylases, 36% chitinases, 36% β-galactosidases, 47% cellulases, and 25% alginate lyases (Ramaiah 1994). Groudieva et al. (2004) found that from 116 strains isolated from the Spitsbergen sea ice 40% possessed the ability to degrade skim milk, casein, lipids, starch and proteins. Enzymes such as dehydrogenase from cold-adapted microorganisms can also be used as biosensors or in biotransformations (Gomes and Steiner 2004). Protease-producing strains from the genera *Pseudoalteromonas*, *Shewanella*, *Colwellia*, and *Planococcus* were isolated from the sub-Antarctic marine sediments of Isla de Los Estados (Olivera et al. 2007).

The enzymes from psychrophilic and psychrotolerant strains can be divided into three categories: (1) heat sensitive, but similar to mesophilic enzymes; (2) heat sensitive and more active at low temperatures than mesophilic enzymes; and (3) heat sensitive exactly as mesophilic enzymes, but more active at lower temperatures (Ohgiya et al. 1999). Another example is a complex of enzymes generated by an Antarctic isolate, *B. subtilis* strain MIUG 6150, which produces α- and β-amylases and proteases (Bahrim and Negoiță 2004). The productivity of the microorganisms showed a strong dependency on the culture media used for growth and other conditions (Bahrim et al. 2007). The cold-adapted strains of *Streptomyces* can be a source of valuable enzymes (Cotârleț et al. 2008).

Proteases. Strains producing proteases belong to the genera *Bacillus* and *Pseudomonas* and were isolated from Antarctic cyanobacterial mats. They showed good production at a temperature of about 20°C, with glucose and maltose as carbon sources (*Pseudomonas* sp.) and soybean meal and peptone as nitrogen sources (Singh and Ramana Venkata 1998). A trypsin-like protease was identified and characterized

which is produced by *Serratia proteamaculans* (Mikhailova et al. 2006). These proteases are used in the dairy industry to enhance the flavour development in cheese. In the chemical industry, the enzymes from psychrophilic strains are used in detergents, food industry, and leather manufacturing (Cavicchioli et al. 2002).

Alkaline phosphatase was isolated and purified from the strain *Shewanella* sp. (Ishida et al. 1998). Interestingly, the enzyme showed a maximum activity at 40°C, 39% of that activity at 0°C, and a tendency to loose activity at 20°C. Two different extracellular alkaline phosphatases were identified from an *Arthrobacter* sp. strain (De Prada et al. 1996). Purified recombinant serine alkaline protease (in *E. coli*) from another *Shewanella* strain showed activity between 5°C and 15°C (Kulakova et al. 1999). The enzymes, such as the microorganisms themselves, face sometimes diverse polyextreme conditions; e.g., the cold-active protease MCP-03 from *Pseudoalteromonas* sp. SM9913 was active and stable also in a high salt environment of about 3 M NaCl/KCl (Yan et al. 2009).

β-Galactosidases. One possible applications of this enzyme is obtaining an ice cream with reduced lactose content for lactose intolerant peoples (Phadtare and Inouye 2008). The removal of lactose from milk is very important for persons with lactose intolerance. The cold-active β-galactosidases (EC3.2.1.23) isolated from psychrophilic yeasts (e.g., *Cryptococus laurentis*; Law and Goodenough 1995) and fungi can be used to hydrolyze lactose and therefore a new method of supplementation of milk with dormant cultures was proposed (Somkutl and Holsinger 1997). The utilization of cold-active β-galactosidase (optimum activity at 10°C) from *Arthrobacter psychrolactophilus* strain F2, which was overexpressed in *E. coli*, for the production of trisaccharides from lactose was also tested for applications in the food industry (Tomoyuki et al. 2007). Some strains contain isoenzymes (C2-2-1 and C2-2-2) such as an *Arthrobacter* strain (Karasová et al. 2002), which was found – as a first example – being able to catalyze transglycosylation reactions in the cold.

Lipases. Cold-active lipases (triacylglycerol acylhydrolases, EC3.1.1.3) were isolated from many psychrophilic strains and can have many industrial applications such as additives for detergents, additives in food products, in bioremediation, and in molecular biology (Joseph et al. 2007, 2008). Strains of *Acinetobacter*, *Achromobacter*, *Moraxella*, *Psychrobacter*, *Pseudoalteromonas*, *Serratia*, and others are lipase producers. An *Aeromonas* strain produces a cold-active lipase (Lee et al. 2003), and fungal lipase producers such as *Candida antarctica*, *Geotrichum*, and *Aspergillus* sp. were described. From 137 anaerobic strains isolated from soils in Schirmacher Oasis, Antarctica, 49 isolates showed lipolytic activity on Tween-agar medium (Ramteke et al. 2005). *Psychrobacter okhotskensis* was isolated from Okhotsk seawater (Yumoto

et al. 2003) and is a producer of cold-active lipases. These cold-active enzymes have a larger K coefficient and high efficiency down to temperatures of zero degree; they are inactivated by raising the temperature. They are used in the food industry and chemical industry; the latter utilizes them for catalyzing reactions with compounds which are unstable at higher temperatures (Suzuki et al. 2001), for example, lipases and esterases from *Pseudomonas* sp. strain B11-1. Many detergents contain a mixture of proteases, lipases, and amylases (Ohgiya et al. 1999). Some lipases can be used to remove fatty stains from various textiles (Araújo et al. 2008). Lipases can also be used for the synthesis of biopolymers, biodiesel, pharmaceuticals, and certain aromatic products (Joseph et al. 2007). The authors reported also that lipases from psychrophilic and psychrotolerant strains can be used in cold environments for the bioremediation areas contaminated by oil and grease, as well as in the detergent industry. From deep-sea sediments of the Pacific ocean, *Photobacterium frigidiphilum*, a lipolytic psychrophilic bacterium, was isolated (Seo et al. 2005). Some yeasts such as *Pichia lynferdii* strain Y-7723 produce a cold-adapted lipase (Kim et al. 2010). Lipases have a wide range of applications reviewed by Joseph et al. (2007, 2008), such as aryl aliphatic glycolipids, synthesis of fine chemicals, production of fatty acids, interesterification of fats, detergent additives, synthesis of biodiesel, removal of hydrocarbons, oils, and lipidic pollutants. Other uses are in the food industry and concern the improvement of food structure and gelling of fish meat (Cavicchioli and Siddiqui 2004). Nielsen et al. (1999) isolated two rather thermotolerant lipases A and B, with uses in the textile industry for the removal of waxes and lipids from fibers. The lipases obtained from microorganisms, which are used in different detergent formulations, are covered by many patents issued for industrial companies (Hasan et al. 2010). Lipase B from *C. antarctica* was immobilized onto epoxy-activated macroporous poly(methyl methacrylate) Amberzyme beads and on nanoparticles, in order to improve contact with the substrate and the reaction activity for polycondensation (Chen et al. 2008a). The enzyme was quickly adsorbed on the polystyrene porous particles (Chen et al. 2008b).

Pectinases. Several cold-adapted yeasts strains were isolated from the soil of Hokkaido Island (Japan), which were taxonomically affiliated with *Cryptococcus cylindricus*, *Mrakia frigida*, and *Cystofilobasidium capitatum*. The strains showed pectinolytic activity at temperatures less than 5°C and can be used for the production of pectinolytic enzymes (pectin methylesterase EC3.1.1.11; endopolygalacturonase EC3.2.1.15) for the clarification of fruit juices at low temperatures (Nakagawa et al. 2004), improving at the same time the filterability and extractability of the juice.

Malate dehydrogenases. A malate dehydrogenase was purified from *Flavobacterium frigidimaris* KUC1 and characterized by Oikawa et al. (2005). It contains lower

amounts of proline and arginine residues compared to other malate dehydrogenases and is dependent on NAD(P)+. The enzyme looses its activity at 55°C within 30 min of incubation. The enzyme can be used for producing malate at low temperatures.

Dextranases. An important problem in the sugar industry is the removal of dextran, a high-molecular-weight polymer of D-glucose, which can lower the recovery of sugar, interfere with material processing and lead to a poor quality of the final product. Bacterial cold-active dextranases can resolve this problem at low temperatures of about 4°C (Doaa Mahmoud and Wafaa Helmy 2009), such as the dextranase from psychrophilic strain *Streptomyces anulatus*.

β-Amylases. The need for cold-active amylases (EC3.2.1.1) and related starch hydrolyzing enzymes, especially for obtaining sweeteners such as palatinose, a disaccharide of glucose and fructose, and cyclodextrin, was reported (Rendleman 1996).

Phytases. A cold-active phytase is produced very efficiently by the Antarctic strain *Cryptococcus laurentii* AL 27 (Pavlova et al. 2008). Phytase is an enzyme which catalyzes the conversion of undigestible phytate to phosphorylated myo-inositol derivatives and inorganic phosphate, which are digestible. Its applications are in the fields of animal food additives and the pharmaceutical industry.

