6.3 Diversity of Psychrophilic Bacteria from Sea Ice - and Glacial Ice Communities

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Introduction

Earth is primarily a cold, marine planet with 90% of the ocean's waters being at 5°C or lower. Frozen soils (permafrost), glaciers and ice sheets, polar sea ice, and snow cover make up 20% of the Earth's surface environments (Deming and Eicken 2007). A great diversity of microorganisms has been found in these habitats. However, only those that are adapted to life in the cold can be active in them and thus influence biogeochemical cycles.

Cold-adapted microbes are termed *psychrophiles* or cold-loving, having minimum, optimum and maximum growth temperatures at or below 0°C, 15°C, and 20°C, respectively or *psychrotolerant* (with growth maxima above 25°C but the capacity to grow to very low temperature (Morita 1975). Recently, additional definitions have been proposed, such as "moderate psychrophiles" with a minimum and maximum growth temperature at or below 0°C and 25°C (Helmke and Weyland 2004), "psychro-active" (organisms growing at or below −1°C, Laucks et al. 2005) and the terms "eurypsychrophile" and "stenopsychrophile" have been suggested (Caviocchiolli 2006 and references therein). Stenopsychrophile (formerly "true psychrophile") refers to a microorganism with a restricted growth-temperature range that cannot tolerate higher temperatures for growth. Eurypsychrophile (the formerly "psychrotolerant" or "psychrotroph") describes a microorganism that prefers permanently cold environments, but can also tolerate a wide range of temperatures reaching up into the mesophilic range (Caviociolli 2006). In this review we use the term *psychrophile* as a general term to describe a microorganism that grows in a cold environment. Specifically, we focus in this review on the diversity of psychrophiles found in the two major marine and terrestrial cold habitats for life on Earth – sea ice and glacier ice.

Marine psychrophiles play a globally significant role in biogeochemical cycling (Helmke and Weyland 2004). In the polar regions, they are important as processors of polar marine primary productivity (Legendre et al. 1992), which serves as the base for the entire polar food web, ultimately feeding krill, fish, whales, penguins, and seabirds. The rate of primary production varies greatly throughout Arctic and Antarctic marine waters and ice, and underlying environmental factors important to this process are investigated (Mock and Thomas 2005; Garneau et al. 2009; Kellogg and Deming 2009; Arrigo et al. 2010). Psychrophilic bacteria are of interest not only because they play important roles in organic carbon and elemental transformations throughout the polar food web (terrestrial and aquatic), but also because of their remarkable ability to thrive under extremely cold and salty conditions (Thomas and Dieckmann 2002). The enzymes and membranes that enable psychrophiles to live at low temperatures are of considerable interest for biotechnological and industrial applications (see Chap. 6.7 Psychrophilic Enzymes: Cool Responses to Chilly Problems).

Cold environments on Earth are extremely sensitive to global warming, especially those in the polar regions. As a result of increasing temperatures, the livelihood of larger organisms that perform critical ecological roles in the food chains of these cold environments (such as Polar Bears) is threatened. How microorganisms that also perform critical ecological roles might respond to these changes is an important and urgent question that requires attention (Kirchman et al. 2009). Furthermore, cold temperatures are prevalent in extraterrestrial environments, and especially in most outer planets and moons in our solar system. Therefore, from an astrobiological perspective, studying frigid environments on Earth can provide important clues and technology for understanding life that may exist elsewhere in our solar system, such as on Mars and Jupiter's moon, Europa.

In the following sections, we present what is known to date about the diversity of cultured and uncultured psychrophilic bacteria in sea ice and glacial ice and describe their habitats. We furthermore discuss briefly the adaptive strategies in *Psychrobacter ingrahamii*, a "true" psychrophile (i.e., stenopsychrophile) and discuss processes that could lead to extended longevity of microbes immured in glacial ice.

Sea Ice Communities

Ecological Aspects

One needs to appreciate the physics and chemistry of sea ice in order to understand the ecology and adaptation mechanisms of the bacteria that live within. Sea ice is one of the largest habitats in polar oceans and plays a crucial role in structuring the whole polar ecosystem (Eicken 1992). At its maximum extent, the ice covers 13% of the Earth surface with the largest expanse occurring in the Southern ocean where, during the winter months, 20 million square kilometers are covered by ice. In recent years, Arctic sea ice has shown a dramatic decrease in thickness and extent due to increasing overall temperatures in Arctic regions caused by global change (Stroeve et al. 2005; Serreze et al. 2007).

Seawater which typically contains about 34 g of dissolved salts and ions (mostly sodium, chloride, sulfate, magnesium, calcium and potassium) begins to freeze when temperatures drop below -1.86°C. Ice crystals begin to form and rise to the surface. These initial crystals (called frazil ice) are of varying shape, from plates to needles; and size, from $\leq$1.0 mm to 1.0 cm in length (Mock and Junge 2007 and references therein; also see Petrich and Eicken 2010 for an in-depth review on ice development, its micro and macrostructure).

Within hours, frazil ice crystals consolidate by wind and water motion to form loosely aggregated disks (called pancakes). After a few days of growth by accumulation of more and more ice crystals that form in the upper water column, pancakes can be several meters across and up to 50 cm thick. They freeze together forming a closed ice cover after 1–2 days (termed pack ice). As temperatures continue to decrease this pack ice thickens, not necessarily by the accumulation of more ice crystals, but by the growth of columnar ice at the ice–water interface. Columnar ice forms by the vertical elongation of frazil ice crystals. The proportion of frazil ice to columnar ice depends largely on the turbulence of the water in which it was formed. The more turbulent the water, the more frazil ice is usually found. Antarctic sea ice contains up to 80% frazil ice as it is formed under more turbulent conditions. In the Arctic, sea ice is formed under more calm conditions and contains up to 80% columnar ice. This difference is important for sea ice biology because frazil ice provides more habitable space for organisms than columnar ice (Spindler 1990).

When ice is formed from seawater, dissolved salts, air and other "impurities" in the seawater, including bacteria, inorganic and organic dissolved and particulate matter, are not incorporated into the freshwater ice crystals and instead are concentrated into a salty brine that persists as inclusions of pockets and channels within the ice or is released into the water below (Eicken 1992). These channels vary in size from a few micrometeres to several millimeters in diameter and represent the main habitat for all sea ice microorganisms (Fig. 6.3.1, Mock and Junge 2007).

