Chapter 4
Bacteria in Subglacial Environments

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4.1 Introduction

Glaciers exist where the annual temperature remains cold enough to allow snowfall to accumulate for an extended period of time and where conditions allow subsequent metamorphosis to ice. Glacial ice forms expansive continental ice sheets in the polar regions, (e.g., in Antarctica and Greenland), and at lower latitudes, ice fields (valley or alpine glaciers) and ice caps (if a volcano or mountain

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range is completely glaciated) exist globally at high altitude. Temperate glaciers comprise <4% of the glacial ice on the planet, but are important freshwater reservoirs and are often the sources for major rivers vital for irrigation, industry, and providing millions of people with drinking water. The Greenland and Antarctic ice sheets currently cover ~10% of the terrestrial surface (>1.5×10^7 km^2) and contain ~75% of the freshwater on Earth (Paterson 1994). The Antarctic ice sheet alone contains ~90% of the planet's ice and, if melted, would result in a sea level rise of ~65 m (The National Snow and Ice Data Center; http://nsidc.org/).

Evidence for liquid water in the basal zones of polar ice sheets (e.g., Ueda and Garfield 1970), the discovery of more than 140 subglacial lakes in Antarctica (Siegert et al. 2005), and expanding perspectives on the tenacity of life under cold conditions (e.g., Priscu and Christner 2004) have motivated research to determine if subglacial environments harbor viable microbial ecosystems. The presence of viable microorganisms has been documented in deep glacier ice (Abyzov et al. 1998; Christner et al. 2000, 2003, 2006; Miteva and Brenchley 2005), subglacial waters (Sharp et al. 1999; Mikucki et al. 2004), basal ice (Skidmore et al. 2000; Sheridan et al. 2003; Miteva et al. 2004; Foght et al. 2004), subglacial sediments (Skidmore et al. 2005), and subglacial lakes and accreted ice (Karl et al. 1999; Priscu et al. 1999; Christner et al. 2001, 2006; Gaidos et al. 2004). Here, we discuss current information on the diversity of environments for life at the base of glaciers and review what is known about subglacial ecosystems, which are inhabited by microbial assemblages that permanently exist at temperatures near the freezing point of water.

4.2 Liquid water in subglacial environments

4.2.1 Water and life

Water is required as a solvent in biochemical reactions, for mass transfer (i.e., the physical transport of molecules), and to establish electrochemical gradients (e.g., proton motive force). Nutrient-rich surface melt waters enter the basal zone of warm and polythermal alpine glaciers, and studies of subglacial outflow and sediments have provided key information on the biogeochemistry and microbial diversity of these environments (e.g., Sharp et al. 1999; Foght et al. 2004; Tranter et al. 2005). The occurrence of subglacial water under polar ice sheets was first discovered in Antarctica (Ueda and Garfield 1970) and was recently documented in Greenland (Anderson et al. 2004). If liquid water and chemical (inorganic or organic) energy sources are available, subglacial microbial habitats may harbor species adapted to one of the most extreme environments in the biosphere.

4.2.2 Liquid water in Arctic and Alpine subglacial environments

Ice masses may be classified into three categories based on the temperature regime of the ice: cold, polythermal, and temperate (Paterson 1994). Cold-based ice masses consist of ice with temperatures below freezing throughout, and liquid water is only present in the veins between ice crystals (Nye 1992), with no significant water layer at the glacier bed. Alpine glaciers in the McMurdo Dry Valleys of Antarctica and smaller glaciers in the Canadian High Arctic are examples of cold based ice masses. Polythermal ice masses are largely found at latitudes above the Arctic Circle, whereas temperate ice masses (i.e., ice is at the melting point from the surface to the base) are located at low and mid-latitudes typically as valley glaciers. Sections of polythermal ice masses are frozen to the bed especially beneath the thinner margins and termini. However, the bed is temperate beneath the thicker, inner zones of the ice mass. Where the basal ice is at the melting point, melting occurs due to geothermal heating and from friction, and there is a layer of liquid water between the basal ice and the substrate (Alley et al. 1997). Temperate-based ice masses have ice at the bed which is at the pressure-melting point throughout and thus the entire glacier bed is wet based. The surface of the ice remains snow covered during the winter and, during the summer ablation season, surface meltwater is delivered to the glacier bed via crevasses and moulins from surface snow and ice melt in both polythermal (Skidmore and Sharp 1999) and temperate ice masses (Nienow et al. 1998).

A number of different subglacial drainage system configurations are possible where the glacier bed is temperate, e.g., linked cavity systems, canals, sheetflow and channelized drainage (for reviews of temperate glacier hydrology and subglacial processes, see Hubbard and Nienow 1997; Fountain and Walder 1998; Clarke 2005). Where basal water is present, microbes have been documented in the subglacial environment at both temperate glaciers (Sharp et al. 1999; Foght et al. 2004; Skidmore et al. 2005) and polythermal glaciers (Skidmore et al. 2000; Wadham et al. 2004; Bhatia et al. 2006). Tranter et al. (2005) review how subglacial hydrological flowpaths control the connectivity of chemical weathering environments, access to atmospheric oxygen, and the redox potential (Eh) of the environments that microbes colonize.

4.2.3 Discovery of subglacial water beneath Antarctica's ice sheets

The Antarctic polar plateau has a mean annual surface temperature of −37°C and was first explored in an expedition led by Robert Falcon Scott in 1902. During the 1950s, geothermal heat flow models were developed that predicted an increase in temperature with depth in the ice, implying that, if the ice sheet is thick enough, then the basal ice in contact with the bedrock is at the melting point of water. Theoretical predictions, together with seismic measurements of the ice sheet thickness,

supported the idea that liquid water existed at the base of the ice sheet in central portions of the Antarctic continent (Zotikov 2006). More than a decade passed before the existence of water beneath Antarctica's ice sheets was confirmed.

The first deep drilling operation in Antarctica was initiated in 1966 at Byrd Station. An ice core was successfully recovered at Byrd Station and bedrock was reached at a depth of 2,164 m below the ice surface in January 1968. The drilling team did not expect to encounter liquid water at the ice-bedrock interface. However, water entered the borehole and raised the drilling fluid level 55 m. Due to the resulting "hydrostatic unbalance" and movement of the aqueous basal fluid into colder portions of the borehole, further coring and collection of subglacial materials were suspended to prevent loss of the drill by freezing (Ueda and Garfield 1970; Zotikov 2006). While an ice core was retrieved that yielded important information on the climate history of West Antarctica and the nature of ice in deep portions of the ice sheet, the implications of this finding for life in Antarctic subglacial environments was not fully realized for another 30 years.