Xylanases. Xylanase from the yeast *Cryptococcus albidus*, isolated from Antarctica, is a glycoprotein; its structure was investigated by mass spectroscopy (Amoresano et al. 2000). The xylanases hydrolyze the heteropolysaccharide xylane (a hemicellulose containing a backbone chain of β-1,4-linked xylanopyranoside residues) and have found wide applications, e.g. improvement of maceration processes, clarification of juices, improvement of filtration efficiency, maceration of grape skins in wine technology, reducing viscosity of coffee extracts, improve drying and lyophilization processes, improving the elasticity of dough and bread textures. Xylanases can also be used to degrade xylane from agricultural wastes in order to obtain energy from biomass. Hydrolyzing xylane from the cell walls of plants at low temperatures will allow energy savings and the production of more accessible feedstock (Lee et al. 2006). Furthermore, xylanases are used for the pulping process in the paper industry and for biobleaching (Beg et al. 2001), thereby reducing the use of alkali. They also improve energy consumption in the textile industry, being used in the microbiological retting of textile materials, which replaces chemical retting. They are useful for obtaining fermentation products, bioethanol, and other chemicals as well as improving the separation of starch and gluten in the starch industry. The glycoside hydrolase family 8 xylanases can be used in baking processes in

order to improve the flexibility of dough and product quality (Collins et al. 2006).

β-Mannanase. β-Mannanase (EC3.2.1.78) was isolated from *Flavobacterium* sp. and showed good activity at 4°C; it can be used to decrease the viscosity in food products (Zakaria et al. 1998).

Nitrile hydratase. Companies such as Nitrochemicals developed many years ago the production of acrylamide with the help of *Rhodoccus* sp. strain N774 (Kobayashi et al. 1992), which produces the enzyme nitrile hydratase (EC4.2.1.84).

Cellulases. Some cellulases are used in bleaching and bio-stoning of textile material (Gomes and Steiner 2004), and alkaline cellulases used in detergents are active toward amorphous cellulose (Ito et al. 1989).

Trehalose. In agriculture, trehalose-producing systems can be used for reducing crop losses due to the lower temperatures with the help of genetic engineering (Phadtare and Inouye 2008).

EPSs. Extracellular polymeric substances released by microorganisms, which promote the formation of biofilms and have presumably protective roles. They can be used in the chemical industry to produce biodegradable plastic materials. Several strains with potential for this type of production were investigated as well as the conditions for production, such as temperature, pressure and pH (Marx et al. 2009). The authors reviewed useful microorganisms isolated from cold Antarctic environments, e.g., *Morixella*, *Psychrobacter*, and *Aeromonas* from polar waters, and psychrotolerants such as *Pseudomonas* and *Photobacterium*. The strains showed a good production of EPS at −4°C to −10°C and resistance under high-pressure conditions between 1 and 200 atm.

Medicinal uses. Besides improving the quality of foods, antifreeze proteins can also improve the preservability of human organs for transplants, for example livers (Kawahara 2008). Frisvad (2008a) reviewed bioactive products from cold-adapted fungi, such as griseofulvin and cycloaspeptide A from *Penicillium soppi* and *P. lanosum* from cold soils; the latter compound can be used as an antimalarial product. Cycloaspeptides were found so far only in cold-adapted fungi.

PUFAs. PUFAs are produced by many different organisms, for instance by strains such as *S. frigidimarina* (Bowman et al. 1997). They can be used as food supplements and medicinal products. Russell and Nichols (1999) showed that the bacterial PUFA-producing strain cannot compete with the fungal PUFA-producing strain, but can be an alternative for feedstock in the food chains used in aquaculture.

Biomining. The biomining industry is developing processes at low temperatures in three-phase systems: the solid phase, which is represented by the mineral ore; a liquid phase, which contains the microorganisms and nutrients; and the gaseous phase (Rossi 1999). Such a process can be performed in stirred tank reactors or in airlift reactors. The process was used for the release and recovery of copper from sulfide minerals, of uranium and for the pretreatment of gold ores (Ovalle 1987).

Bioremediation. Psychrophilic strains can be used to degrade the organic pollutants from soils and waters at low temperatures. Many strains possessing biodegrading properties were isolated from polar and alpine areas, from soils and waters, but more research of some aspects is required, such as the stability of the bacterial community, the accessibility of the pollutant for microorganisms, and the low removal rate (Margesin and Schinner 2001). Petroleum spills can produce catastrophic damages and their cleanup is an important goal. Petroleum is a complex mixture of water-soluble and -insoluble compounds (linear cyclic alkanes, aromatic hydrocarbons, paraffin, asphalt, and waxy oils; Brakstad 2008), being very hard to degrade. About 200 bacterial, cyanobacterial, fungal, and algal genera possess the capacity to do it (Prince 2005). The main bacterial genera able to degrade petroleum are *Acinetobacter*, *Arthrobacter*, *Colwellia*, *Cytophaga*, *Halomonas*, *Marinobacter*, *Marinomonas*, *Pseudoalteromonas*, *Oleispira*, *Rhodococcus*, and *Shewanella* (Brakstad 2008). Both anaerobic and aerobic degradation are possible and bioremediation can occur by stimulation of the local hydrocarbon degraders (using dispersants and nutrients) and less by bioaugmentation (inoculation of cultures of hydrocarbonoclastic bacteria; Margesin and Schinner 1999). *Dietzia psychralkaliphila* can grow on defined culture media containing *n*-alkenes as sole carbon source (Yumoto et al. 2002). The strain *Rhodococcus* sp. Q15 is able to degrade short- and long-chain aliphatic alkenes from diesel fuel at low temperatures of about 5°C (Whyte et al. 1998). Two strains of *Rhodococcus*, *R. ruber*, and *R. erythrococcus*, were used by Russian researchers for the product "Rhoder," which is applied for the removal of oil pollution (Murygina et al. 2000). Some strains isolated from alpine soils are able to degrade phenol and phenolic compounds (bacteria such as *Rhodococcus* spp., *Arthrobacter psychrophenolicus*, and *Pseudomonas*; yeasts such as *Rhodotorula psychrophenolica*, *Trichosporon dulcitum*, and *Leucosporidium watsoni*) and hydrocarbons from oil at low temperatures (Margesin 2007; Margesin et al. 2007), even though a complete biodegradation cannot be obtained. Polychlorophenols are toxic and persistent pollutants which are used as biocidal wood preservatives (Langwaldt et al. 2008). The authors list several genera such as *Ralstonia*, *Burkholderia*, *Arthrobacter*, *Rhodococcus*, *Mycobacteria*, and anaerobes such as *Desulfomonile* and *Desulfitobacterium*, which are able to degrade polychlorophenol compounds at low temperatures.

Polaromonas sp. strain JS666 is an isolate which can grow on *cis*-1,2-dichloroethene as carbon source; the investigation of its genome showed genes for the metabolism of aromatic compounds, alkanes, alcohols and others (Mattes et al. 2008). Another strain, *Polaromonas naphthalenivorans*, was isolated from a contaminated freshwater environment and is capable of degrading naphthalene (Jeon et al. 2004). The isolation of the genes for naphthalene dehydrogenase from cold environments was reported (Flocco et al. 2009). For bioremediation, the genus *Shewanella*, which can use a wide range of electron acceptors, is important, since many members of this group show capabilities for degrading several pollutants. The genus showed the possibility to be used in bioremediation of radionuclide and reduction of elements such as Co, Hg, Cr, and As, as well as for the removal of organics such as halogenated compounds, e.g., tetrachloromethane or nitramine (an explosive contaminant), as reported in the review by Hau and Gralnick (2007). Many strains such as *Brevibacterium antarcticum* have demonstrated a polyresistance to heavy metals in high concentrations, resisting concentrations of Cu^{2+}, Hg^{2+}, and CrO_4, up to 6000 ppm (Tashyrev 2009). Several psychrophilic microorganisms are able to degrade natural organic polymers (starch, agar, and gelatin) such as *Flavobacterium limicola*, which was isolated from freshwater sediments (Tamaki et al. 2003).

Wastes and wastewater treatments. The anaerobic treatment of wastewaters in treatment plants, using expanded granular sludge bed reactors at temperatures of 5–10°C, looks very promising (Lettinga et al. 2001). A mixture of microorganisms such as *Methanobrevibacter* sp., *Methanosarcina* sp., and *Methanosaeta* sp. has been explored (Lettinga et al. 1999). The psychrophilic treatment of landfill leachates using anoxic/oxic biofilters appears to be a good solution to prevent water and soil pollution with such leachates containing organic matter and also heavy metals (Kalyuzhnyi et al. 2004). *Oleispira antarctica* is able to degrade hydrocarbons in cold marine waters (Yakimov et al. 2003). Aerobic treatment of wastewater in cold lagoons has been performed in Canada's cold areas with success (Smith and Emde 1999). The cold-adapted xylanases can be used for the hydrolysis of agricultural and food industry wastes. A selection of cold-active degrading microorganism for wastewater treatment was performed (Gratia et al. 2009), where *A. psychrolactophilus* Sp 31.3 was isolated, which had the desired characteristics and was used further.