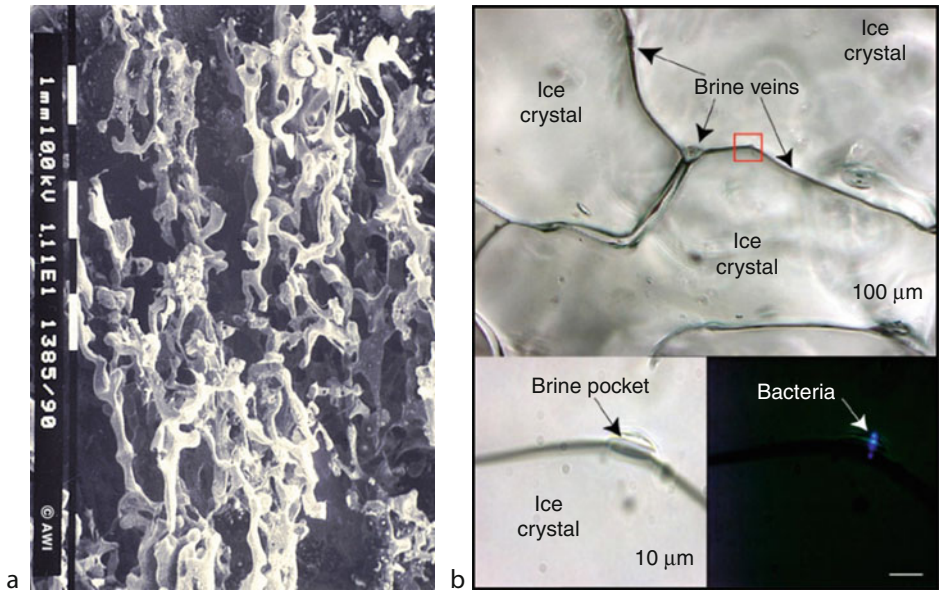

Fig. 6.3.1

(a) SEM picture of the brine channels system in columnar sea ice made visible by filling the system with epoxy resin under a vacuum. Picture by Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven, Germany (Junge and Mock 2007, reprinted with permission from Springer Verlag). (b) In situ microscopic images of (a) ice crystals and brine pockets and (b) detail of a brine pocket in (a) that harbors bacteria stained with the blue DNA stain DAPI (Adapted from Junge et al. 2001)

Temperature determines the volume of brine channels and the concentration of salts within them; with decreasing temperatures, brine volumes decrease and salt content (salinity) increases. Thus, the coldest ice contains brine channels with the saltiest brines, and overall fewer, smaller and less interconnected channels than warmer ice. Ice at the sea ice–air interface is usually colder than ice in contact with the underlying water resulting in vertical temperature-(salinity and brine volume) gradients throughout the ice (*Fig. 6.3.2*). The ice is considered permeable at temperatures above −5°C (with corresponding bulk melt salinities of 5‰ [in situ salinities of 90‰] and brine volume fractions above 5%). Below −5°C sea ice is effectively impermeable with no convective fluid flow occurring (Golden et al. 1998).

The majority of sea ice is ephemeral, with melting during the summer season releasing the community into the underlying water. Melting forms ponds on the surface of the ice (mostly in the Arctic), which develop their own unique ice-algae assemblages and microbial communities (Brinkmeyer et al. 2004). When melting continues due to increasing water temperatures and solar irradiance on top of the ice, the ice gets thinner and more porous, and the majority melts completely. If ice survives the summer, refreezing occurs during the following winter that makes the ice even thicker. The longevity of the ice depends on the geographic location, wind and ocean currents. Sea ice of northern Greenland and the Canadian archipelago can be several years old with an average thickness of 6–8 m. The ice in the Southern Ocean is considerably

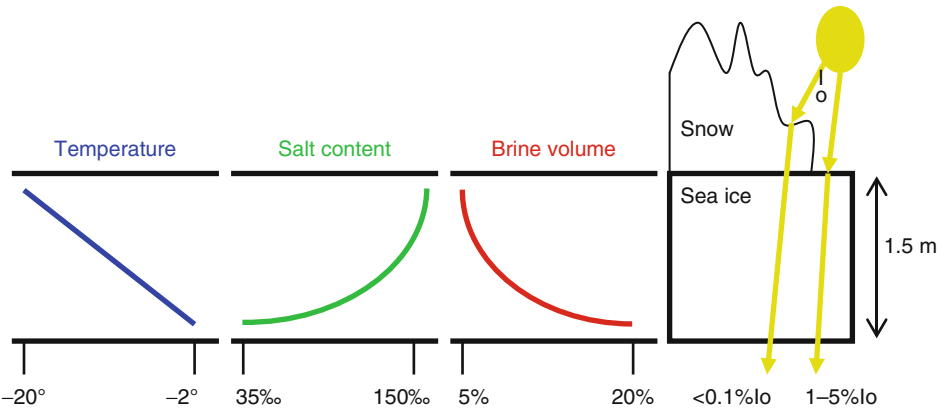

Fig. 6.3.2

Vertical gradients of temperature, salt content, brine volume and irradiance through sea ice. These general patterns may vary due to changes in temperature (Adapted from Mock and Junge 2007)

thinner with an average thickness of only 1 m. Such differences in physical properties of the ice also result in differences in the abundance, activity and composition of the microbial communities within (Mock and Junge 2007).

Psychrophilic bacteria that grow in sea ice must be adapted to cope with these ever-changing physical and chemical conditions of their environment after they are introduced into the ice as it forms. Along with salts, other dissolved impurities and particulate material, the cells are excluded from the newly formed ice crystals and reside in the brine channels (Junge et al. 2001). However, they may also become incorporated into the ice when attached to algae that adhere to ice crystals when they rise through the water as it freezes in autumn (Ackley and Sullivan 1994).

In the following sections we review the state of knowledge on the phylogenetic diversity of psychrophilic sea ice bacteria both from cultivation studies and whole community analyses. A discussion on the discovery of gas-vacuolate sea ice bacteria and the biogeography of sea ice bacteria follows. We end with a brief description of *Psychrobacter ingrahamii* as a representative of the stenopsychrophilic sea ice bacteria.