4.2.4 Liquid water under the Greenland ice sheet

Several ice core drilling projects have drilled to bedrock in Greenland since the mid 1960s: Camp Century, the Greenland Ice Sheet Project (GISP and GISP2), the Greenland Ice Core Project (GRIP), and the North Greenland Ice Core Project (NGRIP). In the deepest portion of these ice cores, referred to as "silty ice", the ice contains numerous organic and inorganic inclusions, which are thought to originate from the subglacial environment rather than from aeolian deposition in snowfall (Gow and Meese 1996). Anderson et al. (2004) encountered pink-colored basal water when drilling at NGRIP in 2003, which entered the borehole and raised the fluid level 45 m. Even though ice sounding radar provided no evidence for liquid water at the base of the ice sheet, the borehole temperature profile indicated that the basal ice is near the pressure melting point of water under the NGRIP site. Importantly, the discovery of basal water at NGRIP revealed that liquid water also exists at certain locations at the bed of the Greenland ice sheet and wet-based conditions may be more prevalent than previously thought.

4.3 Subglacial lakes

4.3.1 Antarctic subglacial lakes

In the late 1950s, pilots with the Soviet Antarctic Expedition noted flat depressions on the polar plateau, which they used for navigation and referred to as "lakes" (Zotikov 2006). Oswald and Robin (1973) used radio echo sounding (RES) to survey portions of East Antarctica and provided evidence for the widespread existence of water under the ice sheet by identifying 17 subglacial lakes. Robin et al. (1977) conducted many

flights in the vicinity of Vostok Station and discovered that a very large lake (Subglacial Lake Vostok) exists beneath the Russian base, which was further supported by satellite radar altimetry (Ridley et al. 1993) and seismic data (Kapista et al. 1996).

In the last ten years, RES data have been used to identify 141 subglacial lakes in East Antarctica and 4 in West Antarctica (Siegert et al. 2005). Most (60%) of the subglacial lakes identified are <200 km from an ice divide and exist under 2,500–4,000 m of ice (Dowdeswell and Siegert 2002). The rapid transport of large amounts of water (1.8 km^3 of water over 16 months) beneath the Antarctic ice sheet through subglacial channels has also been documented (Wingham et al. 2006), providing evidence for hydrological connectivity between subglacial lakes. Thus, subglacial lakes may not be isolated systems and it is possible that periodic floods could transfer microorganisms, carbon, and nutrients between lakes. Estimates indicate that the volume of known Antarctic subglacial lakes is approximately 10,000 km^3 (Dowdeswell and Siegert 2002). This volume represents ~15% of all freshwater on non-glaciated continents, enough to cover the whole Antarctic continent with a uniform water layer thickness of ~1 m.

4.3.2 Subglacial Lake Vostok

Of all the subglacial Antarctic lakes identified to date, Subglacial Lake Vostok (hereafter referred to as Lake Vostok) is by far the largest with a surface area >14,000 km^2, volume of 5,400 ± 1,600 km^3, and maximum depth of ~800 m (Kapista et al. 1996; Studinger et al. 2004). The lake consists of a northern basin (water depth of ~500 m) and a larger southern basin (~800 m), which are separated by a bedrock sill (Studinger et al. 2004). The variation in ice sheet thickness between the north (~4,200 m) and south portion (~3,900 m) of the lake produces a 0.3°C difference in the pressure melting point of water. This gradient results in glacial ice melting into the lake in the north, and refreezing (i.e., accretion) to the bottom of the ice sheet in the south, which has important repercussions for circulation and vertical mixing within the lake (Siegert et al. 2001).

The lake water of Lake Vostok has not been directly sampled. However, molecular, microbiological, and biogeochemical analysis of the basal portion (3,539–3,623 m) of an ice core drilled at Vostok Station has provided valuable data to predict limnological conditions within surface waters of the lake (Karl et al. 1999; Priscu et al. 1999; Christner et al. 2001, 2006; Bulat et al. 2004). Based on ice sheet flow (Bell et al. 2002) and the concentration of particle inclusions within the accretion ice (Souchez et al. 2002; Royston-Bishop et al. 2005; Christner et al. 2006), ice cores recovered between 3,539 and 3,609 m (referred to as Type I accretion ice) are inferred to have formed from lake water that accreted in a shallow embayment in the southwestern portion of the lake, whereas accretion ice between 3,610 and 3,623 m (Type II accretion ice) is much cleaner and probably formed over the deep central portion of the lake's southern basin (de Angelis et al. 2004). Thus, the accretion ice profile represents a transect of surface waters from the shallow depths in the east to that over deep waters in the vicinity of Vostok Station.

Heterotrophic activity has been reported within melted samples of the accretion ice (Karl et al. 1999; Christner et al. 2006) and amplification and sequencing of small subunit (16S) rRNA genes from extracted DNA and isolated cultures imply the lake is inhabited by bacteria related to the Proteobacteria (alpha, beta, gamma and delta subdivisions), Firmicutes, Actinobacteria, and Bacteroidetes (Priscu et al. 1999; Christner et al. 2001, 2006; Bulat et al. 2004). Using a quantitative decontamination protocol (Christner et al. 2005; Fig. 4.1), Christner et al. (2006) examined 20 depths in the Type I and II accretion ice (between 3,540 and 3,623 m) and predicted that the average concentration of organic carbon, prokaryotic cells, and total dissolved solids in surface waters of the shallow embayment and open lake are 86 and 160 μM, 150 and 460 cells ml^{-1}, and 1.5 and 34 mM, respectively. The input of organic carbon from the ice sheet has been estimated to be insufficient to support reproductive growth of the entire lake community, and a sustained ecosystem would likely require a supplemental chemical energy source (Christner et al. 2006). Supplemental energy needed to support a sustained chemolithotrophic-based ecosystem (Fig. 4.2) may originate from sulfide and iron mineral substrates in subglacial debris (e.g., Bottrell and Tranter 2002; Tranter et al. 2002) entering the lake (Christner et al. 2006) and perhaps by geothermal input from deep faults within the bottom of the lake (Bulat et al. 2004).

