Prokaryotic diversity of arctic ice shelf microbial mats

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Summary

The prokaryotic diversity and respiratory activity of microbial mat communities on the Markham Ice Shelf and Ward Hunt Ice Shelf in the Canadian high Arctic were analysed. All heterotrophic isolates and > 95% of bacterial 16S rRNA gene clone library sequences from both ice shelves grouped within the phyla *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. Clone library analyses showed that the bacterial communities were diverse and varied significantly between the two ice shelves, with the Markham library having a higher estimated diversity (Chao1 = 243; 105 operational taxonomic units observed in 189 clones) than the Ward Hunt library (Chao1 = 106; 52 operational taxonomic units observed in 128 clones). Archaeal 16S rRNA gene clone libraries from both ice shelves were dominated by a single *Euryarchaeota* sequence, which appears to represent a novel phylotype. Analyses of community activity by radio-respiration assays detected metabolism in mat samples from both ice shelves at temperatures as low as –10°C. These findings provide the first insight into the prokaryotic biodiversity of Arctic ice shelf communities and underscore the importance of these cryo-ecosystems as a rich source of microbiota that are adapted to extreme cold.

Introduction

The areas of Earth that sustain life are dominated by cold environments, with approximately 75% of the biosphere existing at temperatures below 5°C (Cavicchioli, 2006). Research of these vast expanses of frigid habitat has recently revealed the importance of these environments as complex, active, ecosystems, home to diverse and

unique microbial communities (Deming, 2002). Studies of ice bound ecosystems are providing insight into how life may have existed when Earth experienced periods of widespread glaciations in the Paleoproterozoic (2.4 billion years ago) and Neoproterozoic (550–800 million years ago) periods (Vincent *et al.*, 2000). Microbial ecosystems in cryo-environments are also of relevance to astrobiology, particularly with knowledge that the polar regions of Mars and the moons of Jupiter and Saturn contain stocks of frozen water capable of supporting life in the past or present (Kivelson *et al.*, 2000; Malin and Edgett, 2000; Jakosky *et al.*, 2003; Lorenz, 2003; Schulze-Makuch *et al.*, 2005). Finally, the study of microbiology in cold environments is highly relevant to biotechnology and bioremediation; cold-adapted organisms and their enzymes are increasingly sought after and exploited in the food, chemical and textile industries, and for the remediation of contaminated cold environments (Gerday *et al.*, 2000). The impetus to increase our understanding of cold ecosystems has led to the description of microbial assemblages in other cryo-environments, including sea ice (Brown and Bowman, 2001; Brinkmeyer *et al.*, 2003; Junge *et al.*, 2004), lake ice (Mosier *et al.*, 2007), permafrost (Vishnivetskaya *et al.*, 2006; Steven *et al.*, 2007a) and glacial systems (Christner *et al.*, 2003; Skidmore *et al.*, 2005; Stibal *et al.*, 2006; Zhang *et al.*, 2007); however, many cold temperature systems remain little characterized to date. Ice shelves are regions of landfast sea ice that exist as perennial ecosystems in the Arctic and Antarctica. In both polar regions, these cryo-environments provide unique habitats for complex microbial mat communities (Vincent *et al.*, 2000; 2004). These communities exist frozen and intact within the ice shelves through most of the year, and emerge seasonally in meltwater pools that form atop the ice shelf in summer months. These communities face many harsh environmental stresses, including constant exposure to low temperatures, fluctuations in salinity and high exposure to UV radiation during the summer months (Mueller *et al.*, 2005). Under these summer conditions, the phototrophic biomass demonstrates high productivity and mats can be seen to make up the majority of the biomass in ice shelf ecosystems (Mueller *et al.*, 2005). In addition to the large abundance of cyanobacteria (Vincent, 2007), the mat ecosystems from both polar regions contain bacteria, diatoms, chlorophytes, flagellates, ciliates, nematodes, tardigrades and rotifers (Vincent *et al.*, 2000). Despite the diversity and complexity of these communities, analyses

of microbial mats from Arctic and Antarctic ice shelves have been predominantly restricted to the cyanobacterial components (Vincent *et al.*, 2004; Jungblut *et al.*, 2005).