Phylogenetic Diversity of Psychrophilic Sea Ice Bacteria

Annually during spring and summer, extensive microbial communities (termed SIMCO for sea ice microbial community) develop within sea ice (for recent reviews see Mock and Thomas 2005, Mock and Junge 2007, Deming 2009). The SIMCO are usually dominated by ice-algae assemblages composed primarily of pennate diatoms (reviewed by Mock and Junge 2007). The populations within the ice are often so rich that the ice appears brown-green to the naked eye. Heterotrophic bacteria represent another major group within these communities (as are viruses; recently reviewed by Deming 2010), as evidenced by (1) measures of bacterial abundance, activity and production, (2) the microbial loop and (3) phylogenetically diverse

bacterial assemblages. Heterotrophic protozoa, amphipods, invertebrate larvae, copepods, euphausids such as krill, nematodes, turbellarians and some fishes are the larger protozoan and metazoan consumers found in sea ice (for reviews on these larger consumers see various chapters in Thomas and Dieckman 2010).

Over the last 2 decades, the phylogenetic diversity of sea ice bacteria has been studied extensively, mostly during spring and summer (e.g., Gosink and Staley 1993; Bowman et al. 1997; Brown and Bowman 2001; Junge et al. 2002, Brinkmayer et al. 2003) and more recently during winter as well (e.g., Brinkmayer et al. 2003; Junge et al. 2004; Collins et al. 2010). Efforts have mainly focused on polar Arctic and Antarctic sea ice, with some research performed in other sea ice-influenced water bodies such as the Baltic Sea (e.g., Petri and Imhoff 2001; Kaartokallio et al. 2008), Okhotsk Sea and Sea of Japan (Romanenko et al. 2008). Culturing efforts both in the Antarctic and Arctic have yielded new genera and species within the divisions of the Proteobacteria phylum including the Alphaproteobacteria, Betaproteobacteria and the Gammaproteobacteria classes, the Bacteriodetes phylum (formerly called

Table 6.3.1

Psychrophilic phyla, genera and species described from sea ice, with their temperature optima or range for growth (list amended from data compiled by Bowman J, personal communication and Deming 2010). Genera only represented by environmental sequences are in bold, genera only known from melt ponds and Baltic Sea ice are indicated by (mp) and (bs), respectively

Phyla and associated genera	Species	T_{opt} or T_{range} for growth (°C)	Reference(s)
Bacteria			
γ-proteobacteria:			
Acinetobacter	*Colwellia psychrerythraea*	9–16	D'Aoust and Kushner (1972), Bowman et al. (1998a), Huston et al. (2000)
Alteromonas	*Colwellia demingiae*	10–15	Bowman et al. (1998a)
Citrobacter	*Colwellia hornerae*	10	Bowman et al. (1998)
Colwellia	*Colwellia rossensis*	10	Bowman et al. (1998a)
Glacieocola	*Glaciecola pallidula*	13–14	Bowman et al. (1998b)
Halomonas	*Glaciecola punicea*	15–18	Bowman et al. (1998b)
Iceobacter	*Psychrobacter glacincola*	15–21	Bowman et al. (1998d)
Marinobacter	*Psychromonas boydii*	0–10	Auman et al. (2009)
Marinomonas	*Psychromonas ingrahamii*	−12 to 10	Auman et al. (2006)
Neptunomonas	*Shewanella gelidimarina*	16	Bowman et al. (1997b)
Oceanospirillum	*Shewanella frigidimarina*	20	Bowman et al. (1997b)
Pseudoalteromonas			
Pseudomonas			
Psychrobacter			
Shewanella			

Table 6.3.1 (Continued)

Phyla and associated genera	Species	T_{opt} or T_{range} for growth (°C)	Reference(s)
Terridinibacter			
Vibrio			
β-proteobacteria:			
Aquaspirillum (mp)	*Polaromonas vacuolatus*	10	Irgens et al. (1996)
Matsuebacter (mp)			
Rhodoferaz (mp)			
Ultramicrobacterium (mp)			
Comamonadaceae (bs)			
Hydrogenophaga (bs)			
α-proteobacteria:			
Octadecabacter	*Octadecabacter arcticus*	10	Gosink et al. (1997)
Roseobacter	*Octadecabacter antarcticus*	8	Gosink et al. (1997)
Ruegeria			
Sphingomonas			
Sulfitobacter			
Devosia (mp)			
Rhodobacter (mp)			
Loktanella (bs)			
Bacteroidetes (former CFB):			
Cellulophaga	*Flavobacterium gillisiae*	20	McCammon and Bowman (2000)
Cytophaga	*Gelidibacter algens*	15–18	Bowman et al. (1997c)
Cyclobacterium (mp)	*Polaribacter irgensii*	8–10	Gosink et al. (1998)
Flavobacterium	*Polaribacter franzmannii*	7	Gosink et al. (1998)
Flexibacteraceae (bs)	*Polaribacter filamentus*	10–12	Gosink et al. (1998)
Gelidibacter	*Psychroflexus torquis*	12	Bowman et al. (1998c)
Hymenobacter (mp)			
Lewinella			
Salegentibacter			
Polaribacter			
Psychroflexus			
Psychroserpens			
Green non-sulfur bacteria			
Planctomycetes			
Planctomycetales (mp)			
Verrucomicrobia			
Verrucomicrobium			
Prosthecobacter			

Table 6.3.1 (Continued)

Phyla and associated genera	Species	T_{opt} or T_{range} for growth (°C)	Reference(s)
Purple sulfur bacteria (bs)			
Firmicutes:			
Halobacillus	*Planomicrobium mcmeekinii*	0–37	Junge et al. (1998), Yoon et al. (2001)
Planomicrobium			
Actinobacteria:			
Corynebacterium	*Arthrobacter*	0–30	Junge et al. (1998)
Clavibacter	*Brachybacterium*	0–30	Junge et al. (1998)
ARCHAEA (only from winter ice)			Collins et al. (2010)
Crenarchaeota			
Marine Group I			
Euryarchaeota			
Group IIb			

the Cytophaga-Flexibacter-Bacteroides or CFB phylum) and the Actinobacteria phylum (see *Table 6.3.1* for a list of psychrophilic sea ice bacterial isolates described to date and associated references). Many of the described Arctic and Antarctic sea ice bacteria are stenopsychrophiles with growth optima or maxima below 15°C. Most bacteria isolated from sea ice have been observed to be pigmented, highly cold-adapted with some able to form gas vesicles (see below and Sullivan and Palmisano 1994; Grossi et al. 1984; Staley et al. 1989; Gosink and Staley 1995).