4.3.3 Sampling Antarctic subglacial lakes

The exploration and direct sampling of Antarctic subglacial lake environments will require a substantial logistical effort (e.g., Priscu et al. 2003; Inman 2005; Siegert et al. 2007) and the implementation of protocols that introduce minimal chemical and microbial contamination to the pristine environment. The deployment of in situ observatories to measure the physical and chemical properties of the lake environment over spatial and temporal scales has been suggested as a prudent first step in the exploration of subglacial lake environments (Priscu et al. 2003). However, to study the physiology and diversity of microbial life in subglacial lake environments, it will be necessary to retrieve lake water column samples and sediments and return them to the surface while maintaining in situ pressures and temperature. The technological and logistical issues, together with concerns for environmental protection, make subglacial lake environments exigent systems for scientific study (National Research Council 2007). Despite these challenges, several projects are currently underway to drill into and sample subglacial lakes located beneath the ice sheet in East and West Antarctica.

The deepest ice core borehole at Vostok Station (designated borehole 5G) was drilled to a depth of 3,623 m in 1998 by a coordinated Russian, French, and American effort. Owing to concerns of contaminating the lake environment, drilling was terminated in a zone of accretion ice ~120 m above the water–ice interface. A solely Russian drilling effort began again in 2006 with

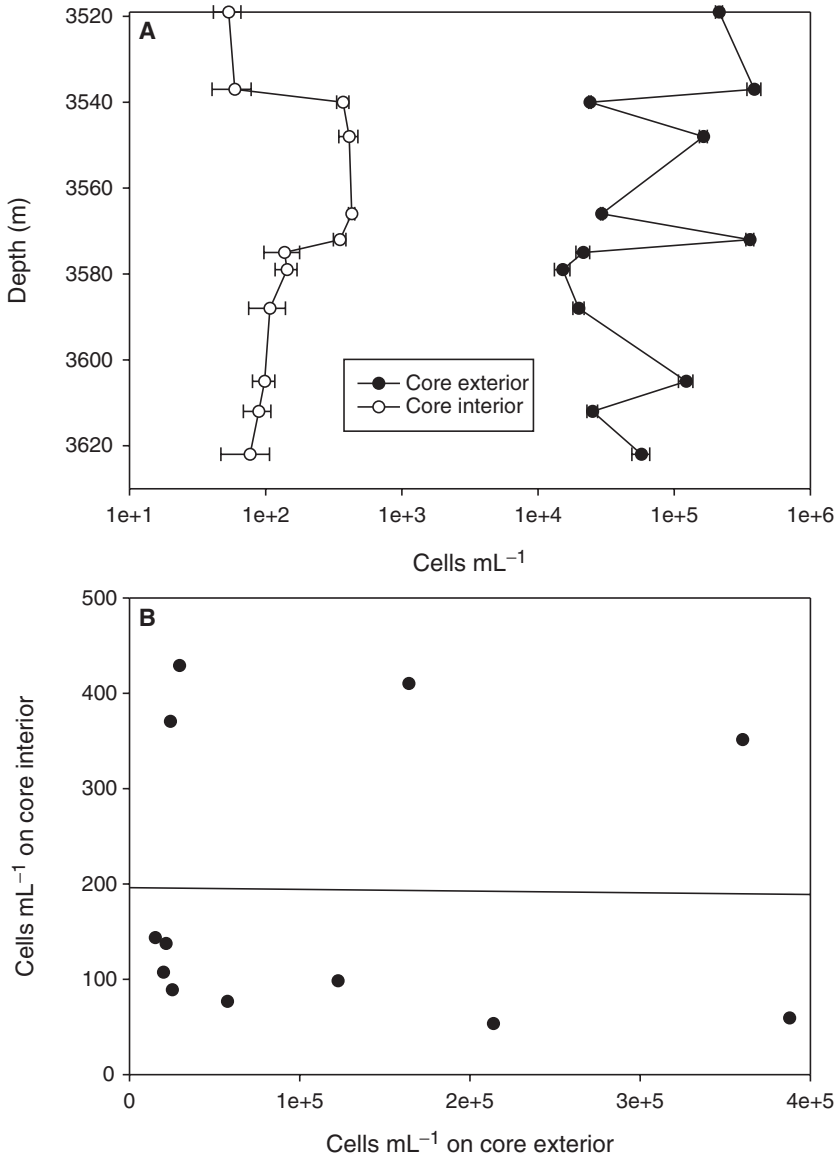

Fig. 4.1 **A** Concentration of cells on the exterior and interior of ice cores from the bottom ~100 m (i.e., accretion ice) of the Vostok 5G ice core. **B** Cell densities on the inside versus the outside of the ice core are statistically different ($r = 0.016$) and the data do not co-vary with depth (paired t-test, $p < 0.050$). The line is a regression plot of the data points. These data are not consistent with cells penetrating the ice core as a result of drilling (i.e. through microfractures in the ice) or via the drilling fluid, supporting the notion that bona fide cell concentrations were detected within the ice core interior. For extensive detail on the decontamination protocol and the cell enumeration method, see Christner et al. (2005, 2006)