The Ward Hunt and Markham ice shelves, located off the northern coast of Ellesmere Island in the Canadian high Arctic, are home to the richest reported Arctic ice shelf microbial mat communities. These ice shelves are among the remnants of a larger ice shelf that has been in place for at least 3000 years, but that has contracted 90% during the last century from 9000 km^2 to its current fragment sizes (Vincent *et al.*, 2004). Meltwater pools atop the ice shelves in summer months resemble dilute seawater (Vincent *et al.*, 2004), which remain predominantly as freshwater systems through the summer melt and become saline during freeze-up (Mueller *et al.*, 2005). Mat communities exist submerged within the meltwater pools and on raised mounds of ice with little or no water cover. Mats at both ice shelves share similar architectures, although the mat material found on the Markham Ice Shelf is generally more developed than that on the Ward Hunt Ice Shelf. Various cyanobacteria (*Nostoc*, *Phormidium*, *Leptolyngbya*, *Gloeocapsa*) and eukaryotic algae (*Chlorosarcinopsis*, *Pleurastrum*, *Palmellopsis*, *Brateococcus*) were identified in these mats and found to be most abundant in the top 100–500 μm of mat material (Vincent *et al.*, 2004). High concentrations of non-cyanobacterial prokaryotes have been observed in these communities, but their phylogenetic diversity has not been assessed. As such, the prokaryotic communities in these mats remain largely uncharacterized.

The objective of this study was to evaluate the prokaryotic diversity, microbial distribution and metabolic activity in the mats on the Ward Hunt and Markham ice shelves using a combination of culture-dependent and culture-independent techniques. This study represents the first detailed description of the prokaryotic assemblages in the microbial mats of High Arctic ice shelves, the first molecular phylogenetic analyses of these mat communities and the first description of subzero metabolic activity in these mat communities.

Results

Site description

During the 2005 sampling, mats were found directly atop the ice at both the Ward Hunt and Markham ice shelves in meltwater pools and on raised mounds of ice. Temperature, pH and conductivity of the water overlaying the mats at each sample site are presented in Table 1. During freeze-up, the mats experience much more extreme chemical and physical conditions due to freeze concentration of solutes and liquid water temperatures below 0°C. During the 2006 sampling period, mats were found

Table 1. Characteristics of water in the meltwater pools at sampling sites on the Markham (MIS) and Ward Hunt (WIS) ice shelves in spring 2005.

Site	Location	Temperature (°C)	pH	Conductivity (mS cm^{-1})
MIS 1	83°01.90′N 71°27.15′W	1.5	7.39	1.83
MIS 2	83°01.90′N 71°29.72′W	3.4	7.20	2.28
WIS 1	83°04.87′N 74°25.84′W	1.0	8.26	0.53
WIS 2	83°04.87′N 74°25.84′W	1.0	7.98	1.22

frozen beneath approximately 45–55 cm of snow on both ice shelves, with snow temperatures directly overlaying mat samples of −7.0°C at the Markham Ice Shelf and −2.0°C at the Ward Hunt Ice Shelf. In both seasons, the mats consisted of dark organic material covered with a 0.5–1 mm layer of highly pigmented orange biomaterial. On the Markham Ice Shelf in 2006, the mat samples collected ranged from 60 to 100 mm in thickness. The dark organic material did not appear to be stratified by macroscopic observation, and anoxic black sediments reported previously in thick mats from Arctic and Antarctic ice shelves (Mueller *et al.*, 2005) were not observed in the samples analysed in this study. The mats sampled at the Markham Ice Shelf were generally classified as 'orange mat' and were more highly pigmented than those found on the Ward Hunt Ice Shelf which were classified as cohesive 'matlet' based on previous mat classifications (Mueller *et al.*, 2006). Microscopic analyses showed that both mats contained a high concentration of mineral particles, as observed previously (Mueller *et al.*, 2006).