Acid mine drainage is the result of oxidation of certain sulfide minerals by exposure to environmental conditions and the activity of microorganisms. For example, ores containing pyrite and chalcopyrites are oxidized in the presence of water and oxygen and form highly acidic, sulfate-rich drainage. Ferrous iron (Fe^{2+}) develops in the process, which can be re-oxidized by acidophilic bacteria and archaea to ferric iron (Fe^{3+}), and the sulfur is oxidized to sulfate. These oxidations and the

concomitant dissolution of sulfide minerals can take place in cold conditions, too. Sulfate reduction at low temperatures occurs with bacteria such as *Desulfofrigus*, *Desulfofaba*, *Desulfotalea*, and *Desulfovibrio* (Kaksonen et al. 2008). *Acidithiobacillus ferrooxidans* is also able to oxidize iron and sulfur compounds at low temperatures (Kaksonen et al. 2008).

Astrobiological models. A special theoretical application concerns astrobiology, since some scientists are considering the Antarctic a model of planet Mars or other planets, with respect to the low temperatures and water activity (Abyzov et al. 1998), and also a model of cold environments where certain microorganisms could possibly live (Deming 2007). At the same time, the protocols for sampling of ice and permafrost and their analysis can be used for the exploration of Mars, and could be relevant and helpful in the isolation of potential Martian microbiota and for the development of future protocols for the decontamination of extraterrestrial samples (Christner et al. 2005).

7 Conclusions

1. Psychrophilic and psychrotolerant microorganisms can be retrieved from very diverse environments – oceans and freshwater, hypersaline cold waters, sediments, soils, permafrost, ice, glaciers, cold deserts, alpine soils, lakes and snow, cold man-made environments, and some microecosystems. The microorganisms in ice layers constitute not real ecosystems, even if some activity at subzero temperatures was proven; instead, most of them are only opportunistic assemblages of mixtures of microorganisms brought together by air and water currents from other environments. The diversity of so-called cold environments is much greater than was thought initially, and many microenvironments can be distinguished. In addition, the cold-adapted members of microbiota can have different other adaptations to extreme conditions – resistance to high radiation, oligotrophy, adaptation to high pressures, and perhaps others.
2. Psychrophiles are found in all the three domains of life and have a very diverse taxonomic origin. The most frequent taxonomic groups in cold environments are *Alpha-*, *Beta-*, *Delta-*, and *Gamma-Proteobacteria*, the phylum *Cytophaga–Flavobacterium–Bacteriodetes*, and Actinobacteria. Together with prokaryotes (Archaea and Bacteria) numerous eukaryotes are present such as algae, yeasts, and fungi.
3. Special adaptations allowing life in the cold include membrane lipids with branched unsaturated fatty acids, proteins and enzymes with a more flexible 3D structure due to the reduction of the number of weak intramolecular bonds, reduction of salt bridges, reduction of aromatic interactions, density of charged surface residues, increased surface hydrophobicity, and increased clustering of

glycine residues. Special proteins are Csps, ice nucleation proteins and antifreeze proteins, which protect the structure of cells from the cold and from formation of ice crystals.

4. The molecular biology of psychrophiles showed that their enzymes have low activation energy requirements due to their structure discussed in the text and to the flexibility of near active sites domains, and that they are easily inactivated by higher temperatures.
5. The different possibilities of adaptation of the microorganisms, either psychrophiles or psychrotolerants, showed complicated mechanisms, combining adaptation features and environmental opportunities. Their adaptation mechanisms are thus much more flexible than we have thought, providing possibilities and strategies of survival in extreme conditions. There is evidence for survival of psychrophiles in such conditions for very long periods of time which suggests the possibility of survival of similar microorganisms on other planets.
6. Psychrophiles produce bioactive and useful compounds, especially enzymes, pharmaceuticals, biodegradable plastics, substances for medical care, agriculture, biomining and bioremediation of wastes; all being usable in low temperature conditions, which entails important energy savings.
7. More laboratory and field bioprospecting should be envisaged for the isolation and identification of appropriate microorganisms for psychrophilic biotechnology.

Acknowledgments

We thank Dr. D.S. Nichols and his co-workers for the kind permission to integrate their data on enzymes from psychrophilic microorganisms in the table of this chapter.

This work benefitted from the research funded by the Executive Unit for Funding of High Level Education and of Universities Scientific Research, Romania (U.E.F.I.S.C.S.U.) in the frame of contract nr 1, Europolar 2010.

References

Abyzov SS, Mitskevich IN, Poglazova MN, Barkov NI, Lipenkov VY, Bobin NE, Koudryashov BB, Pashkevich VM (1998) Antarctic ice sheet as a model in search of life on other planets. Adv Space Res 22:363–368

Allen MA, Lauro FM, Williams TJ, Burg D, Siddiqui KS, De Francisci D, Chong KW, Pilak O, Chew HH, De Maere MZ, Ting L, Katrib M, Ng C, Sowers KR, Galperin MY, Anderson IJ, Ivanova N, Dalin E, Martinez M, Lapidus A, Hauser L, Land M, Thomas T, Cavicchioli R (2009) The genome sequence of the psychrophilic archaeon, *Methanococcoides burtonii*: the role of genome evolution in cold adaptation. ISME J 3:1012–1035

Amoresano A, Andolfo A, Corsaro MM, Zocchi I, Petrescu I, Gerday I, Marino G (2000) Structural characterization of a xylanase from psychrophilic yeast by mass spectrometry. Glycobiology 10:451–458

Andersson RE, Hedlund CB, Jonsson U (1979) Thermal inactivation of a heat-resistant lipase produced by the psychrotrophic bacterium *Pseudomonas fluorescens*. J Dairy Sci 62:361–367

Araújo R, Casal M, Cavaco-Paulo A (2008) Application of enzymes for textile fibres processing. Biocatal Biotransform 26:332–349

Auborn KJ, Fan S, Rosen EM, Goodwin L, Chandraskaren A, Williams DE, Chen D, Carter TH (2003) Indole-3-carbinol is a negative regulator of estrogen. J Nutr 133(Suppl):2470S–2475S

Ayala-del-Río HL, Chain PS, Grzymski JJ, Ponder MA, Ivanova N, Bergholz PW, Di Bartolo G, Hauser L, Land M, Bakermans C, Rodrigues D, Klappenbach J, Zarka D, Larimer F, Richardson P, Murray A, Thomashow M, Tiedje JM (2010) The genome sequence of *Psychrobacter arcticus* 273-4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. Appl Environ Microbiol 76:2304–2312

Ayub ND, Tribelli PM, Lopez N (2009) Polyhydroxyalkanoates are essential for maintenance of redox state in the Antarctic bacterium *Pseudomonas* sp. 14-3 during low temperature adaptation. Extremophiles 13:59–66

Babalola OO, Kirby BM, Le Roes-Hill M, Cook AE, Craig CS, Burton SG, Cowan DA (2008) Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environ Microbiol 11:566–576

Bae E, Phillips GN Jr (2004) Structures and analysis of highly homologous psychrophilic, mesophilic, and thermophilic adenylate kinases. J Biol Chem 279:28202–28208

Bahrim G, Negoiță TG (2004) Effects of inorganic nitrogen and phosphorous sources on hydrolase complex production by the selected *Bacillus subtilis* Antarctic strain. Rom Biotechnol Lett 9: 1925–1932

Bahrim GE, Scântee M, Negoiță TG (2007) Biotechnological conditions of amylase and protease complex production and utilization involving filamentous bacteria. In: Annals Univers "Dunărea de Jos" Galați, Fasc VI Food Technol, pp 76–81

Bakermans C (2008) Limits for microbial life at subzero temperatures. In: Margesin R, Schinner R, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 17–28

Beg QK, Kapoor M, Mhajan L, Hoondal GS (2001) Microbial xylanases and their industrial applications – a review. Appl Microbiol Biotechnol 56:326–338

Benešova E, Markova M, Kralova B (2005) Alpha glucosidase and beta glucosidase from psychrophilic strain *Arthrobacter* sp. C2-2. Czech J Food Sci 23:116–120

Białkowska AM, Cieslinski H, Niowakowska KM, Kur J, Turkievich M (2009) A new beta-galactosidase with a low temperature optimum isolated from the Antarctic *Arthrobacter* sp. 20B: gene cloning, purification, characterization. Arch Microbiol 191:825–835

Blees J, Wenk C, Niemann C, Schubert C, Zopfi J, Veronesi M, Simona M, Lehmann M (2010) The isotopic signature of methane oxidation in a deep south-alpine lake Geophysical Research Abstracts 12, EGU2010–788

Bowman JP (2008) Genomic analysis of psychrophilic prokaryotes. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 265–284

Bowman JP, McCammon SA, Nichols DS, Skerratt JH, Rea SM, Nichols PD, McMeekin TA (1997) *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with

the ability to produce eicosapentaenoic acid (20:5 omega 3) and grow anaerobically by dissimilatory Fe(III) reduction. Int J Syst Bacteriol 47:1040–1047