Sea ice bacteria have been found to be unusually easy to cultivate - with up to 60% of the total bacterial population being culturable (Junge et al. 2002; Brinkmayer et al. 2003). This stands in marked contrast to the culturability of most seawater bacteria (~0.01% of the total cell count, Amann et al. 1995) and might be due to the exceptionally high concentrations of labile dissolved organic matter (DOM) released by ice algae reported for sea ice brines in the Arctic, Antarctic and Baltic Sea (to exceed surface water concentration by factors of up to 500; Thomas and Mock 2005 and references therein). This likely explains why sea ice bacteria are so readily cultivated both in low and high nutrient media (Junge et al. 2002) and why groups commonly associated with marine algae are repeatedly found in sea ice bacterial culture collections.

Small subunit rRNA gene sequence analyses of whole communities have demonstrated an unexpectedly strong congruence with cultivation data. Mostly, the same phyla and genera were found to be present (Brown and Bowman 2001; Brinkmayer et al. 2003), except for a few clones grouping among the Verrucomicrobia, closest to the prosthecate aerobic genera *Verrucomicrobium* and *Prosthecobacter* (Brown and Bowman 2001). No evidence of uncultured bacterial clone groups such as SAR11, SAR86 or archaeal groups that commonly occur in oceanic non-polar and polar seawater samples had been found in the Arctic and Antarctic spring/summer ice or melt ponds studied (Brinkmeyer et al. 2004).

Molecular and cultivation studies on Arctic summer time ice-melt ponds revealed another group that had not been found previously in the interior of the ice. β-Proteobacterial genera known only from freshwater habitats were found to dominate in the mostly freshwater ponds (along with gram-positives species, α- and γ-Proteobacterial genera occurring in more saline ponds and Bacteriodetes members in sediment containing ponds; Brinkmeyer et al. 2004).

Anaerobic phototrophic purple sulpfur bacteria have also been found in the interior of Baltic sea ice (Petri and Imhoff 2001), indicating that oxygen deficient and anoxic zones are present in sea ice. High amounts of mucopolysaccharide gels and exopolymeric substances (EPS) are suggested to provide oxygen-depleted microhabitats for these species within the sea ice habitat (Mock and Thomas 2005). Other Baltic sea ice studies showed denitrification to occur in zones of ice with accumulated nitrite (Kaartokallio 2001). In Arctic sea ice, anaerobic bacterial denitrification with high numbers of anaerobic nitrate reducing bacteria and ammonium oxidation was found in zones with high levels of nitrate, ammonium ions and DOM (Rysgaard and Glud 2004; Rysgaard et al. 2008), which are characteristics of many sea ice habitats (reviewed by Thomas and Dieckmann 2002).

Archaea were found in low numbers (up to 4% of the population) in Arctic wintertime sea - ice samples (Junge et al. 2004) but not in any of the Spring- and Summer time Arctic and Antarctic ice samples cited above. More recently, the presence of Archaea in winter time sea ice was confirmed during a seasonal study of community composition over the course of the Arctic winter (Collins et al. 2010). Surprisingly it was also found that communities of Bacteria and Archaea in the ice resembled that in the underlying water. The microbial community consisted primarily of SAR-11 clade alpha Proteobacteria and Marine Group 1 Crenarcheaota, neither of which is known from Spring and Summer time sea ice. The wintertime ice bacterial clone library contained γ-proteobacteria from oligotrophic seawater clades (e.g., OM60, OM182) and no clones from γ-proteobacteria genera commonly detected in spring and summertime ice (e.g., *Colwellia, Psychrobacter*) were observed. It was concluded that selection during ice formation and mortality during winter played minor roles in the process of microbial succession that leads to distinctive spring and summer sea ice bacterial communities (Collins et al. 2010). Seasonal studies that explore the winter/spring/summer transitions are needed to explore further how distinctive summer and spring time communities develop but factors such as extensive algal growth resulting in increased highly labile DOM levels are likely determining factors.

Discovery of Gas Vacuolate Sea Ice Bacteria

Gas vesicles, which have been hypothesized to be an early organelle of prokaryotic motility (Staley 1980), are intracellular structures found in many aquatic Bacteria and Archaea (Walsby 1994). They appear as bright areas in cells that contain them when they are observed by light microscopy. To distinguish them from other bright, intracellular areas in cells, they need to be visualized by transmission electron microscopy. Each gas vacuole observed by TEM contains many subunits that are termed gas vesicles. The membranes of the gas vesicles consist of a hydrophobic protein with a molecular weight of about 7,500, which is the subunit of the gas vesicle. The subunits are arrayed in a manner that produces a central cylindrical structure capped at each end by conical tips.

The function of the gas vesicles is that they to provide buoyancy to the organism. This is accomplished by excluding water into the interior of the vesicles because of the hydrophobic

vesicle membrane. However, gases are freely permeable through the vesicle membranes so that gases in the ambient environment accumulate in the interior of the vesicle. As a result, the vesicles decrease the density of the cells thereby providing buoyancy.

Gas vacuolated bacteria are very common in freshwater habitats, especially those that have a thermally stratified water column. Typically in these habitats the gas vacuolated cyanobacteria that produce blooms in lakes are found in the surface waters where they receive abundant light for photosynthesis. In the deeper hypolimnion of stratified lakes, blooms of anoxygenic photosynthetic bacteria, many of which contain gas vacuoles, occur. Gas vacuolated bacteria and archaea are also found in the hypolimnion and even in freshwater sediments.

In 1989, marine gas vacuolate bacteria were reported from euphotic waters off the Palmer Peninsula (Irgens et al. 1989). Based on this discovery, it was hypothesized that gas vacuolate bacteria, which are typically found in stratified aquatic habitats might be indigenous to the SIMCO because it is a dramatic example of a stratified marine habitat in which underlying cold water is capped by the SIMCO ice layer.

To test this hypothesis, more intensive studies were performed at McMurdo Sound, where the sea ice community is more amenable to study. Sure enough, gas vacuolate bacteria were encountered, in some instances at very high concentrations relative to total cultivable bacteria, in the SIMCO layer, underlying frazil ice layer and water column (Staley et al 1989). It is still not known whether the gas vesicles are expressed when they are in the SIMCO layer but it is thought that they are likely expressed after the summer thaw when the sea ice microbial community is dispersed in the water column. The evidence for this is that the gas vacuolated strains were only isolated from samples taken from the surface waters and to depths down to 50 m whereas none were isolated from lower depths, i.e., 100, 200, and 500 m.