Microbial enumeration and characterization

Cells with intact membranes, which were scored as live cells, were enumerated by direct microscopic counts of LIVE/DEAD *Bac*Light-stained samples. Mat material collected from the Markham Ice Shelf and the Ward Hunt Ice Shelf in the 2005 field season were found to contain 3.6×10^8 ($\pm 2.3 \times 10^7$) live cells per gram of mat and 4.0×10^8 ($\pm 6.9 \times 10^7$) live cells per gram of mat respectively. Samples collected in the 2006 field season revealed the presence of 2.3×10^8 ($\pm 1.6 \times 10^7$) live cells per gram of mat in Markham Ice Shelf samples and 6.9×10^8 ($\pm 5.0 \times 10^7$) live cells per gram of mat in Ward Hunt Ice Shelf samples. Cells with compromised membranes were not enumerated due to difficulties in distinguishing these cells from background fluorescence resulting from mat pigments and their degradation products, other organic matter and sediments captured on the filters.
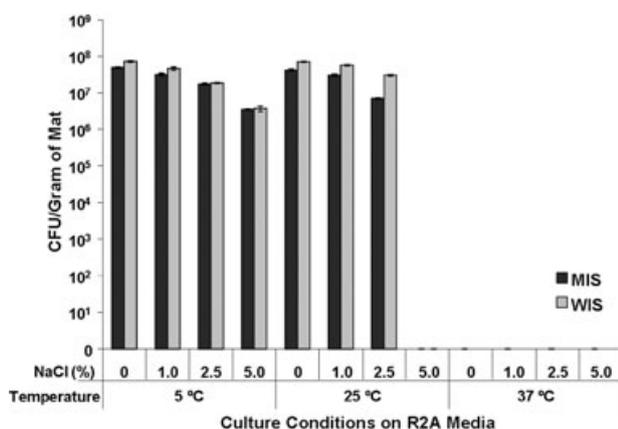
Fig. 1. Heterotrophic plate counts of microbial mat communities from the Markham (MIS) and Ward Hunt (WIS) ice shelves. Culturing was performed on R2A agar supplemented with 0%, 1.0%, 2.5% and 5.0% w/v NaCl and incubated at 5°C, 25°C and 37°C. Error bars indicate standard error.

Heterotrophic plate counts on R2A inoculated in the field in 2005 revealed the presence of 6.3×10^7 and 7.8×10^7 colony-forming units (cfu) per gram of mat at Markham Ice Shelf and Ward Hunt Ice Shelf respectively. Heterotrophic plate counts prepared in the laboratory from samples collected in 2005 revealed similar trends in salt sensitivity and temperature sensitivity in the cultured communities from both ice shelves (Fig. 1). No growth was observed in samples plated on Subzero medium, regardless of incubation temperature. Isolates obtained grouped into 34 phylotypes (defined as isolates sharing greater than 97% 16S rRNA gene sequence similarity). Of the phylotypes obtained, 8 were isolated solely from the Markham Ice Shelf samples, 13 were isolated from the Ward Hunt Ice Shelf samples and 12 were isolated from samples from both ice shelves. Phylogenetic groupings and observations of growth at various temperatures on R2A supplemented with various salt concentrations for the isolates are presented in Table 2.

Denaturing gradient gel electrophoresis (DGGE) analyses

Denaturing gradient gel electrophoresis (DGGE) analyses of bacterial 16S rRNA genes were performed to compare the microbial composition of the ice shelf microbial mats, and the DGGE banding patterns were used to construct dendrograms. DGGE analysis revealed many similarities in banding patterns between all of the mat samples analysed (Fig. 2). Although replicate samples collected from within the same ice shelf showed the highest degree of similarity to each other, the banding patterns of samples collected from the two different ice shelves were also similar. The DGGE profiles of the top and bottom layers of samples from each ice shelf also showed little variation.

Bacterial 16S rRNA gene clone libraries

Bacterial 16S rRNA gene clone libraries from both the Markham and Ward Hunt ice shelves were analysed in order to characterize the microbial biodiversity of the two ice shelves. Of the 189 clones analysed from the Markham library, 105 phylotypes were identified when grouping sequences with greater than 97% 16S rRNA gene sequence similarity. Of the 128 clones analysed from the Ward Hunt library, 52 phylotypes were identified. Statistical analyses of the two libraries revealed differences in composition, species richness and diversity (Table 3). The two libraries were determined to have significantly different compositions ($P < 0.05$) by analysis with WEBLIBSHUFF; the differences in composition were supported by comparing the libraries by the Sorensen index (Sorensen index = 0.53). Common measures of community richness and diversity were used to compare aspects of the communities between the two ice shelves. Calculations of community richness by the Chao1 (Markham Chao1 = 243.00; Ward Hunt Chao1 = 106.38) and ACE richness estimates (Markham ACE = 244.85; Ward Hunt ACE = 99.82) and community diversity by Shannon's index of diversity (Markham $H' = 4.41$; Ward Hunt $H' = 3.50$) and the reciprocal of Simpson's index of diversity (Markham $1/D = 95.52$; Ward Hunt $1/D = 25.52$) showed that the Markham Ice Shelf bacterial library had greater richness and diversity than that of the Ward Hunt Ice Shelf. Estimates of library coverage (Markham library coverage = 63.49%; Ward Hunt library coverage = 76.56%) and analysis of rarefaction curves (Fig. S1) also indicated higher diversity in the Markham library than the Ward Hunt library.
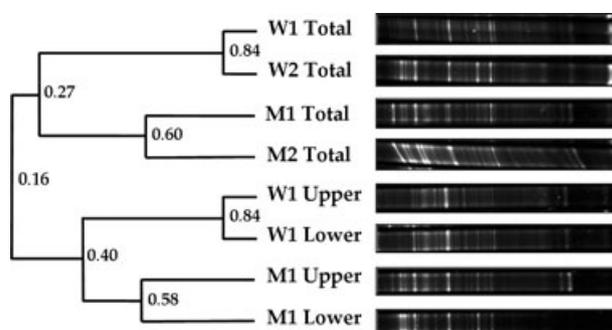