Bowman JP, Gosink JJ, McCammon SA, Lewis TE, Nichols DS, Nichols PD, Skeratt JH, Staley JT, McMeekin TA (1998) *Colwellia demingiae* sp. nov., *Colwellia hornerae* sp. nov., *Colwellia rossensis* sp. nov. and *Colwellia psychrotropica* sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22:6ω3) Int J Syst Bacteriol 48:1171–1180

Bozal N, Montes MJ, Tudela E, Jimenez F, Guinea J (2002) *Shewanella frigidimarina* and *Shewanella livingstonensis* sp. nov. isolated from Antarctic coastal areas. Int J Syst Evol Microbiol 52:195–205

Brakstad OG (2008) Natural and stimulated biodegradation of petroleum in cold marine environments. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 389–428

Breezee J, Cady N, Staley JT (2004) Subfreezing growth of the sea ice bacterium *Psychromonas ingrahamii*. Microbial Ecol 47:300–304

Brenchley JE (1996) Psychrophilic microorganisms and their cold-active enzymes. J Ind Microbiol Biotechnol 17:432–437

Busse H-J, Denner EBM, Buczolits S, Salkinoja-Salonen M, Bennasar A, Kämpfer P (2003) *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. Int J Syst Evol Microbiol 53:1253–1260

Cavicchioli R (2006) Cold adapted Archaea. Nat Rev Microbiol 4:331–343

Cavicchioli R, Siddiqui KS (2004) Cold-adapted enzymes. In: Paney A, Webb C, Socol CR, Larroche C (eds) Enzyme technology. Asia Tech Publishers, New Delhi, pp 615–638

Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low temperature extremophiles and their applications. Curr Opin Biotechnol 13:253–261

Chen B, Hu J, Miller EM, Xie W, Cai M, Gross RA (2008a) *Candida antarctica* lipase B chemically immobilized on epoxy-activated micro- and nanobeads: catalysts for polyester synthesis. Biomacromolecules 9:463–470

Chen B, Miller ME, Gross RA (2008b) Immobilization of *Candida antarctica* lipase B on porous polystyrene resins: protein distribution and activity. In: Polymer biocatalysis and biomaterials II, ACS symposium series, vol 999. American Chemical Society, Washington, DC, pp 165–177

Chintalapati S, Kiran MD, Shivaji S (2004) Role of membrane lipid fatty acids in cold adaptation. Cell Mol Biol 50:631–642

Christner BC (2002) Incorporation of DNA and protein precursors into macromolecules by bacteria at −15°C. Appl Environ Microbiol 68:6435–6438

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2003a) Bacterial recovery from ancient glacial ice. Environ Microbiol 5:433–436

Christner BC, Kvitko BH, Reeve JN (2003b) Molecular identification of bacteria and eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177–183

Christner BC, Mikucki JA, Foreman CM, Denson J, Priscu JC (2005) Glacial ice cores: a model system for developing extraterrestrial decontamination protocols. Icarus 174:572–584

Christner BC, Skidmore ML, Priscu JC, Tranter M, Foreman C (2008) Bacteria in subglacial environments. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 51–71

Cohen Z (1990) The production potential of eicosapentaenoic acid and arachidonic acid of the red algae *Porphyridium cruentum*. J Am Oil Chem Soc 67:916–920

Collins T, Dutron A, Georis J, Genot B, Dauvrin T, Arnaut F, Gerday C, Feller G (2006) Use of glycoside hydrolase family 8 xylanases in baking. J Cereal Sci 43:79–84

Collins T, Roulling F, Piette F, Marx JC, Feller G, Gerday C, D'Amico S (2008) Fundamentals of cold adapted enzymes. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 211–228

Copeland A, Lucas S, Lapidus A, Barry K, Detter JC, Glavina del Rio T, Hammon N, Israni S, Dalin E, Tice H, Pitluck S, Fredrickson JK, Kolker E, McCuel LA, DiChristina T, Nealson KH, Newman D, Tiedje JM, Zhou J, Romine MF, Culley DE, Serres M, Chertkov O, Brettin T, Bruce D, Han C, Tapia R, Gilna P, Schmutz J, Larimer F, Land M, Hauser L, Kyrpides N, Mikailova N, Richardson P (2006) Complete sequence of *Shewanella frigidimarina* NCIMB 400. Submitted (Aug. Sept. 2006). Released 09/14/2006 by the DOE Joint Genome Institute

Cotârleț M, Negoiță TG, Bahrim G, Stougaard P (2008) Screening of polar streptomycetes able to produce cold-active hydrolytic enzymes using common and chromogenic substrates. Rom Biotechnol Lett 13:69–80 [special issue, edited for Int Conf Ind Microbiol Appl Biotechnol]

Cowan DA, Casanueva A (2007) Stability of ATP in Antarctic mineral soils. Polar Biol 30:1599–1603

D'Amico S, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7:385–389

de los Ríos A, Grube M, Sancho LG, Ascaso C (2007) Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. FEMS Microbiol Ecol 59:386–395

De Prada P, Loveland-Curtze J, Brenchley JE (1996) Production of two extracellular alkaline phosphatases by a psychrophilic *Arthrobacter* strain. Appl Environ Microbiol 62:3732–3738

Deming JW (2007) Life in ice formations at very cold temperatures. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 133–145

Di Prisco G (2007) Lake Vostok and subglacial lakes of Antarctica: do they host life? In: Gerday C, Glansdorff F (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 145–154

Doaa Mahmoud AR, Wafaa Helmy A (2009) Application of cold-active dextranase in dextran degradation and isomaltotriose synthesis by micro-reaction technology. Aust J Basic Appl Sci 3:3808–3817

Feller G (2007) Life at low temperatures: is disorder the driving force? Extremophiles 11:211–216

Feller G, Gerday C (1997) Psychrophilic enzymes: molecular basis of cold-adaptation. Cell Mol Life Sci 53:830–841

Feller G, Le Bussy O, Gerday C (1998) Expression of psychrophilic genes in mesophilic hosts: assessment of the folding state of a recombinant α-amylase. Appl Environ Microbiol 64: 1163–1165

Finster K (2008) Anaerobic bacteria and archaea in cold ecosystems. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 103–119

Flocco CG, Newton C, Gomes M, MacCormack W, Smalla K (2009) Occurrence and diversity of naphthalene dioxygenase genes in soil microbial communities from the maritime Antarctic. Environ Microbiol 11:700–714

Frisvad JC (2008a) Cold adapted fungi as source of valuable Metabolites. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 381–388

Frisvad JC (2008b) Fungi in cold environment. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 137–156

Galante YM, Formantici C (2003) Enzyme applications in detergency and in manufacturing industries. Curr Org Chem 7:1399–1422

Ganzert L, Jurgens G, Münster U, Wagner D (2007) Methanogenic communities in permafrost-affected soils of the Laptev Sea Coast, Siberian Arctic, characterized by 16S rRNA gene fingerprints. FEMS Microbiol Ecol 59:476–488

Georlette D, Damien B, Blaise V, Depiereux E, Uversky VN, Gerday C, Feller G (2003) Structural and functional adaptations to extreme temperatures in psychrophilic, mesophilic and thermophilic DNA ligases. J Biol Chem 278:37015–37023

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

Gianese G, Argos P, Pascarella S (2001) Structural adaptation of enzymes to low temperatures. Prot Eng 14:141–148

Giaquinto L, Curmi PMG, Siddiqui KS, Poljak A, DeLong E, DasSarma S, Cavicchioli R (2007) Structure and function of cold shock proteins in archaea. J Bacteriol 189:5738–5748

Gilichinsky DA (2002) Permafrost. In: Bitton G (ed) Encyclopedia of environmental microbiology. John Wiley & Sons, New York, pp 2367–2385

Gilichinsky D, Vishnivetskaya T, Petrova M, Spirina E, Mamkyn V, Rivkina E, (2008) Bacteria in Permafrost. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 83–102

Gomes J, Steiner W (2004) The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol 42:223–235

Goto S, Sugiyama J, Iizuka HA (1969) A taxonomical study of Antarctic yeasts. Mycologia 61:748–774

Gounot AM (1999) Microbial life in permanently cold soils. In: Margesin R, Schinner F (eds) Cold-adapted organisms. Springer, Berlin, Heidelberg, New York, pp 3–15

Gratia E, Weekers F, Margesin R, D'Amico S, Thonart P, Feller G (2009) Selection of a cold-adapted bacterium for bioremediation of wastewater at low temperatures. Extremophiles 13:763–768

Groudieva T, Kambourova M, Yusef H, Royter M, Grote R, Trinks H, Antranikian G (2004) Diversity and cold-active hydrolytic enzymes of culturable bacteria associated with Arctic sea ice, Spitzbergen. Extremophiles 8:475–488

Grzymski JJ, Carter BJ, DeLong EF, Feldman RA (2006) Comparative genomics of DNA fragments from six Antarctic marine planktonic bacteria. Appl Environ Microbiol 72:1532–1541