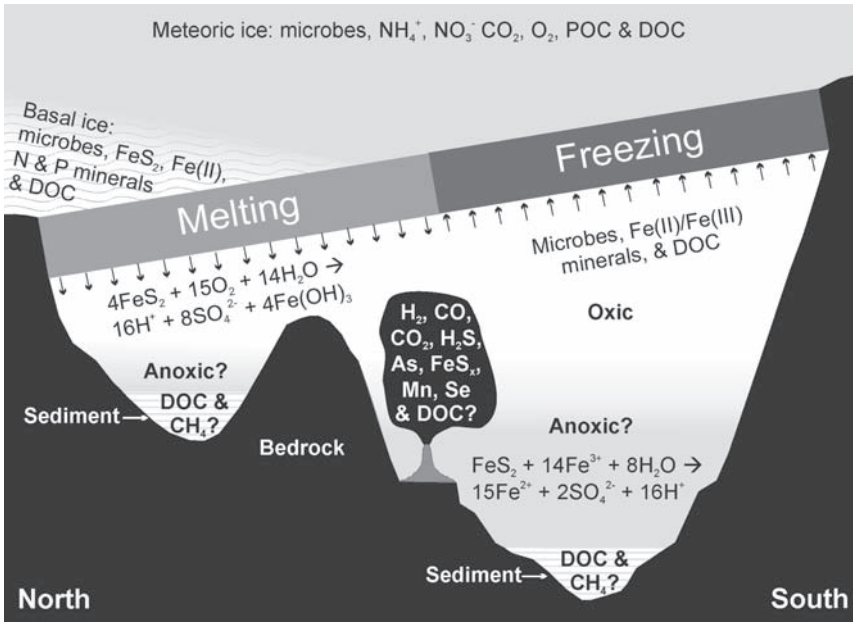

Fig. 4.2 Hypothetical scenario for chemically-driven biogeochemical reactions that could be used for bioenergetics in Lake Vostok. Inputs to the system (northern portion of the lake; see text) are through the melting of basal ice, which contains crushed sulfide and iron minerals and organic material from the bedrock, and glacial ice, which provides a constant supply of oxidants (O_2 and NO_3^-), nutrients, and organic material. Microbes, minerals, and organic carbon are removed from the lake via the accretion ice (southern portion of the lake). Shown are oxic and anoxic chemolithotrophic reactions (i.e., metal sulfide oxidation) that have been documented in Alpine and Arctic subglacial environments (Bottrell and Tranter 2002; Tranter et al. 2002; Wadham et al. 2004). Fault vents may be present in the shallow embayment of the lake (Bulat et al. 2004), which could introduce significant amounts of thermal energy, geochemical energy, and organic carbon to the lake. If biotic and/or abiotic oxygen sinks exist in the lake, then the deep waters and sediments would be expected to be anaerobic. POC = particulate organic carbon; DOC = dissolved organic carbon

the removal of an additional 27 m of ice core (3,624–3,651 m; V. Lukin, personal communication). The Russian Antarctic Expedition plans to mechanically drill another ~75 m, replace the existing fluid in the bottom of the borehole with an "ecologically clean drilling fluid", and then penetrate the lake with a thermal drill (Pomelov 2003). Lake water entering the borehole will eventually freeze and this material will be drilled and recovered for analysis in a subsequent field season.

An international collaboration of scientists is also preparing to drill into a subglacial lake in West Antarctica, named Ellsworth Subglacial Lake. The Lake Ellsworth Consortium (Siegert et al. 2007) have proposed to enter this lake by hot water drilling through the overlying 3,400 m of ice, lower an instrument probe to measure the biological, chemical and physical characteristics of the

lake water and sediments, and then return water and sediment samples to the surface for analysis. Hot water drilling (e.g., Fig. 4.3C–E) uses melted snow as the water source, which is heated to 80–90°C and pumped out of the drill tip. The surface snow naturally turns into firn and then glacier ice through burial and

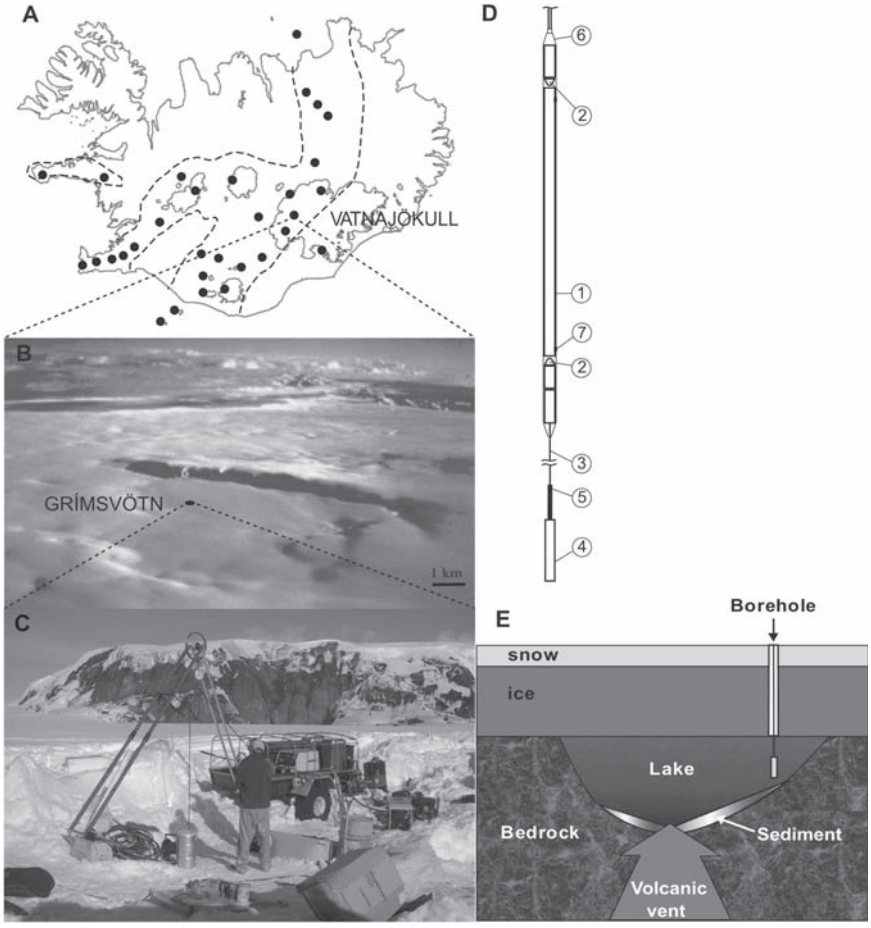

Fig. 4.3 **A** Location of zones of volcanic activity (*dotted lines*), volcanoes (*dots*), and major glaciers (including Vatnajökull) on Iceland. **B** Aerial view of the Grímsvötn caldera and ice shelf from the northwest. The approximate location of the drilling and sampling site. **C** Drilling operation on the Grímsvötn ice shelf. Modified from Gaidos et al. (2004). **D** Gas tight and water tight sampling device consists of a 400-ml sample chamber (*1*), open at both ends; a closure mechanism (*2*), comprising conical end-plugs driven by coil springs and a triggering mechanism consisting of a spring-loaded ratchet held closed by tension on a line (*3*) below which is suspended a weight (*4*) on a shock cord (*5*) and a load-bearing frame (*6*) which runs from the attachment point to the deployment cable to the triggering device beneath the sample bottle. The sample is retrieved using the sampling port (*7*). Modified from Gaidos et al. (2007). **E** Schematic cutaway diagram of the subglacial caldera lake, with borehole and water sampler. (Not to scale)