Fig. 2. Denaturing gradient gel electrophoresis (DGGE) analysis of bacterial 16S rRNA genes amplified from samples from two sites on the Markham Ice Shelf (M1 and M2), two sites on the Ward Hunt Ice Shelf (W1 and W2), as well as the upper and lower halves of core samples from M1 and W1. DGGE was run on an 8% acrylamide gel with a gradient of urea and formamide from 35% to 65% (left to right). A similarity matrix based on band position was used to construct a neighbour joining tree for comparison of banding patterns between lanes using Quantity One imaging software version 4.2.1 (Bio-Rad Laboratories, Hercules, CA).

Table 2. Growth characteristics and 16S rRNA gene analyses of isolates obtained from samples from the Markham and Ward Hunt ice shelves.

Isolate	Growth temperature -5°C	5°C	25°C	Growth at various NaCl concentrations in R2A 0%	1.0%	2.5%	5.0%	Closest cultured BLAST match	Origin of BLAST culture	Similarity to BLAST match	DP grouping
M73/W2	+	+	+	+	+	+	+	*Cryobacterium* sp. SCO M20 (DQ628922)	John Evans Glacier	100% (691/691)	*Microbacteriaceae* (family)
M22/W18	−	+	+	+	+	+	+	*Caulobacteraceae* bacterium (DQ628966)	John Evans Glacier	100% (665/665)	*Brevundimonas* (genus)
M2/W32	−	+	+	+	+	+	−	*Caulobacteraceae* bacterium (DQ628967)	John Evans Glacier	100% (664/664)	*Brevundimonas* (genus)
M71/W64	+	+	+	+	+	+	+	*Cryobacterium* sp. KAR22 (EF451652)	Arctic permafrost	99% (712/716)	*Cryobacterium* (genus)
M3/W48	+	+	+	+	+	+	+	*Cryobacterium* sp. PIC-C9 (DQ227776)	Puruogangri glacier	99% (700/704)	*Microbacteriaceae* (family)
M13/W25	+	+	+	+	+	+	−	*Sphingomonadaceae* bacterium (DQ497241)	Svalbard snow cover	99% (657/659)	*Sphingomonadaceae* (family)
M38/W43	+	+	+	+	+	+	+	*Actinobacterium* gap-f-58 (DQ530475)	Arctic sea ice	99% (652/656)	*Leifsonia* (suborder)
M7/W20	−	+	+	+	+	+	+	*Cellulomonas* sp. RODSPM19 (EF451735)	Arctic permafrost	99% (679/683)	*Micrococcineae* (suborder)
M1/W26	−	+	+	+	+	+	+	Denitrifying bacterium W99 (AB162104)	Upland soil	97% (671/691)	*Comamonadaceae* (family)
M15/W66	−	+	+	+	+	−	−	*Cellulomonas fermentans* (X83805)	Urban waste	96% (651/675)	*Micrococcineae* (suborder)
M27/W9	−	+	+	+	+	+	+	*Lysobacter* sp. Dae08 (AB249682)	NA	95% (659/692)	*Xanthomonadaceae* (family)
M11/W23	−	+	+	+	+	−	+	*Sphingobacterium* sp. OM-E81 (AB020206)	NA	92% (634/687)	*Pedobacter* (genus)
W1	−	+	+	+	+	+	+	α-*Proteobacterium* (DQ234536)	Mire pond sediment	100% (672/672)	*Sphingomonas* (genus)
W27	−	+	+	+	+	+	−	*Panaciterramonas fulva* (AB245357)	Ginseng field	99% (702/703)	*Aquabacterium* (genus)
W24	−	+	+	+	+	+	+	γ-*Proteobacteria* SOCA20 (DQ628970)	John Evans Glacier	99% (697/704)	*Lysobacter* (genus)