Guffogg SP, Thomas-Hall S, Holloway P, Watson K (2004) A novel psychrotolerant member of the hymenomycetous yeasts from Antarctica: *Cryptococcus watticus* sp. nov. Int J Syst Evol Microbiol 54:275–277

Hamamoto T (1993) Psychrophilic microorganisms from deep sea environments. Riken Rev 3:9–10

Hasan F, Shah AA, Javed S, Hameed A (2010) Enzymes used in detergents: lipases. African J Biotechnol 9:4836–4844

Hau HH, Gralnick JA (2007) Ecology and biotechnology of the genus *Shewanella.* Ann Rev Microbiol 61:237–258

Helmke E, Weyland H (2004) Psychrophilic versus psychrotolerant bacteria – occurrence and significance in polar and temperate marine habitats. Cell Mol Biol 50:553–561

Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu J, Laybourn-Parry J, Sattler B (2008) Glacial ecosystems. Ecol Monogr 78:41–67

Hollibaugh JT, Lovejoy C, Murray AE (2007) Microbiology in polar ocean. Oceanography 20: 140–147

Hou S, Saw JH, Lee KS, Freitas TA, Belisle C, Kawarabayasi Y, Donachie SP, Pikina A, Galperin MY, Koonin EV, Makarova KS, Omelchenko MV, Sorokin A, Wolf YI, Li QX, Keum YS, Campbell S, Denery J, Aizawa S, Shibata S, Malahoff A, Alam M (2004) Genome sequence of the deep-sea gamma-proteobacterium *Idiomarina loihiensis* reveals amino acid fermentation as a source of carbon and energy. Proc Natl Acad Sci USA 101:18036–18041

Hoyoux, IJ, Dubois P, Genicot S, Dubail F, Franc JM, Baise Ois E, Feller G, Gerday C (2001) Cold-adapted beta-galactosidase from the Antarctic psychrophile *Pseudoalteromonas haloplanktis*. Appl Environ Microbiol 67:1529–1535

Humphry DR, George A, Black GW, Cummings SP (2001) *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. Int J Syst Evol Microbiol 51:1235–1243

Huston AL (2008) Biotechnological aspects of cold adapted enzymes. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 347–364

Ishida Y, Tsuruta H, Tsuneta ST, Uno T, Watanabe K, Aizono I (1998) Characteristics of psychrophilic alkaline phosphatase. Biosci Biotechnol Biochem 62:2246–2250

Ito S, Shikata S, Ozaki K, Kawai S, Okamoto K, Inoue S, Takei A, Ohta Y, Satoh T (1989) Alkaline cellulases for laundry detergents production by *Bacillus* sp. KSM 635 and enzymatic properties. Agric Biol Chem 53:1275–1281

Ivanova EP, Gorshkova NM, Bowman JP, Lysenko AM, Zhukova NV, Sergeev AF, Mikhailov VV, Nicolau DV (2004) *Shewanella pacifica* sp. nov., a polyunsaturated fatty acid-producing bacterium isolated from sea water. Int J Syst Evol Microbiol 54:1083–1087

Jeon CO, Park W, Ghiorse WC, Madsen EL (2004) *Polaromonas naphthalenivorans* sp. nov., a naphthalene-degrading bacterium from naphthalene-contaminated sediment. Int J Syst Evol Microbiol 54:93–97

Joseph B, Ramteke PW, Thomas G, Shrivastava N (2007) Standard review. Cold-active microbial lipases: a versatile tool for industrial applications. Biotechnol Mol Biol Rev 2:39–48

Joseph B, Ramteke PW, Thomas G (2008) Cold active microbial lipases: some hot issues and recent developments. Biotechnol Adv 26:457–470

Junge K, Eicken, H, Deming JW (2003) Motility of *Colwellia psychrerythraea* strain 34H at subzero temperatures. Appl Environ Microbiol 69:4282–4284

Kaksonen AH, Dopson M, Karnachuk O, Tuovinen OH, Puhakka JA (2008) Biological iron oxidation and sulfate reduction in the treatment of acid mine drainage at low temperatures. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 429–454

Kalyuzhnyi SV, Gladchenko M, Epov A (2004) Combined anaerobic-aerobic treatment of landfill leachates under mesophilic, submesophilic and psychrophilic conditions. Water Sci Technol 48:311–318

Karasová P, Spiwok V, Malá Š, Králová B, Russel NJ (2002) Beta-galactosidase activity in psychrotrophic microorganisms and their potential use in food industry. Czech J Food Sci 20:43–47

Karr EA, Sattley WM, Jung DO, Madigan MT, Achenbach LA (2003) Remarkable diversity of phototrophic purple bacteria in permanently frozen Antarctic lake. Appl Environ Microbiol 69:4910–4914

Karr EA, Ng JM, Belchik SM, Sattley WM, Madigan MT, Achenbach LA (2006) Biodiversity of methanogenic and other archaea in the permanently frozen lake Fryxwell, Antarctica. Appl Environ Microbiol 72:1662–1666

Katayama T, Tanaka M, Moriizumi J, Nakamura T, Brouchkov A, Douglas TA, Fukuda M, Tomita F, Asano K (2007) Phylogenetic analysis of bacteria preserved in a permafrost ice wedge for 25,000 years. Appl Environ Microbiol 73:2360–2363

Kato Y, Sakala RM, Hayashidani H, Kiuchi A, Kaneuchi C, Ogawa M (2000) *Lactobacillus algidus* sp. nov., a psychrophilic lactic acid bacterium isolated from vacuum-packaged refrigerated beef. Int J Syst Evol Microbiol 50:1143–1149

Kawahara H (2008) Cryoprotectant and ice binding proteins. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 229–246

Khachane AN, Timmis KN, Martins dos Santos VAP (2005) Uracil content of 16S rRNA of thermophilic and psychrophilic prokaryotes correlates inversely with their optimal growth temperatures. Nucleic Acids Res 33:4016–4022

Kim H-R, Kim I-H, Hou CT, Kwon K-Il, Shin B-S (2010) Production of a novel cold-active lipase from *Pichia lynferdii* Y-7723. J Agric Food Chem 58:1322–1326

Kitamoto D, Ikegami T, Suzuki GT Sasaki A, Takeyama Y, Idemoto Y, Koura N, Yanagishita H (2001) Microbial conversion of n-alkanes into glycolipid biosurfactants, annosylerythritol lipids by *Pseudozyma* (*Candida antarctica*). Biotechnol Lett 23:1709–1714

Knoblauch C, Sahm K, Jorgensen BB (1999) PsychrophiIic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov. and *Desulfotalea arctica* sp. nov. Int J Syst Bact 49:631–643

Kobayashi M, Nagasawa T, Yamada H (1992) Enzymatic synthesis of acrylamide manufacturing process using microorganisms. Trends Biotechnol 10:402–408

Krembs C, Deming JW (2008) The role of exopolymers in microbial adaptation to sea ice. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 247–286

Krishnan KP, Sinha RK, Krishna K, Nair S, Singh SM (2009) Microbially mediated redox transformations of manganese (II) along with some other trace elements: a study from Antarctic lakes Polar Biol 32:1765–1778

Kulakova L, Galkin A, Kurihara T, Yoshimura T, Esaki N (1999) Cold-active serine alkaline protease from the psychrotrophic bacterium *Shewanella* strain Ac10: gene cloning and enzyme purification and characterization. Appl Environ Microbiol 65:611–617

Kurihara T, Esaki N (2008) Proteomic studies of psychrophilic microrganisms. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 333–346

Kwon KK, Lee HS, Yang SH, Kim SJ (2005) *Kordiimonas gwangyangensis* gen. nov., sp. nov., a marine bacterium isolated from marine sediments that forms a distinct phyletic lineage (*Kordiimonadales* ord. nov.) in the '*Alphaproteobacteria*'. Int J Syst Evol Microbiol 55:2033–2037

Langwaldt JH, Tirola M, Puhakka JA (2008) Microbial adaptation to boreal saturated subsurface: implication in bioremediation of polychlorophenols. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 409–428

Larose C, Berger S, Ferrari C, Navarro E, Dommergue A, Schneider D, Vogel TM (2010) Microbial sequences retrieved from environmental samples from seasonal Arctic snow and meltwater from Svalbard, Norway. Extremophiles 14:205–212

Law BA, Goodenough PW (1995) Enzymes in milk and cheese production. In: Tucker GA, Woods LFJ (eds) Enzymes in food processing, 2nd edn. Blackie Academic and Professional, Bishopbriggs, Glasgow, UK, pp 114–143

Lee HK, Ahn MJ, Kwak SH, Song WH, Jeong BC (2003) Purification and characterization of cold active lipase from psychrotrophic *Aeromonas* sp. LPB 4. J Microbiol 41:22–27

Lee CC, Smith MR, Accinelli R, Williams TG, Wagschal KC, Wong D, Robertson GH (2006) Isolation and characterization of a psychrophilic xylanase enzyme from *Flavobacterium* sp. Curr Microbiol 52:112–116