compaction and is eventually melted at the base of the ice into the lake. Thus, the hot water drilling is simply speeding up the natural process of transferring snow into the lake. Heating the water to >80°C in the drilling system likely causes macromolecular degradation, and when combined with inline filtering, the number of viable cells introduced into the lake via drilling can be minimized. Siegert et al. (2007) argue that Lake Ellsworth is a good initial candidate for subglacial lake exploration due to the lake's small size (10 km length × 10 km width × 250 m deep; lake volume ~25 km^3), comparable pressure and temperature to other subglacial lakes, accessibility to existing infrastructure (United Kingdom and United States field operations), and lower ice surface altitude (compared with the East Antarctic Ice Sheet), which reduces issues associated with human and mechanical performance at higher altitudes and colder temperatures. The sampling efforts described above are sure to make the next decade a very exciting time for subglacial lake research. Current investigations and techniques for sampling ice-entrapped lakes occurring elsewhere provide a training ground for testing sampling technology and an indication of the microbial species and metabolic lifestyles that may exist in the numerous lakes beneath the Antarctic ice sheet.

4.3.4 Subglacial caldera lakes

Smaller subglacial lakes have also been found in volcanic calderas beneath Icelandic ice caps. Hydrothermal vent activity results in melting of the overlying ice and filling of the subglacial caldera with water to form a subglacial lake (Fig. 4.3A). The overlying ice on Vatnajokull is ~250–300 m thick and water depths in the lakes vary from 20–140 m (Gaidos et al. 2004). These lakes typically drain catastrophically beneath the ice resulting in an outburst flood, which is termed a jokulhlaup. The draining and filling cycle for these subglacial lakes over the past four decades has ranged from one to less than ten years (T. Thorsteinsson, personal communication).

Two Icelandic subglacial lakes, one in the Grímsvötn Caldera (Fig. 4.3B) and one in the western Skaftárkatlar Cauldron, have been accessed via hot water drilling of a borehole into the lake from the ice surface. Analysis of the water column samples demonstrated that Grímsvötn Subglacial Lake contains a viable microbial assemblage (Gaidos et al. 2004). Molecular analysis of the Grímsvötn water samples indicates that the lake assemblage is distinct from the assemblages of organisms in the borehole water (before lake penetration) and overlying ice and snow. Sequencing of selected 16S rRNA gene fragments amplified from DNA extracted from the lake water revealed phylotypes with high identity to psychrophilic organisms (Gaidos et al. 2004). The uptake of ^{14}C-labeled bicarbonate in dark, low-temperature incubations of lake water samples indicates the presence of chemoautotrophs (Gaidos et al. 2004).

4.4 Adaptations, bioenergetics, and cosmopolitan genera in subglacial environments

4.4.1 Molecular adaptations for survival in icy environments

Microorganisms in subglacial environments are susceptible to the physical and osmotic stress associated with freezing and thawing during regelation. Ice crystals initially form in the extracellular phase and solute exclusion draws water from the cell, damaging the integrity of the cell membrane. Many plants, animals, and microbes adapted to freezing conditions therefore produce compatible solutes (e.g., proline, betaine, glycine, and trehalose) which reduce the shock of an osmotic imbalance (Tanghe et al. 2003). Thermal hysteresis antifreeze proteins may also be important to survival in subglacial environments, as these cold-induced proteins function to prevent damage initiated by intra- and extra-cellular ice crystal formation.

It is also possible that microorganisms entrapped within the solid basal ice of a glacier may remain metabolically active. This is an important transient phase in the subglacial environment due to melting and refreezing of water at the glacier bed.

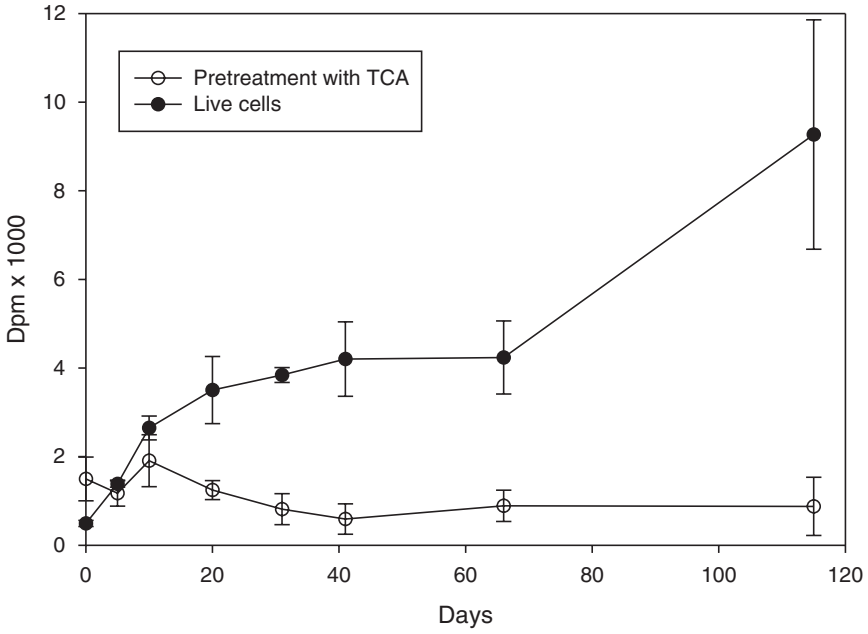

Fig. 4.4 Incorporation of [^{3}H]thymidine into TCA-precipitable material by frozen suspensions of *Sporosarcina* species TGTB5-5B at −15°C. The experiment was conducted for 115 days in a media with a total dissolved solid concentration of ~10^{-4} M. Freezing conditions and experimental details were as described by Christner (2002)

During freezing, cells are excluded into the interstitial liquid veins that exist between three grain boundaries in polycrystalline ice (Mader et al. 2006), similar to chemical impurities in the ice (Nye 1992). Figure 4.4 shows the incorporation of thymidine under frozen conditions (−15°C) by a psychrophilic bacterium isolated from the basal ice of Taylor Glacier, Antarctica. Based on these results and because the in situ temperature of the basal ice at Taylor Glacier is −17°C, it is possible that bacteria entrapped in basal ice metabolize in the aqueous veins between grain boundaries (e.g., Price 2000).