The taxonomy of the gas vacuolated bacteria followed their isolation. The first described gas vacuolated bacterium from Antarctica, *Polaromonas vacuolata*, is a member of the Proteobacteria (Irgens et al. 1996). Although *Polaromonas vacuolata*, is likely a member of the SIMCO, this bacterium was isolated from open waters during the summer months so its association with the SIMCO is uncertain. However, many gas vacuolated bacteria have been isolated from the SIMCO layer and identified.

The gas vacuolate bacteria isolated and described taxonomically from sea ice fall into two major phylogenetic groups, the phyla, Proteobacteria and Bacteroidetes (Staley et al 1989; Gosink and Staley 1995). Examples of gas-vacuolate sea ice bacteria that have been named belong to Proteobacterial genera, *Octadecabacter* (Gosink et al. 1997), *Psychromonas* (Auman et al. 2006; Auman et al. 2009) and the Bacteroidetes genus *Polaribacter* (Gosink et al. 1998).

Biogeography of Polar Sea Ice Bacteria

One of the reasons for studying the bi-polar distribution of sea ice bacteria, is to evaluate whether the same species are found at each pole. Baas-Becking's hypothesis (1934) that "everything is everywhere, the environment selects" indicates that polar sea ice bacteria should be cosmopolitan. Therefore, the two polar environments should contain the same species of bacteria. Staley has hypothesized that this is improbable and therefore psychrophilic bacteria from each pole should be endemic, separate species (Staley, ASM News; Staley and Gosink 1999). He reasoned that the great distances between the two poles and the difficulty of dispersing stenopsychrophiles, which quickly die at room temperature, across tropical areas through various means, such as animal vectors like the arctic tern or long transport via deep

ocean circulation under deep sea pressures, might make it improbable that the same species would be found at each pole.

To test this hypothesis, a number of strains of unique psychrophilic gas vacuolated bacteria were isolated from each pole and compared. The closest matches that were found were strains were from the genera *Octadecobacter* and *Polaribacter*. This indicates, that at the level of the genus, these bacteria are cosmopolitan in their distribution.

The 16S rRNA gene sequences of the two most closely related strains of *Octadecobacter* isolated from the two poles, were $<1\%$. Therefore, in order to determine whether they were separate species, DNA-DNA hybridization was performed. The reassociation value at 42% was well below the $>70\%$ required for them to be the same species. Therefore the new species were named *O. arcticus* and *O. antarcticus* to reflect the two different poles from which they were isolated (Gosink et al. 1997).

Likewise, four *Polaribacter* strains isolated from the two separate poles were also compared. The two most closely related strains based on DNA-DNA hybridization showed 34% relatedness, again significantly lower than the 70% required for them to be the same species (Gosink et al. 1998). Each strain was therefore named as a separate species of the genus.

Junge et al. (2002) reported on an Arctic isolate (*Shewanella frigidimarina*) which showed 100% sequence similarity to a *Shewanella frigidimarina* isolate from Antarctica, suggesting bipolar distribution for bacteria even at the species level and thus possibly reversing the earlier suggestion of endemism by Staley and Gosink (1999). However, DNA-DNA hybridization tests are needed to confirm this suggestion. Furthermore, despite the implication of its species name, *frigidimarina*, the type species of *Shewanella frigidimarina* is not a stenopsychrophile because its maximum temperature for growth is 30°C. In contrast the gas vacuolated sea ice bacteria tested for bipolar endemism were true stenopsychrophiles that do not grow above 10–15°C and cannot survive at room temperature and are therefore highly unlikely to survive transport across the equator.

Thus to date no definitive evidence of cosmopolitan bi-polar strains of stenopsychrophilic bacteria from the SIMCO habitat has yet been established. Multiple-locus sequence analysis (MLSA) is an alternative approach to resolving this question (Whitaker et al. 2002). This approach would require the isolation and comparison of several closely related strains of a presumed "species" from each pole for a more complete comparative analysis.

Are Sea Ice Bacteria "Good" Psychrophiles?

Not all organisms that grow in polar environments are steno-psychrophiles. For example, typical bacteria isolated from Antarctic soils may grow in the laboratory at temperatures above 25°C. This may be due to the large annual fluctuation in temperatures in soils. In contrast, polar marine environments are consistently cold. Temperatures in many areas do not exceed 2°C during the year and temperatures in the sea ice are colder. Therefore, in general, the sea ice environment has been an excellent source of psychrophilic bacteria. The question arises, are there features that are typically found in organisms that make them steno-psychrophiles?

Several attributes of bacteria make them good candidates for psychrophily. For example low growth temperatures provide a basis for predicting a psychrophilic life style. It should be noted that low temperature for growth as contrasted with low temperature for metabolism provides a better indicator of psychrophily. As with thermophiles, the maximum temperature for growth is the hallmark of thermophily. Therefore, using the same rationale, the lowest

temperature for growth should be the maxim for psychrophily. Furthermore, it is very difficult to accurately identify and assess in situ microbial activities in frozen habitats (for advances in this area see Junge et al. 2004 and 2006).

Low maximum growth temperature. One feature is that stenopsychrophiles have a low maximum temperature for growth. Most of the sea ice bacteria do not grow at room temperature and many do not grow at temperatures above 10°C. This feature is an important predictor for psychrophily, because most bacteria have a limited temperature range for growth. *E. coli* for example, grows at a low of 8°C and at a high of 44°C. Therefore its temperature range is 36°C. Likewise, extreme thermophiles, such as species in the genus *Pyrococcus*, which grow from about 80–110°C, have a growth temperature range of about 30°C.

Therefore, if we extrapolate this information to psychrophiles, if they have a maximum growth temperature of 10°C, they could actually grow at temperatures as low as −20°C or perhaps lower.

Compatible solute production. Another feature of a typical steno psychrophile is its ability to produce an intracellular solute to counterbalance the osmotic effect of higher salinities in brine pockets where organisms reside, that are encountered as sea ice freezes.

Habitat. The lowest temperatures encountered in sea ice occur in the winter months at the air–ice interface. Therefore, it would seem logical that organisms that are located in the upper layers of the sea ice are good candidates for psychrophily.