Lees RS (1990) Impact of dietary fats on human health. Food Sci Technol 37:1–38

Lettinga G, Rebac S, van Lier J, Zeman G (1999) The potential of sub-mesophilic and/or psychrophilic anaerobic treatment of low strength wastewaters. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, pp 221–234

Lettinga G, Rebac S, Zeeman G (2001) Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnol 19:363–370

Liebner S, Wagner D (2007) Abundance, distribution and potential activity of methane oxidizing bacteria in permafrost soils from the Lena Delta, Siberia. Environ Microbiol 9:107–117

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

Loveland-Curtze J, Miteva V, Brenchley JE (2009) *Herminiimonas glaciei* sp. nov., a novel ultramicrobacterium from 3042 m deep Greenland glacial ice. Int J Syst Evol Microbiol 59:1272–1277

Loveland-Curtze J, Miteva V, Brenchley J (2010) Novel ultramicrobacterial isolates from a deep Greenland icecore represent a proposed new species, *Chryseobacterium greenlandense* sp. nov. Extremophiles 14:61–69

Madigan MT, Jung DO (2003). Extremophiles. Worldbook Encyclopedia, Science Year 2004 Annual, pp 74–89

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

Margesin R, Schinner F (1999) Biodegradation of organic pollutants at low temperature. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, New York, pp 271–290

Margesin R, Schinner F (2001) Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. Appl Environ Microbiol 67:3127–3133

Margesin R, Schumann P, Spröer C, Gounot AM (2004) *Arthrobacter psychrophenolicus* sp. nov., isolated from an alpine ice cave. Int J Syst Evol Microbiol 54:2067–2072

Margesin R, Fonteyne PA, Schinner F, Sampaio JP (2007) *Rhodotorula psychrophila* sp. nov., *Rhodotorula psychrophenolica* sp. nov. and *Rhodotorula glacialis* sp. nov., novel psychrophilic basidiomycetous yeast species isolated from alpine environments. Int J Syst Evol Microbiol 57:2179–2184

Marx JG, Carpenter SD, Deming JW (2009) Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychrerythraea* strain 34H under extreme conditions. Can J Microbiol 55:63–72

Matsuyama H, Hirabayashi T, Kasahara H, Minami H, Hoshino T, Yumoto I (2006) *Glaciecola chathamensis* sp. nov., a novel marine polysaccharide-producing bacterium. Int J Syst Evol Microbiol 56:2883–2886

Mattes TE, Alexander AK, Richardson PM, Munk AC, Han CS, Stothard P, Coleman NV (2008) The Genome of *Polaromonas* sp. strain JS666: insights into the evolution of a hydrocarbon- and

xenobiotic-degrading bacterium, and feature of relevance to biotechnology. Appl Environ Microbiol 74:6405–6416

McCammon SA, Innes BH, Bowman JP, Franzmann PD, Dobson SJ, Holloway PE, Skerratt JH, Nichols PD, Rankin LM (1998) *Flavobacterium hibernum* sp. nov., a lactose-utilizing bacterium from a freshwater Antarctic lake. Int J Syst Bacteriol 4:1405–1412

Médigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin PN, Cheung F, Cruveiller S, D'Amico S, Duilio A, Fang G, Feller G, Ho C, Mangenot S, Marino G, Nilsson J, Parrilli E, Rocha EP, Rouy Z, Sekowska A, Tutino ML, Vallenet D, von Heijne G, Danchin A (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res 15:1325–1335

Methé BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, Moult J, Madupu R, Nelson WC, Dodson RJ, Brinkac LM, Daugherty SC, Durkin AS, DeBoy RT, Kolonay JF, Sullivan SA, Zhou L, Davidsen TM, Wu M, Huston AL, Lewis M, Weaver B, Weidman JF, Khouri H, Utterback TR, Feldblyum TV, Fraser CM (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci USA 102:10913–10918

Michaux C, Massant J, Kerff F, Frere J-M, Docquier J-D, Vandenberghe I, Samyn B, Pierrard A, Feller G, Charlier P, Van Beeumen J, Wouters J (2008) Crystal structure of a cold-adapted class C β-lactamase. FEBS J 275:1687–1697

Mikhailova G, Likhareva V, Khairullin RF, Lubenets NL, Rumsh LD, Demidyuk IV, Kostrov SV (2006) Psychrophilic trypsin-type protease from *Serratia proteamaculans*. Biochem Sci 71:563–570

Milne PJ, Hunt AL, Rostoll K, Van Der Walt JJ, Graz CJM (1998) The biological activity of selected cyclic dipeptides. J Pharm Pharmacol 50:1331–1337

Miteva V (2008) Bacteria in snow and glacier ice. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 31–50

Mock T, Thomas DN (2008) Microalgae from Polar Regions: functional genomics and physiology. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 347–368

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Molec Biol Rev 70:222–252

Morita Y, Nakamura T, Hasan Q, Murakami Y, Yokoyama K, Tamiya E (1997) Cold-active enzymes from cold-adapted bacteria. J Am Oil Chem Soc 74:441–444

Moyer CL, Morita RY (2007) Psychrophiles and psychrotrophs. In: Encyclopedia of life sciences. John Wiley & Sons, Ltd. www.els.net

Murray E, Preston CM, Massana R, Taylor LT, Blakis A, Wu K, Delong EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl Environ Microbiol 64:2585–2595

Murygina V, Arinbasarov M, Kalyuzhnyi S (2000) Bioremediation of oil polluted aquatories and soils with novel preparation "Rhoder". Biodegradation 11:385–389

Naganuma T, Hua PN, Okamoto T, Ban S, Imura S, Kanda H (2005) Depth distribution of euryhaline halophilic bacteria in Suribati Ike, a meromictic lake in East Antarctica. Polar Biol 28:964–970

Nakagawa T, Nagaoka T, Taniguchi S, Miyaji T, Tomizuka N (2004) Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes. Lett Appl Microbiol 38:383–387

Nakagawa T, Ikehata R, Myoda T, Miyaji T, Tomizuka N (2007) Overexpression and functional analysis of cold-active β-galactosidase from *Arthrobacter psychrolactophilus* strain F2. Protein Expr Purif 54:295–299

Napolitano MJ, Shain DH (2004) Four kingdoms on glacier ice: convergent energetic processes boost energy levels as temperatures fall. Proc R Soc Lond B 271:S273–S276

Negoiță TG, Ştefanic G, Irimescu Orzan ME, Palanciuc V, Oprea G (2001a) Chemical and biological characterization of soils from the Antarctic East Coast. Polar Biol 24:565–571

Negoiță TG, Ştefanic G, Irimescu Orzan ME, Palanciuc V, Oprea G (2001b) Microbial chemical and enzymatic properties in Spitsbergen soils. Polar Forschung 71:41–46

Nguyen KT, Xiaowei H, Alexander DC, Li C, Gu J-Q, Mascio C, Van Praagh A, Mortin L, Chu M, Silverman JA, Brian P, Baltz RH (2010) Genetically engineered lipopeptide antibiotics related to A54145 and daptomycin with improved properties. Antimicrob Agents Chemother 54:1404–1413

Nichols DS, Bowman J, Sanderson C, Nichols CM, Lewis T, McMeekin T, Nichols PD (1999) Developments with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. Curr Opin Biotechnol 10:240–246

Nichols DS, Sanderson K, Buia A, van de Kamp J, Holloway P, Bowman JP, Smith M, Mancuso C, Nichols PD, McMeekin T (2002) Bioprospecting and biotechnology in Antarctica. In: Jabour-Green J, Haward M (eds) The Antarctic: past, present and future. Antarctic CRC Research Report #28. Hobart, pp 85–103

Nielsen TB, Ishii M, Kirk O (1999) Lipases A and B from the yeast *Candida antarctica*. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, New York, pp 48–61

Niemann H, Elvert M, Wand U, Samarkin VA, Lehmann MF (2010) First Lipid Biomarker evidence for aerobic methane oxidation in the water column of Lake Untersee (East Antarctica). Geophysical Research Abstracts Vol. 12, EGU2010–5921

Nogi Y (2008) Bacteria in deep sea: psychropiezophiles. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 73–82

Ohgiya S, Hoshino T, Okuyama H, Tanaka S, Ishizaki K (1999) Biotechnology of enzymes from cold adapted microorganisms, In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, New York, pp 17–35

Oikawa T, Yamamoto N, Shimoke K, Uesato S, Ikeuki T, Fujioka T (2005) Purification, characterization and overexpression of psychrophilic and thermolabile malate dehydrogenase from a novel Antarctic psychrotolerant *Flavobacterium frigidimaris* KUC 1. Biosci Biotechnol Biochem 59:2146–2154

Olivera NL, Sequeiros C, Nievas ML (2007) Diversity and enzyme properties of protease-producing bacteria isolated from sub-Antarctic sediments of Isla de Los Estados, Argentina. Extremophiles 11:517–526