W5	−	+	+	+	+	+	+	Arctic sea ice bacterium ARK10037 (AF468359)	Arctic sea ice	99% (602/603)	*Devosia* (genus)
W28	−	+	+	+	+	+	+	*Aequorivita anatarctica* isolate S4-8 (AY771732)	Antarctic marine site	98% (690/703)	*Aequorivita* (genus)
W8	−	+	−	+	+	−	−	Unidentified bacterium oxSCC-37 (AJ387875)	Paddy soil	98% (703/712)	*Polaromonas* (genus)
W21	−	+	−	+	−	−	−	*Sphingomonas* sp. SIA181-1A1 (AF395032)	Vostok accretion ice	98% (643/652)	*Sphingomonas* (genus)
W53	−	+	+	+	+	+	−	α-*Proteobacterium* AP-22 (AY145559)	Weser Estuary	98% (642/649)	*Cryobacterium* (genus)
W19	−	+	+	+	+	+	−	Antarctic bacterium R-7666 (AJ440984)	Antarctic lake mat	96% (688/714)	*Flavobacterium* (genus)
W70	−	+	+	+	+	+	+	Glacial ice bacterium CanDirty14 (AF479331)	Glacial ice	95% (657/686)	*Flavobacteriaceae* (family)
W3	−	+	+	+	+	+	+	*Algibacter gromonii* KMM6038 (DQ312294)	Sea urchin	93% (646/688)	*Algibacter* (genus)
W22	−	+	−	+	+	+	−	*Pseudoxanthomonas taiwanensis* (AB210278)	Activated sludge	93% (559/599)	*Xanthomonadaceae* (family)
W6	−	+	+	+	+	+	+	*Ginsengisolibacter pocheensis* (AB267722)	Ginseng field	92% (593/640)	*Pedobacter* (genus)
M31	−	+	−	+	+	+	−	Arctic sea ice bacterium ARK10281 (AF468446)	Arctic sea ice	99% (707/711)	*Rhodoferax* (genus)
M20	−	+	+	+	+	+	−	Arctic sea ice bacterium ARK10157 (AF468345)	Arctic sea ice	99% (684/687)	*Oxalobacteraceae* (family)
M33	−	+	+	+	+	+	+	Arctic sea ice bacterium ARK10173 (AF468440)	Arctic sea ice	99% (696/698)	*Leifsonia* (genus)
M19	−	+	+	+	+	+	+	Antarctic bacterium R-9284 (AJ441011)	Antarctic lake mat	98% (712/724)	*Hydrogenophaga* (genus)
M6	−	+	+	+	+	+	+	*Actinobacterium* SOC A20 (DQ628959)	John Evans Glacier	98% (660/670)	*Micrococcineae* (suborder)
M10	−	+	−	+	+	+	+	*Dokdonella koreensis* DS-140 (AY987369)	Soil	93% (649/694)	*Xanthomonadaceae* (family)
M8	−	+	+	+	+	+	+	*Flavosolibacter ginsengiterrae* (AB267477)	Ginseng field	93% (661/688)	*Chitinophaga* (genus)
M16	−	+	+	+	+	+	+	Bacterium DG10219 (AY258128)	Marine dinoflagellate	91% (526/574)	*Bacteroidetes* (phylum)

Cultured representatives from the BLAST analysis whose isolation sources were not available in GenBank were marked NA.

Table 3. Statistical analyses of bacterial 16S rRNA gene clone libraries constructed from samples from the Markham (MIS) and Ward Hunt (WIS) ice shelves.

	MIS	WIS
Clones analysed	189	128
Operational taxonomic units	105	52
Library coverage (%)	63.49	76.56
Chao1 richness estimate	243.00	106.38
ACE richness estimate	244.85	99.82
Shannon's diversity index (*H'*)	4.41	3.50
Simpson's diversity index 1/*D*	95.52	25.52
Sorensen index	0.53	

Grouping of operational taxonomic units and all statistical analyses were calculated based on groupings of sequences at 0.02% difference in DOTUR.