Low mol% G+C content. From a purely theoretical standpoint, DNA replication may be aided at low temperatures when there are fewer hydrogen bonds. DNA with a low G+C content would have more double-bonded AT nucleotides and fewer GC nucleotides thereby aiding in replication. Consistent with this argument is that several psychrophilic bacteria have mol% G+C contents below 50%. It should be noted, however, that some thermophilic bacteria also have mol% G+C values below 50%, e.g., in *Sulfolobus* species it ranges from about 35–42 mol%, so this pattern may not apply to thermophiles.

A good example for a sea ice bacterial species exhibiting all of the features listed above that are consistent with psychrophilic growth is *P. ingrahamii*. Its maximum growth temperature is 10°C. Therefore, its minimum growth temperature, in theory could be at least −20°C, if we accept the modest estimate of a 30°C temperature range for its growth. The lowest growth temperature recorded for this organism is −12°C (Breezee et al. 2008). However, the authors noted that it is likely that the organism can grow at even lower temperatures, but the conditions they used for lower temperatures resulted in freezing the cultures which resulted in cessation of growth.

P. ingrahamii was isolated from the upper half of a sea ice column in Elson lagoon, near Pt. Barrow, Alaska. This lagoon has a slightly higher salinity than typical seawater thereby also providing conditions to lower the freezing point of lagoon's water.

The mol% G+C of *P. ingrahamii* is 40.1, lower than that of many other members of the Gammaproteobacteria. Finally, genomic analyses of this organism indicate that it produces a compatible solute, betaine choline aiding its growth under high salinity conditions encountered in brine pockets in the ice (Riley et al. 2008).

Genomes of Psychrophilic Bacteria

Several genomes of psychrophilic bacteria have been published. They include *Colwellia psychroerythraea* 34H (Methé et al. 2005), *Idiomarina loihiensis* L2TR, *Pseudoalteromomas*

haloplanktis TAX 125 in addition to *Psychromonas ingrahamii* (Riley et al. 2008). For further information on this subject we refer the reader to an excellent review of the genomes of psychrophilic bacteria written by Bowman (2008).

Glacial Ice Microbiology

Ecological Aspects

The ice sheets of Greenland and Antarctica represent most of the glacial ice on Earth, which cover about 10% of Earths terrestrial surface and contain 77% of the freshwater on the planet (Miteva 2008). Temperate, high altitude non-polar glaciers are found all over the world (e.g., China has more than 46,000 glaciers) and represent an important freshwater source for millions of people. Glaciers can range from a few hundred meters to 4 km in depth, with temperatures increasing with depth (e.g., the glacier overlying Lake Vostok in Antarctica exhibits −60°C to −2°C from surface to base).

The physicochemical characteristics and structure of the polycrystalline glacier ice as a microbial habitat have recently received more attention where it is now understood that cells reside either in liquid filled veins (cells need to be less than 2 μm; *Fig. 6.3.3*; Mader et al. 2006) containing variable substrates suitable to support bacterial metabolism and survival or in the thin liquid films on the surface of mineral grains (see below; Miteva 2008). Furthermore, clear glacier ice and basal ice show significant differences (with basal ice containing much higher concentrations of inorganic and organic ions and minerals) resulting in significant differences in the microbial populations within (with much higher abundances in the latter and a possible function of basal ice microbes in geochemical cycling reactions within the ice,

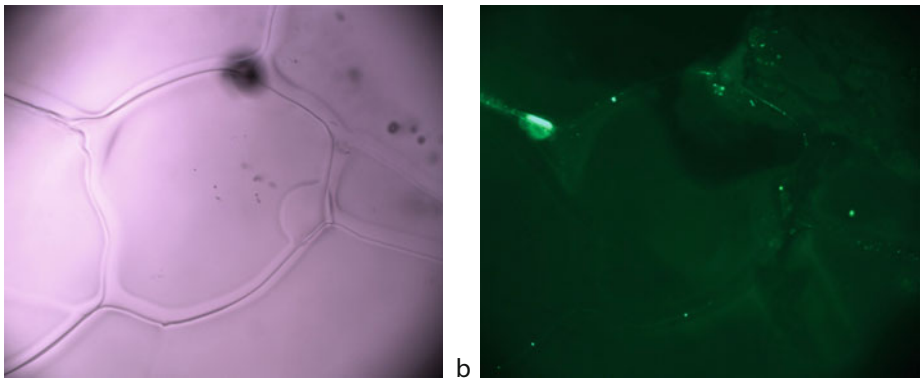

Fig. 6.3.3

Cryostage microscopic image at −20°C of microbial cells within an ice vein habitat. A thin section of glacial ice from the Taylor Glacier (McMurdo Dry Valleys, Antarctica) was mounted on a microscope slide and overlain with a saline solution of SYBR Green I. (a) Bright field image of the ice; (b) the same area under epifluorescence showing DNA-containing cells in the interstitial habitat between ice crystals. Each micrograph is ~1,000 mm in width. Images: K. Suematsu, visiting researcher, LSU

see Chap. 6.5 Ecological Distribution of Microorganisms in Terrestrial, Psychrophilic Habitats; Miteva 2008). In the following we focus on what is currently known about the diversity of glacial microbes, discuss the glacial environment as a habitat or archive and the longevity of microbes in ice.

Diversity of Microbial Species Immured in Glacial Ice

Glacial ice sheets entrap and preserve aerosolized biological material (i.e., insects, plant fragments, seeds, pollen grains, fungal spores and microorganisms) deposited in snowfall. The presence of viable bacteria and fungi in ancient glacier ice has been widely documented in polar and non-polar locations (e.g., Abyzov et al. 1993, 1998; Christner et al. 2000, 2003, 2006; Miteva and Brenchley 2005; Bidle et al. 2007; D'Elia et al. 2009; Miteva et al. 2009). Recent reviews by Priscu and Christner (2004) and Miteva (2008), and Christner et al. (2008c) and Priscu et al. (2008), provide good detailed overviews of the diversity of bacteria reported in a range of glacial and subglacial environments. Remarkably, many isolates obtained from geographically diverse glacier samples of polar and non-polar origin belong to the same bacterial genera (summarized in *Table 6.3.2*), including representatives of the Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes (Miteva 2008). Isolates of the Actinobacteria and Firmicutes are usually found to be predominant, followed by members of the Proteobacteria and Bacteroidetes. Studies that have succeeded in characterizing glacial microbial communities directly through culture-independent 16S rRNA based methods found sequences primarily relating to the same groups (Miteva 2008).