Determining the molecular and physiological adaptations of microbial life in subglacial environments will yield information that is vital for understanding the evolution and ecology of microorganisms inhabiting the deep cold biosphere, and may well yield biotechnologically relevant information for the identification of enzymes with improved cold-active properties.

4.4.2 Resistance to high oxygen concentrations

The precursor to glacial ice is firn, which is composed of granularized and compacted snowflakes. As the overlying snowfall accumulates and applies pressure, firn crystals glide and bond to other crystal planes, effectively squeezing intervening air spaces together into ice-entrapped bubbles that comprise ~10% (by volume) of the ice (Paterson 1994). At increasing depth, the gas bubbles are compressed into clathrate hydrates, which consist of a cage of water molecules around a gas molecule and are only stable at high pressure and low temperature.

In Lake Vostok, glacial ice melts into the northern portion of the lake, continually introducing air clathrates to the system. The accretion ice is essentially gas-free relative to the overlying glacial ice and very high (2.51 kg^{-1} water) dissolved gas levels have been estimated to exist in Lake Vostok and the dissolved oxygen concentration has been predicted to be ~50 times higher than air-equilibrated water (McKay et al. 2003). Metabolic activity at high oxygen concentrations inevitably results in the formation of hydrogen peroxide and superoxide by O_2-utilizing enzymes, which damages proteins, lipids, and DNA. Microorganisms capable of tolerating oxygen possess detoxifying enzymes such as catalase and superoxide dismutase. Resistance to oxidative stress is important for surviving freezing (Tanghe et al. 2003), and this may also be a vital trait for survival at the high oxygen concentrations predicted in Lake Vostok.

4.4.3 Survival under oligotrophic conditions

The low organic carbon concentration in the subglacial environment may select for heterotrophic species capable of surviving in oligotrophic conditions and extended periods of no growth. Low concentrations of dissolved organic carbon have been

reported in Lake Vostok (17–250 µM, estimated from the accretion ice: Priscu et al. 1999; Karl et al. 1999; Christner et al. 2006) and in subglacial meltwaters and basal ice in the high Arctic (8–100 µM: Skidmore et al. 2000; Barker et al. 2006) and glacial meltwaters of the Canadian Rocky Mountains (13–64 µM: Lafreniere and Sharp 2004). Miteva and Brenchley (2005) report that cells <0.1 μm^3 dominated the populations of cells in the GISP2 ice core and they also cultivated a number of bacterial isolates that are close phylogenetic relatives of known "ultramicrobacteria". *Sphingopyxis alaskensis* (formerly *Sphingomonas alaskensis*) is the most extensively studied ultramicrobacterium (e.g., Eguchi et al. 2001) and this microorganism is highly resistant to environmental stress (i.e., heat, hydrogen peroxide, and ethanol), is capable of growth at low organic carbon concentrations (~65 µM dissolved organic carbon), and maintains a small cell size (0.03–0.07 um^3) even when it is exposed to rich culture media.

Particle analysis, using flow cytometry, of two glacial and one accretion ice section from the Vostok core, revealed a pattern that contrasts the data of Miteva and Brenchley (2005). Total particles densities in the Vostok ice samples ranged from 4.4×10^4 to 1.9×10^5 ml^{-1} in glacial ice from 2,334 and 1,686 m, and 1.2×10^4 ml^{-1} in accretion ice from 3,612 m (Table 4.1). These data corroborate published data (Christner et al. 2006) that the accretion ice has lower total particle densities than the overlying glacial ice. The mean and median sizes of the biotic particles were 3.8, 3.4 and 4.6 µm for these same cores. The large biotic particles detected may, however, be illusory, representing cells that have aggregated or are attached to larger abiotic particles. We have observed cellular aggregation when viewing SYBR Gold stained ice core samples with epifluorescence microscopy and have shown with SEM that many of the bacteria in Vostok accretion ice are attached to particles of lithogenic origin (Fig 4.5; Priscu et al. 1999). A better representation of biotic particle size may be given by the mode. The modes for the ice cores from 1,686, 2,334 and 3,612 m were 0.7, 3.5 and 0.7 µm, respectively, which were 20%, 93%, and 15% of the biotic population means. The majority of the particles from the Vostok ice cores were abiotic with biotic counts ranging from only 2.5 to 19.2% of the total particles.

Table 4.1 Particle size distribution (mean, median, mode; µm) and particle density (particles ml^{-1} ice; percent of total particles) for the biotic and abiotic fractions at three depths in the Vostok ice core. The size distributions were determined on the raw data and from Weibull distributions (parentheses)

Depth (m)	Mean (µm)	Median (µm)	Mode (µm)	Particles ml^{-1}	Particles ml^{-1} (% of total)
1686					
Biotic	3.41 (2.45)	2.27 (2.04)	0.74 (1.02)	4,610	2.5
Abiotic	0.67 (0.60)	0.58 (0.59)	0.52 (0.56)	181,536	97.5
2334					
Biotic	3.81 (3.74)	3.43 (3.36)	3.54 (2.41)	8,512	19.2
Abiotic	2.65 (2.63)	2.26 (2.22)	0.51 (1.18)	35,804	80.8
3612					
Biotic	4.60 (4.60)	3.52 (3.51)	0.68 (0.72)	1,278	10.3
Abiotic	2.76 (1.24)	1.27 (1.03)	0.53 (0.52)	11,090	89.7