Onofri S, Zucconi L, Selbmann L, de Hoog S, de los Ríos A, Ruisi S, Grube M (2007) Fungal associations at the cold edge of life in algae and cyanobacteria in extreme environments. In: Seckbach J (ed) Cellular origins, life in extreme habitats and astrobiology, vol 11. Springer, Netherlands, pp 735–757

Ovalle AW (1987) In-place leaching of a block carving mine. In: Cooper WC, Lagos GE, Ugarte G (eds) Copper 87, vol 3: Hydrometallurgy and electrometallurgy of copper. Universidad de Chile, Santiago, Chile, pp 17–37

Papaleo E, Pasi M, Riccardi L, Sambi I, Fantucci P, De Gioia L (2008) Protein flexibility in psychrophilic and mesophilic trypsins. Evidence of evolutionary conservation of protein dynamics in trypsin-like serine-proteases. FEBS Lett 582:1008–1018

Park SC, Kim MS, Baik KS, Kim EM, Rhee MS, Seong CN (2008) *Chryseobacterium aquifrigidense* sp. nov., isolated from a water-cooling system. Int J Syst Evol Microbiol 58:607–611

Parrilli E, Duillio A, Tutino ML (2008) Heterologous protein expression on psychrophilic hosts. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 365–380

Pavlova K, Gargova S. Hristozova T, Tankova Z (2008) Phytase from Antarctic yeast strain *Cryptococcus laurentiis* AL 27. Folia Microbiol 23:29–34

Pernthaler J, Glöckner FO, Unterholzner S, Alfreider A, Psenner R, Amann R (1998) Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. Appl Environ Microbiol 64:4299–4306

Perovich DK, Grenfell TC, Light B, Hobbs PV (2002) Seasonal evolution of the albedo of multiyear Arctic sea-ice. J Geophys Res 107(C10):8044. DOI: 10.1029/2000JC000438

Phadtare S, Inouye M (2008) Cold shock proteins. In: Margesin R, Schinner F, Marx JC, Gerday G (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 191–210

Prabagaran SR, Manorama R, Delile D, Shivaji S (2007) Predominance of *Roseobacter*, *Sulfitobacter*, *Glaciecola* and *Psychrobacter* in seawater collected from Ushuaia, Argentina, sub Antarctica. FEMS Microbiol Ecol 59:342–355

Price BP (2006) Microbial life in glacial ice and implications for a cold origin of life. FEMS Microbiol Ecol 59:217–231

Prince RC (2005) The microbiology of marine oil spills bioremediation. In: Olivier B, Margot M (eds) Petroleum microbiology. ASM Press, Washington, DC, pp 317–335

Priscu JC, Christner BC (2004) Earths icy biosphere. In: Bull AT (ed) Microbial diversity and bioprospecting. ASM Press, Washington, DC, pp 130–145

Rabus R, Ruepp A, Frickey T, Rattei T, Fartmann B, Stark M, Bauer M, Zibat A, Lombardot T, Amann J, Gellner K, Teeling H, Leuschner WD, Glockner F-O, Lupas AN, Amann R, Klenk H-P (2004) The genome of *Desulfotea psychrophila* a sulphate reducing bacterium from permanently cold Arctic sediment. Environ Microbiol 6:887–902

Raja A, Prabakaran P, Gjalakshmi P (2010) Isolation and screening of antibiotic producing actinomycetes and its nature from Rothang Hill soil against *Viridans Streptococcus* sp. Res J Microbiol 5:44–49

Ramaiah N (1994) Production of certain hydrolytic enzymes by psychrophilic bacteria from the Antarctic krill, zooplankton and seawater. In: Ninth Indian Expedition to Antarctica, Scientific Report, National Institute of Ocenography, Department of Ocean Development, Goa, India, Technical Publication 6:107–114

Ramteke PW, Joseph B, Kuddus M (2005) Extracellular lipases from anaerobic microorganism of Antarctic. Ind J Biotechnol 4:293–294

Rashidah A, Sabrina S, Ainihayati A, Shanmugapriya P, Nazalan N, Razip S (2007) Psychrophilic enzymes from the Antarctic isolates. In: Proceeedings of the international symposium Asian collaboration in IPY 2007–2008, Science Council of Japan, Tokyo 1st March 2007, National Institute of Polar Research, Tokio, Japan, pp 116–119

Raymond JA, Fritsen C, Shen K (2007) An ice-binding protein from an Antarctic sea ice bacterium. FEMS Microbiol Ecol 61:214–221

Rendleman JA Jr (1996) Enzymatic conversion of malto-oligosaccharides and maltodextrin into cyclodextrin at low temperature. Biotechnol Appl Biochem 24:129–137

Riley M, Staley JT, Danchin A, Wang TZ, Brettin TS, Hauser LJ, Land M, Thompson LS (2008) Genomics of an extreme psychrophile, *Psychromonas ingrahamii*. BMC Genomics 9:210

Rivkina E, Gilichinsky D, Wagener S, Tiedje J, McGrath J (1998) Biogeochemical activity of anaerobic microorganisms from buried permafrost sediments. Geomicrobiol J 15:187–193

Rivkina EM, Friedmann EI, McKay CP, Gilichinsky DA (2000) Metabolic activity of permafrost bacteria below the freezing point. Appl Environ Microbiol 66:3230–3233

Rodrigues-Diaz F, Ivanova N, He Z, Huebner M, Zhou J, Tiedje JM (2008) Architecture of thermal adaptation in an *Exiguobacterium sibiricum* strain isolated from 3 million year old permafrost: a genome and transcriptome approach. BMC Genomics 9:547

Rossi G (1999) Biohydrometallurgical processes and temperature. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, New York, pp 291–308

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

Russell NJ, Cowan DA (2005) Handling of psychrophilic microorganisms. In: Rainey FA, Oren A (eds) Extremophiles. Methods in Microbiology, vol 35. Elsevier, pp 371–393

Russell NJ, Nichols DS (1999) Polyunsaturated fatty acids in marine bacteria – a dogma rewritten. Microbiology 145:767–779

Rybalka N, Andersen RA, Kostikov I, Mohr KI, Massalski A, Olech M, Friedl T (2008) Testing for endemism, genotypic diversity and species concepts in Antarctic terrestrial microalgae of the *Tribonemataceae* (*Stramenopiles*, *Xanthophyceae*). Environ Microbiol Rep 11:554–565

Rysgaard S, Glud RN, Sejr MK, Blicher ME, Stahl HJ (2008) Denitrification activity and oxygen dynamics in Arctic sea ice. Polar Biol 31:527–537

Sanchez LA, Gomez FF, Delgado OD (2009) Cold-adapted microorganisms as a source of new antimicrobials. Extremophiles 13:111–120

Sattley WM, Madigan MT (2006) Isolation, characterization and ecology of cold-active, chemolithotrophic, sulfur-oxidizing bacteria from perennially ice-covered Lake Fryxell, Antarctica. Appl Environ Microbiol 72:5562–5568

Sattley WM, Madigan MT (2007) Cold-active acetogenic bacteria from surcial sediments of perennially ice-covered Lake Fryxell, Antarctica. FEMS Microbiol Lett 272:48–54

Saunders NFW, Thomas T, Curmi PMG, Mattick JS, Kuczek E, Slade R, Davis J, Franzmann PD, Boone D, Rusterholtz K, Feldman R, Gates C, Bench S, Sowers K, Kadner K, Aerts A, Dehal P, Detter C, Glavina T, Lucas S, Richardson P, Larimer F, Hauser L, Land M, Cavicchioli R (2003) Mechanisms of thermal adaptation revealed from the genomes of the Antarctic Archaea *Methanogenium frigidum* and *Methanococcoides burtonii*. Genome Res 13:1580–1588

Sælensminde G, Halskau Ø Jr, Jonassen I (2009) Amino acid contacts in proteins adapted to different temperatures: hydrophobic interactions and surface charges play a key role. Extremophiles 13:11–20

Säwström C, Laybourn-Parry J, Granéli W, Anesio AM (2007) Heterotrophic bacterial and viral dynamics in Arctic freshwaters: results from a field study and nutrient – temperature manipulation experiments. Polar Biol 30:1407–1415

Seo HJ, Bae SS, Lee J-H, Kim S-J (2005) *Photobacterium frigidiphilum* sp. nov., a psychrophilic, lipolytic bacterium isolated from deep-sea sediments of Edison Seamount. Int J Syst Evol Microbiol 55:1661–1666

Sheridan PP, Brenchley JE (2000) Characterization of a salt-tolerant family 42 beta-galactosidase from a psychrophilic Antarctic *Planococcus* isolate. Appl Environ Microbiol 66: 2438–2444

Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–433

Siddiqui KS, Poljak A, Guilhaus M, De Francisci D, Curmi PM, Feller G, D'Amico S, Gerday C, Uversky VN, Cavicchioli R (2006) Role of lysine versus arginine in enzyme cold-adaptation:

modifying lysine to homo-arginine stabilizes the cold-adapted alpha-amylase from *Pseudoalteromonas haloplanktis*. Proteins 1:486–501