Microorganisms entrapped at increasing depth, and therefore, increasing age in glacier ice remain viable for hundreds of thousands (Abyzov et al. 1993, 1998; Christner et al. 2003, 2006; Miteva and Brenchley 2005) to perhaps millions of years (Bidle et al. 2007). Metagenomic analysis of a primarily bacterial assemblage in glacial ice from the German Alps indicates the potential for high metabolic diversity and molecular evidence for adaptations to increase survival at low temperature and during freezing (Simon et al. 2009). Some of the ice-related phenotypes found in species deposited in glacial environments may even have a role in precipitation generation and/or their precipitation from the atmosphere in snowfall (e.g., Christner et al. 2008a, b). Comprehensive studies of microbial assemblages immured chronologically within an ice core provide a unique approach to geobiology, e.g., by examining the influence of climate conditions on the species that were distributed in the atmosphere at different times in history (e.g., Christner et al. 2000; Miteva et al. 2009). Miteva et al. (2009) used a molecular approach to study the microbial assemblage in the GISP 2D (Greenland) ice core and found that higher bacterial and fungal diversity in ice deposited during cold climate conditions. Efforts to characterize the unique properties of psychrophiles from glaciers and other permanently frozen environments have increased significantly in recent years, and an assortment of properties have been identified that would be useful to cells that persist in a frozen matrix. Loveland-Curtze et al. (2010) described the species *Chryseobacterium greenlandense*, which was isolated from a depth of 3,043 m in the GISP 2D (Greenland) ice core. The small cells (<0.1 mm^3) of this species enable a high cell surface-to-volume ratio, which enhances nutrient uptake and diffusion of substances throughout the cell. A species in the genus *Chryseobacterium* has also been reported from a deep Antarctic ice core (3,519 m in the Vostok ice core) that has the ability to alter the physical structure of ice (Raymond et al. 2008). This microbe produces an extracellular protein which binds to the

Table 6.3.2

Examples of bacteria isolated from various polar and nonpolar glacial ices

Phylum	Genus	Source	Reference
Actinobacteria	*Arthrobacter*	Antarctica, China, Greenland, and New Zealand	Christner et al. (2000), Miteva et al. (2004), Foght et al. (2004), Xiang et al. (2005)
Actinobacteria	*Clavibacter*	China and Bolivia	Christner et al. (2000), Xiang et al. (2005)
Actinobacteria	*Mycobacterium*	Bolivia and Greenland	Christner et al. (2000), Miteva et al. (2004)
Alphaproteobacteria	*Methylobacterium*	Antarctica, Greenland, and China	Christner et al. (2000, 2001, 2008), Miteva et al. (2004, 2009)
Alphaproteobacteria	*Sphingomonas*	Antarctica, Greenland, China, and New Zealand	Christner et al. (2000, 2001, 2008), Foght et al. (2004), Miteva et al. (2004), Xiang et al. (2005), Miteva et al. (2009)
Gammaproteobacteria	*Acinetobacter*	Antarctica, Bolivia, and China	Christner et al. (2000), Xiang et al. (2005)
Bacteroidetes	*Chryseobacterium*	Antarctica and Greenland	Raymond et al. (2008), Loveland-Curtze et al. (2010)
Bacteroidetes	*Flavobacterium*	Antarctica, China, and New Zealand	Christner et al. (2000), Zhu et al. (2003), Foght et al. (2004), Xiang et al. (2005), Zhang et al. (2006)
Firmicutes	*Bacillus*	Antarctica, Bolivia, China, and Greenland	Abyzov et al. (1993), Christner et al. (2000), Miteva and Brenchley (2005), Xiang et al. (2005), Miteva et al. (2009)
Firmicutes	*Exiguobacterium*	Bolivia, Greenland, and India	Christner et al. (2000), Miteva et al. (2004), Chaturvedi and Shivaji (2006)
Firmicutes	*Paenibacillus*	Antarctica, Bolivia, China, Greenland, and New Zealand	Christner et al. (2000, 2001), Foght et al. (2004), Miteva et al. (2004), Xiang et al. (2005)
Firmicutes	*Plannococcus*	Bolivia and China	Christner et al. (2000), Xiang et al. (2005)

prism faces of ice crystals and prevents recrystallization, i.e., an ice-binding protein. Bacteria in the genus *Psychrobacter* have been recovered frequently in enrichments targeting heterotrophs in icy environments (e.g., Gilichinsky et al. 2003, 2005; Bakermans et al. 2003, 2006; Mosier et al. 2007). In particular, studies of *Psychrobacter* species have provided valuable insight into their growth (Bakermans et al. 2003) and gene and protein expression patterns (Bakermans et al. 2007; Bergholz et al. 2009) at subzero temperatures, as well as their capability to metabolize within an ice matrix (Christner 2002; Amato and Christner 2009a).

Glacial Ice: Archive or Habitat?

It was initially assumed that cells surviving within ancient glacial ice did so in a state of anabiosis (e.g., Abyzov et al. 1998). In freshwater and saline ice, various studies have shown that cells are physically located in the aqueous interstitial veins that exist at ice grain boundaries (Junge et al. 2004; Mader et al. 2006; Amato et al. 2009b; *Figs. 6.3.1* and *6.3.3*), supporting Price's (2000) hypothesis that ice veins provide a habitat for cells in glacial ice. Sub-nanometer thin films of liquid water have been reported down to temperatures of −196°C (Pearson and Derbyshire 1974); however, the increased ionic concentration of unfrozen water at decreasing temperatures in ice likely constrains biological activity by creating chemical conditions incompatible with a microorganism's physiology. Laboratory studies have provided a new perspective on the potential for functional biological processes under frozen conditions (Carpenter et al. 2000; Rivkina et al. 2000; Christner 2002; Amato et al. 2009b). As of yet, the low temperature limit for metabolism is not known and is probably <-40°C (e.g., Junge et al. 2006; Panikov et al. 2006; Amato and Christner 2009). Hence, the physiological potential for metabolism under conditions in glacial ice has been established.

Analysis of the gases entrapped in ice cores from Antarctica, Bolivia, and Greenland have revealed ice horizons where the CO_2, N_2O and CH_4 concentrations are significantly elevated above atmospheric concentrations, and a number of investigators have concluded that these anomalies are the result of in situ microbial activity (Sowers 2001; Campen et al. 2003; Tung et al. 2006). Gas anomalies are commonly observed in basal ice, and Souchez et al. (1995) suggest that anomalously high CO_2 and CH_4 concentrations entrapped within GRIP silty ice may have originated from "flow-induced mixing"; however, in situ production of these gases still remains a plausible explanation (e.g., Tung et al. 2006). If microbial consortia remain metabolically active in the ice, then the World's glaciers represent active biomes, substantially expanding the known realm for life in the biosphere.