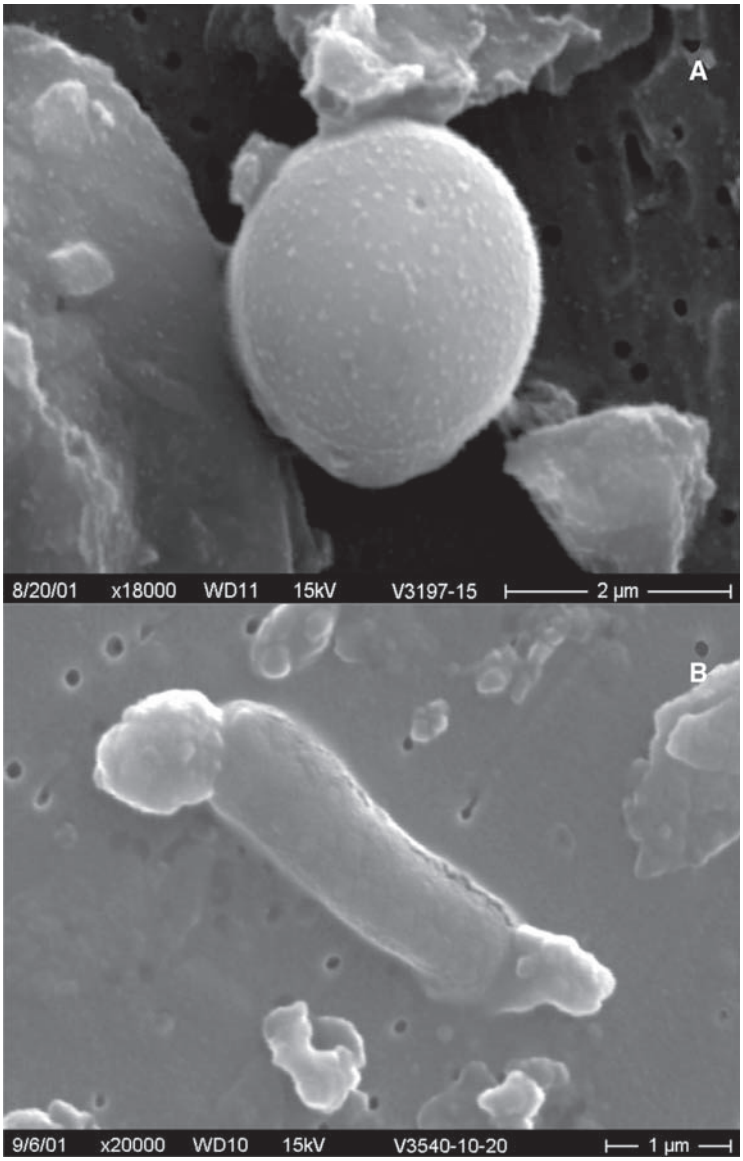

Fig. 4.5 SEM images showing bacterial cells aggregated with non-cellular material in Vostok cores from 3,197 m (**A**; glacial ice) and 3,540 m (**B**; accretion ice). Cryogenic SEM (JEOL-6100 SEM) and energy dispersive spectrometry (EDS) were used to image and analyze particles; EDS revealed that the non-cellular material was organic in origin

The ability to efficiently scavenge nutrients (i.e., small cells with high surface to volume ratios) and endure stress is likely to be valuable in terms of extended survival in all extreme environments, including those that exist deep beneath glacier ice. However, it is also possible that alternative energy sources and survival strategies are important in subglacial ecosystems.

4.4.4 Chemolithotrophy in subglacial environments

Glacier flow results in the comminution of mineral matrices in the underlying bedrock, releasing carbonate, sulfide, iron, and organic matter into the subglacial environment (Tranter et al. 2005). Geochemical evidence implies that the microbial oxidation of metal sulfides in glacial flour occurs in oxic and anoxic glacier bed environments (Bottrell and Tranter 2000; Tranter et al. 2002). Under oxic conditions, sulfide oxidation and heterotrophic activity will consume oxygen, eventually creating anoxia. Sulfide oxidation with Fe(III) as an oxidant can occur in the absence of oxygen, and sulfate reduction and methanogenesis are potential biogeochemical pathways for the anaerobic mineralization of organic matter. Thus, glacial physical processes may be sufficient to supply an energy source to microbes existing in subglacial environments.

Christner et al. (2006) suggest that sulfide and iron oxidation could serve as the basis for a chemolithotrophic food web in Lake Vostok (Fig. 4.2). Electron acceptors such as oxygen and nitrate are continually introduced into the lake through the melting of basal ice, and SO_4^{2-} is produced through the chemical weathering of sulfide minerals in the bedrock. There has been speculation regarding geothermal energy input from high-enthalpy mantle processes or seismotectonic activity (Bulat et al. 2004), which could introduce significant amounts of thermal energy and support an ecosystem similar to those found in deep-sea hydrothermal vents. However, since documented glaciological processes could supply subglacial lake ecosystems with nutrient and redox couples for microbial metabolism, the search for viable subglacial communities need not be exclusive to environments with geothermal input.

4.4.5 Do subglacial environments harbor endemic microbial species?

Microbiological investigations of glacier environments (i.e., cryoconite holes, subsurface glacial ice, accreted ice from Lake Vostok, subglacial sediment, and subglacial outflow and streams) in polar and non-polar locations indicate that strong phylogenetic relationships exist between bacteria from geographically distant environments (e.g., Priscu and Christner 2004). In plants and animals, allopatric speciation can occur when a geographically isolated population diverges from the parent population, resulting in the emergence of a genetically distinct species. Biogeographical relationships exist between cyanobacteria in hot springs (e.g., Papke and Ward 2004). However, the question remains as to whether geographically-separated glacier environments possess endemic or cosmopolitan species. .

Figure 4.6 shows the phylogenetic relationships (based on 16S rRNA gene analysis) between Alphaproteobacteria of the genera *Methylobacterim* and *Sphingomonas* isolated from glacial and subglacial environs in Antarctica (glacial ice, Lake Vostok accretion ice, cryoconite holes), Greenland (glacial and subglacial "silty" ice), China (glacial ice), and New Zealand (subglacial sediment). Also included in Fig. 4.6 are related strains and cloned sequences recovered globally from the deep sea, high mountain lakes, snow, sea ice, endolithic assemblages, cold