Singh L, Ramana Venkata K (1998) Introduction. Isolation and characterization of psychrotrophic Antarctic bacteria from blue-green algal mats and their hydrolytic enzymes. In: Fourteenth Indian Expedition to Antarctica, Scientific Report, Depart Ocean Develop, Techn Public No 12, pp 199–206

Singh SM, Puja G, Bhat DJ (2006) Psychrophilic fungi from Schirmacher Oasis, East Antarctica. Curr Sci 90:1388–1392

Sizova M, Panikov N (2007) *Polaromonas hydrogenivorans* sp. nov., a psychrotolerant hydrogen-oxidizing bacterium from Alaskan soil. Int J Syst Evol Microbiol 57:616–619

Skidmore ML, Foght JM, Sharp MJ (2000) Microbial life beneath a high arctic glacier. Appl Environ Microbiol 66:3214–3220

Smith DW, Emde KME (1999) Effectiveness of wastewater lagoons in cold regions. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, New York, pp 235–256

Somkutl GA, Holsinger VH (1997) Microbial technologies in the production of low-lactose dairy foods. Food Sci Technol Int J 3:163–169

Sonan GK, Receveur-Brechot V, Duez C, Aghari N, Czjzek M, Haser R, Gerday C (2007) The linker region plays a key role in the adaptation to cold of the cellulase from an Antarctic bacterium. Biochem J 407:293–302

Spring S, Merkhoffer B, Weiss N, Kroppenstedt RM, Hippe H, Stackebrandt E (2003) Characterization of novel psychrophilic clostridia from an Antarctic microbial mat: description of *Clostridium frigoris* sp. nov., *Clostridium lacusfryxellense* sp. nov., *Clostridium bowmanii* sp. nov. and *Clostridium psychrophilum* sp. nov. and reclassification of *Clostridium laramiense* as *Clostridium estertheticum* subsp. *laramiense* subsp. nov. Int J Syst Evol Microbiol 53: 1019–1029

Ştefanic G (1994) Biological definition, quantifying method and agricultural interpretation of soil fertility. Rom Agric Res 2:107–116

Steven B, Briggs G, McKay CP, Pollard WH, Greer CW, Whyte LG (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. FEMS Microbiol Ecol 59:513–523

Suzuki T, Nakayama T, Kurihara T, Nishino T, Esaki N (2001) Cold active lipolytic activity of psychrotrophic *Acinetobacter* sp. strain no. 6. J Biosci Bioeng 92:144–148

Takeuchi N, Kohshima S (2004) A snow algal community on Tyndall glacier in the Southern Patagonia Icefield, Chile. Arct Antarct Alp Res 36:92–99

Tamaki H, Hanada S, Kamagata Y, Nakamura K, Nomura N, Nakano K, Matsumura M (2003) *Flavobacterium limicola* sp. nov., a psychrophilic, organic-polymer-degrading bacterium isolated from freshwater sediments. Int J Syst Evol Microbiol 53:519–526

Tashyrev OB (2009) The complex researches of structure and function of Antarctic terrestrial microbial communities. Ukr Antarctic J 8:343–357

Thomas-Hall S, Watson K (2002) *Cryptococcus nyarrowii* sp. nov., a basidiomycetous yeast from Antarctica. Int J Syst Evol Microbiol 52:1033–1038

Thomas-Hall S, Hall R, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K (2010) Cold-adapted yeasts from Antarctica and the Italian Alps – description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. Extremophiles 14:47–59

Tian F, Yu Y, Chen B, Li H, Yao YF, Gua XK (2009) Bacterial, archaeal and eukaryotic diversity in Arctic sediment as revealed by 16S rRNA and 18S rRNA gene clone libraries analysis. Polar Biol 32:93–103

Tkaczuk KL, Buinicki JM, Bialkowska A, Bielecki S, Turkievicz M, Cieslinski H, Kur J (2005) Molecular modelling of a psychrophilic beta-galactosidase. Biocat Biotransf 23:201–209

Tomoyuki N, Ikehata R, Myoda T, Miyaji T, Tomizuka N (2007) Overexpression and functional analysis of cold-active beta-galactosidase from *Arthrobacter psychrolactophilus* strain F2. Prot Expr Purif 54:295–299

Turkiewicz M, Pazgier M, Donachie SP, Kalinowska H (2005) Invertase and glucosidase production by the endemic Antarctic marine yeast *Leucosporidium antarcticum*. Pol Polar Res 26:125–136

Tutino ML, Duilio A, Parrilli R, Remaut E, Sannia G, Marino G (2001) A novel replication element from an Antarctic plasmid as a tool for the expression of proteins at low temperature. Extremophiles 5:257–264

Vishniac HS, Kurtzman CP (1992) *Cryptococcus antarcticus* sp. nov., and *Cryptococcus albidosimilis* sp. nov., basidioblastomycetes from Antarctic soils. Int J Syst Bact 42:547–553

Vorobyova E, Soina V, Gorlenko M, Minkovskaya M, Zalinova N, Mamukelashvili A, Gilichinsky D, Rivkina E, Vishnivetskaya T (1997) The deep cold biosphere: facts and hypothesis. FEMS Microbiol Rev 20:277–290

Voytek MA, Priscu JC, Ward BB (1999) The dicversity and abundance of ammonia oxidizing bacteria in lake of the McMurdo Dry Valley, Antarctica. Hydrobiologia 401:113–130

Wagner-Döbler I, Rheims H, Felske A, El-Ghezal A, Flade-Schröder D, Laatsch H, Lang S, Pukall R Tindall BJ (2004) *Oceanibulbus indolifex* gen. nov., sp. nov., a North Sea alphaproteobacterium that produces bioactive metabolites. Int J Syst Evol Microbiol 54:1177–1184

Wartiainen I, Hestnes AG, McDonald IR, Svenning Mette M (2006) *Methylocystis rosea* sp. nov., a novel methanotrophic bacterium from Arctic wetland soil, Svalbard, Norway (786°N). Int J Syst Microbiol 56:541–547

Wells LE (2008) Cold active viruses. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 157–176

Wharton RA Jr, McKay CP, Simmons GM Jr, Parker CB (1985) Cryoconite holes on glaciers. Bioscience 35:499–503

Whyte LG, Hawari J, Zhou E, Bourbonnere L, Inniss WE, Greer CW (1998) Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus*. Appl Environ Microbiol 64:2578–2584

Wierzchos J, de los Ríos A, Sancho LG, Ascaso C (2004) Viability of endolithic microorganisms in rocks from the McMurdo Dry Valleys of Antarctica established by confocal and fluorescence microscopy. J Microsc Oxford 216:57–61

Woo JH, Hwang YO, Kang SG, Lee HS, Cho JC, Kim SJ (2007) Cloning and characterization of three epoxide hydrolases from a marine bacterium, *Erythrobacter litoralis* HTCC2594. Appl Microbiol Biotechnol 76:365–375

Yakimov MM, Giuliano L, Gentile G, Crisafi E, Chernikova TN, Abraham W-R, Lünsdorf H, Timmis K.N, Golyshin PN (2003) *Oleispira antarctica* gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. Int J Syst Evol Microbiol 53:779–785

Yan BQ, Chen XL, Hou XY, He H, Zhou BC, Zhang YZ (2009) Molecular analysis of the gene encoding a cold-adapted halophilic subtilase from deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913: cloning, expression, characterization and function analysis of the C-terminal PPC domains. Extremophiles 13:725–733

Yang SH, Lee JH, Ryu JS, Kato C, Kim SJ (2007) *Shewanella donghaensis* sp. nov., a psychrotrophic, piezosensitive bacterium producing high levels of polyunsaturated fatty acid, isolated from deep-sea sediments. Int J Syst Evol Microbiol 57:208–212

Yu Y, Li H, Zeng Y, Chen B (2009) Extracellular enzymes of cold-adapted bacteria from Arctic sea ice, Canada Basin. Polar Biol 32:1539–1547

Yumoto I, Nakamura A, Iwata H, Kojima K, Kusumoto K, Nodasaka Y, Matsuyama H (2002) *Dietzia psychralkaliphila* sp. nov., a novel, facultatively psychrophilic alkaliphile that grows on hydrocarbons. Int J Syst Evol Microbiol 52:85–90

Yumoto I, Hirota K, Sogabe Y, Nodasaka Y, Yokota Y, Hoshino T (2003) *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. Int J Syst Evol Microbiol 53:1985–1989

Zachariassen KE, Lundheim R (1999) Application of antifreeze proteins. In: Margesin R, Schinner F (eds) Biotechnological applications of cold adapted organisms. Springer, Berlin, Heidelberg, pp 319–332

Zakaria MM, Ashiuchi M, Yamamoto S, Yagi T (1998) Optimization for beta-mannanase production of a psychrophilic bacterium, *Flavobacterium* sp. Biosci Biotechnol Biochem 62:655–660

Zakhia F, Jungblut AD, Taton A, Vincent WF, Wilmotte A (2008) Cyanobacteria in cold ecosystems. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, Heidelberg, pp 121–135