Limits to Longevity in Glacial Ice

For a microorganism to remain viable over an extended period of frozen dormancy, the macromolecular damage (e.g., to DNA) incurred by the cell must not exceed a level where effective repair is no longer possible. Viable microorganisms have been recovered from ancient glacial ice (Christner et al. 2003; Miteva and Brenchley 2005; Bidle et al. 2007) and permafrost (Vishnivetskaya et al. 2006; Gilichinsky et al. 2007; Johnson et al. 2007) samples ranging from hundreds-of-thousands to millions of years old. McKay (2001) predicted that background ionizing radiation in terrestrial permafrost (i.e., from the decay of potassium-40, thorium-232, and uranium-238) would inactivate dormant bacteria in 10–100 million years. In *Fig. 6.3.4*, we predicted cellular damage based on the introduction of lethal DNA double-stranded breaks (DSB) to the genome from ionizing radiation dosages in the Antarctic subsurface and "low background" environments (10^{-3} to 10^{-4} Gy y^{-1}; Luckey 1991). The susceptibility of bacteria to ionizing radiation is expressed as the number of DNA DSB at the D_{37}, which is the ionizing radiation dosage at which 37% of the cells survive, which on average, is sufficient to inactivate a single colony-forming unit of the irradiated population (Battista 1997). These data and related calculations imply that it would take 300,000–3,000,000 years of exposure to acquire an ionizing radiation dosage equivalent to the D_{37} of *Escherichia coli*, but a radiation-resistant bacterium such as *Deinococcus radiodurans* could remain in the ice for

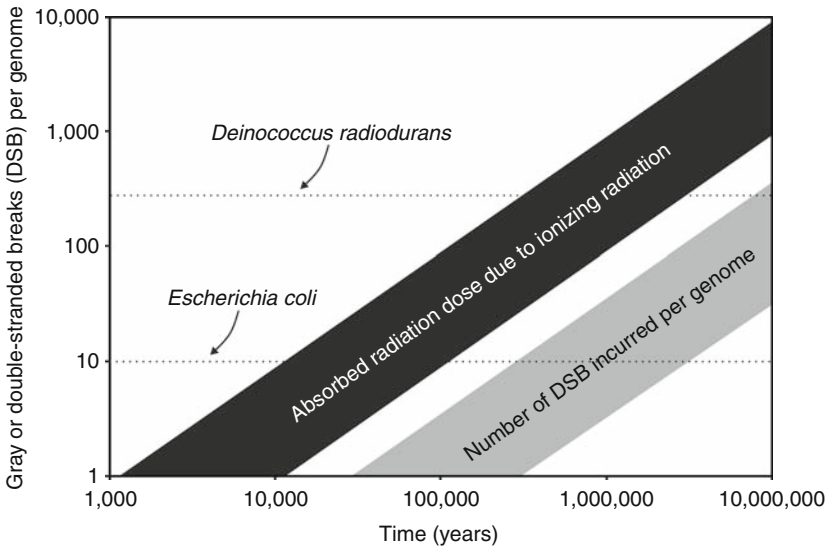

Fig. 6.3.4

Estimated number of DNA double-stranded breaks (DSB) in a microbial genome over time from ionizing radiation sources within glacial ice. Data range estimates for dosage from Luckey (1991). Values for DNA DSB damage due to ionizing radiation dosage (1 DSB per 30 Gy) from Battista (1997). The dotted horizontal lines are examples for the number of DNA DSB at the D37 for Escherichia coli and Deinococcus radiodurans

>8,250,000 years before reaching the D_{37} (*Fig. 6.3.4*). Our calculations are consistent with the exponential decline in average community DNA size ($t_{1/2}$ = 1.1 million years) observed by Bidle et al. (2007) in a time sequence of ice samples up to 8 million years old. It is important to note that these estimates assume that microorganisms are metabolically dormant when entrapped within frozen matrices, and therefore, are incapable of repairing cellular damage as it is incurred.

Christner (2002a) demonstrated the ability of bacteria to conduct DNA synthesis at −15°C and hypothesized that the metabolic activity observed was directed towards repairing damage sustained during the freezing process (i.e., DNA breakage) or for maintenance metabolism (Price and Sowers 2004). Johnson et al. (2007) provided evidence for DNA repair in permafrost samples up to 600,000 years old and concluded that "bacteria with an active DNA repair mechanism are most likely to persevere." If laboratory measurements of microbial metabolism under frozen conditions are a true reflection of their physiological potential in natural icy environments, a slow metabolic rate may be sufficient to offset cellular damage, allowing survival in frozen substrates for extended time frames. In contrast, ionizing radiation-resistant bacteria, which are often also resistant to UV radiation and desiccation (i.e., Rainey et al. 2005), might be capable of remaining viable in the absence of metabolism due to their extremely efficient DNA repair mechanisms (e.g., Battista 1997). Testing the ionizing radiation tolerances of viable microorganisms recovered from ancient glacial ice would provide a means to formulate hypotheses regarding the particular means of survival (i.e., dormancy and efficient DNA repair versus metabolic activity and repair under frozen conditions) used by species that survive for geological timeframes in ice.

Conclusions

Sea ice and glacier ice harbor unique microbial communities with many steno- and euro psychrophilic members. These psychrophiles exhibit a rich diversity with many of the same groups represented in both habitats indicating that environmental conditions in the ice (temperature, concentration of solutes, etc.) select for certain types of organisms. It has now been shown that psychrophilic microbes residing both in saline and freshwater ice veins might not be merely persisting but can also carry out low levels of metabolic activity. For glacial ice microbes, this could possibly allow for DNA damage repair while being immured in the ice over thousands to hundreds of thousands and possibly even millions of years.

Cross-References

- 6.4 Adaptation Mechanisms of Psychrotolerant Bacterial Pathogens
- 6.5 Ecological Distribution of Microorganisms in Terrestrial, Psychrophilic Habitats
- 6.7 Psychrophilic Enzymes: Cool Responses to Chilly Problems
- 9.4 Genetics, Genomics, Evolution

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