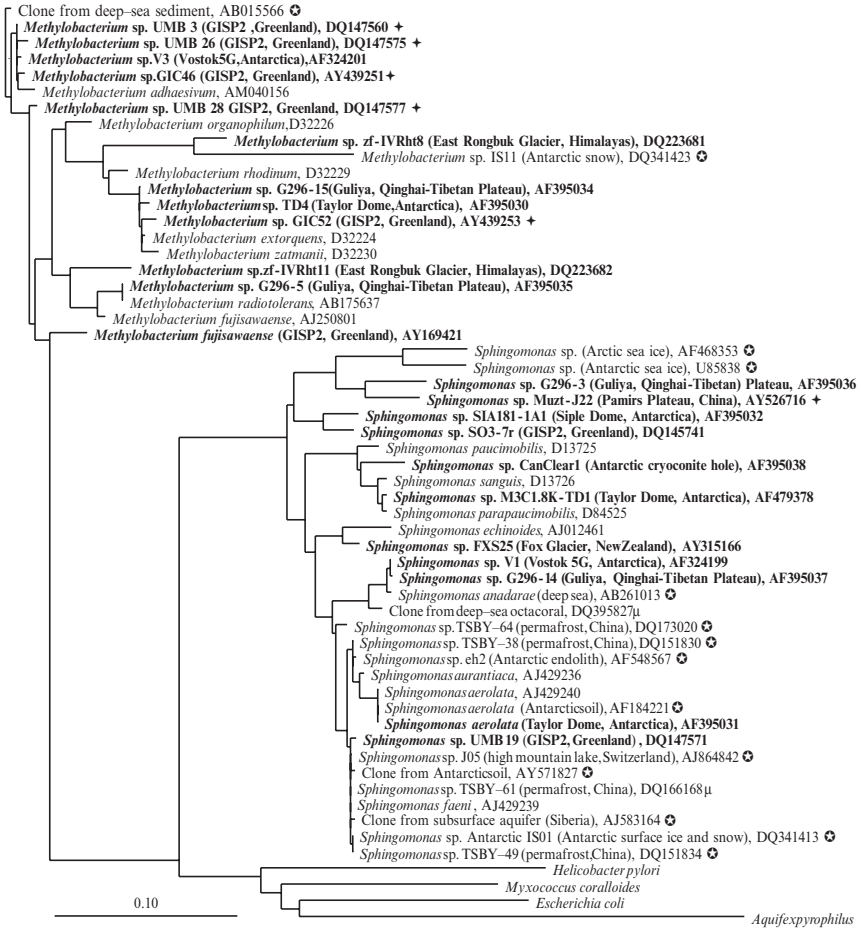

Fig. 4.6 Phylogenetic analysis of small subunit (16S) rRNA gene sequences from *Methylobacterium* and *Sphingomonas* species isolated from glacial and subglacial environments (in bold). The sequences were aligned on the basis of secondary structure and a 1,220-nucleotide mask (120-1,377, *Escherichia coli* 16S rRNA gene numbering) was used to generate the tree using the maximum likelihood method. The *scale bar* represents 0.1 fixed substitutions per nucleotide position. Branches labeled with a *white star* in a *black circle* are isolates and cloned sequences obtained from permanently cold and frozen environments; a *black star* designates lineages for which only short sequences (488–720 nucleotides) are available, and these sequences were added to the tree using the ARB parsimony insertion tool (http://arb-home.de/). The source environment is shown in parentheses, followed by the GenBank accession number

soil, and permafrost. The distribution of related bacteria in worldwide glacial and subglacial environments implies that some members of these genera evolved under cold circumstances and likely possess similar strategies to survive freezing and, possibly, to metabolize at low temperatures. While the phylogeny of a single gene (i.e., 16S rRNA; Fig. 4.6) is not sufficient to resolve fine scale evolutionary relationships, analysis of multiple loci and recent advances in genomic sequencing

technology make these type of experiments now feasible and cost-effective. Due to the isolated nature of subglacial environments, these systems may represent promising evolutionary models for investigating bacterial endemism and to test theory-based species concepts (e.g., Cohan 2002).

4.5 Conclusions

Despite the fact that >80% of the biosphere (by volume) is permanently below 5°C and most of the biomass is microbial (Priscu and Christner 2004), very little is known about the biology of microorganisms inhabiting permanently cold environments. Biologists have studied life on the margins and surfaces of glaciers for nearly a century, but until recently, the subglacial environment was thought to be inhospitable for life. The discovery of active microbial assemblages beneath glaciers and realization that large quantities of liquid water exist beneath polar ice sheets has resulted in a new paradigm in the study of life on Earth.

Knowledge of microbial life in subglacial ecosystems is limited due to sparse data and the technological, financial, and environmental challenges associated with sampling such cold and remote subsurface environments. Considerable progress has been made over the last 10 years in the exploration and study of subglacial environments, permitting a glimpse of the microbial life that exists under conditions of high pressure, cold temperature, low nutrient input, and no sunlight. Priscu et al. (2007) estimate that the number of cells and organic carbon content in Earth's glaciers and subglacial environs ($4 \times 10_{29}$ cells and 10 Pg C) exceeds that reported for the Earth's surface freshwater lakes and rivers ($1.3 \times 10_{26}$ cells and 0.5 Pg C) and is close to that for the open ocean. These tentative estimates imply that glaciated environments contain a considerable pool of cells and organic carbon, and the deep cold biosphere may represent a significant and previously unknown global source of CO_2 and CH_4 (Sharp et al. 1999). As such, biogeochemical cycling models assuming zero rates of microbial mineralization in glacially-overridden soils may underestimate the flux of CO_2 and CH_4 released to the atmosphere during glacial to interglacial transitions.

The study of ecosystems in the cold deep biosphere also has implications for the natural history and evolution of life on Earth, as well as on icy planets and moons in the solar system. Geological evidence indicates that a long period of low latitude pervasive global glaciation occurred during the late Proterozoic, referred to as a "Snowball Earth". Hoffman et al. (1998) argue that the planet was completely covered in ice for at least 10 million years, and liquid water only existed in the ocean under a thick ice cover. If this scenario is accurate, such a long period of global freeze would have had drastic consequences on ecosystems established prior to this event, and subglacial environments may have provided an important refuge for life during such an extended ice age. Polar ice caps composed of water ice exist on Mars, there is evidence for glaciers at lower latitudes during times of higher obliquity (Head et al. 2005), and the jovian moon Europa is thought to maintain a 50- to 100-km-deep liquid ocean under a 3- to 4-km-thick ice shell (Turtle and Pierazzo 2001).

Thus, the study of cold, dark, subglacial environments on Earth will provide insight as to the likelihood of microbial life surviving and persisting in icy extraterrestrial environments. Furthermore, the challenge of identifying appropriate extraterrestrial sites for exploration and developing technology to sample icy subsurface environs will directly benefit from the experience gained by studying earthly analogs.

Subglacial environments remain one of the last unexplored frontiers on our planet. While the study of microbial communities that function near the freezing point of water is inherently interesting, these ecosystems are also clearly relevant to determining the boundaries for life in the biosphere, biogeochemical cycling, the natural history of life on Earth, and astrobiology. We can therefore expect subglacial exploration to be at the forefront of cryospheric research in the future and the years to follow should prove to be an interesting time of discovery.

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