Despite the apparent difference in diversity, there were some similarities in community structure between the two sites, with representatives of the same phyla found to dominate both libraries (Fig. 3). The Markham library was dominated by the three phyla *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*. Sequences grouping in the phyla *Gemmatimonadetes*, *Firmicutes* and *Verrucomicrobia* were also present in the library and represented less than 3% of the sequences in the library. Approximately 3% of the sequences in the library did not group well with any phylum. Members of the orders *Sphingobacteriales* and *Flavobacteriales* were found to comprise 71% and 9% of the *Bacteroidetes* phylum, respectively, while 20% of the sequences grouped in this phylum were unclassified. Approximately 94% of *Actinobacteria* sequences were of the subclass *Actinobacteridae*, and 6% of the sequences remained unclassified in the phylum. The phylum *Proteobacteria* had representatives of the classes γ-*Proteobacteria* (15%), δ-*Proteobacteria* (21%), β-*Proteobacteria* (38%) and α-*Proteobacteria* (23%). Approximately 86% of γ-*Proteobacteria* sequences grouped within the order *Xanthomonadales*, and

sequences grouping in the order *Pseudomonadales* and unclassified γ-*Proteobacteria* each made up 7% of the class. Approximately 86% of β-*Proteobacteria* sequences were found to group in the order *Burkholderiales*, and 3% of sequences grouped in the order *Hydrogenophilales* and 11% of sequences remained ungrouped in this class. The classes δ-*Proteobacteria* and α-*Proteobacteria* had representatives of multiple orders, with the δ-*Proteobacteria* being comprised of sequences representative of the orders *Desulfobacterales* (50%), *Desulfuromonales* (15%), *Myxococcales* (10%) and unclassified δ-*Proteobacteria* (25%). The α-*Proteobacteria* were comprised of sequences from the orders, *Rhizobiales* (36%), *Rhodobacterales* (27%), *Sphingomonadales* (23%), *Rhodospirillales* (9%) and *Caulobacterales* (5%). A total of 4% of the sequences grouping within the phylum *Proteobacteria* were unclassified within the phylum.

The Ward Hunt library was also found to be dominated by the phyla *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, with all sequences grouping within these phyla except for 2% of sequences that grouped in the phylum *Fibrobacteres* and 4% of sequences which did not group well with any phylum. Members of the order *Sphingobacteriales* made up 78% of the *Bacteroidetes* phylum, with the other 22% of the phylum composed of unclassified *Bacteroidetes* sequences. Members of the subclass *Actinobacteridae* made up 95% of the *Actinobacteria* phylum, while sequences grouping in the *Rubrobacteridae* subclass and unclassified *Actinobacteria* each made up ~3% of the sequences in the phylum. *Proteobacteria* had representatives of the classes γ-*Proteobacteria* (7%), δ-*Proteobacteria* (9%), β-*Proteobacteria* (48%) and α-*Proteobacteria* (35%). All representatives of the γ-*Proteobacteria* grouped within the order *Xanthomonadales* while ~77% of β-*Proteobacteria* sequences grouped in the order *Burkholderiales* and the remaining 23% of sequences in this class represented unclassified
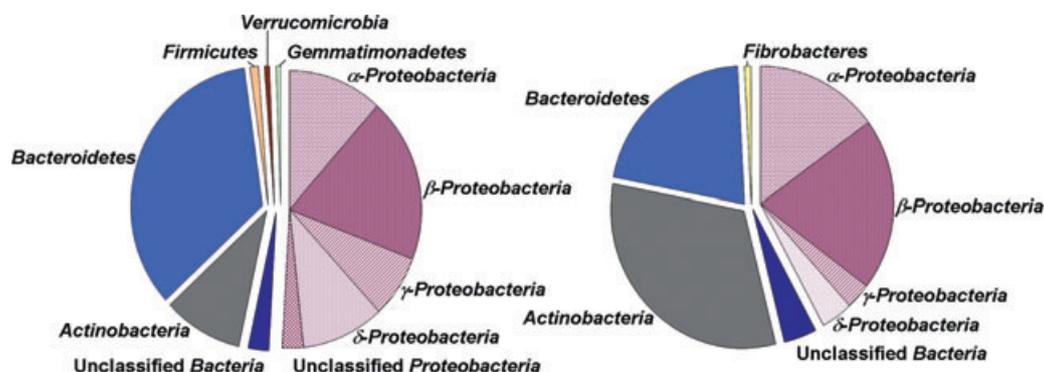

Fig. 3. Phylogenetic groupings of sequences from 16S rRNA gene clone libraries constructed from samples from the Markham (left) and Ward Hunt (right) ice shelves. The number of sequences in each phylum is represented as a percentage of the total 189 sequences analysed from the Markham library and 128 sequences analysed from the Ward Hunt library. Sequences were grouped using the RDP Classifier function of the Ribosomal Database Project-II release 9 with a confidence threshold of 80%.

β-*Proteobacteria*. The class δ-*Proteobacteria* contained members of the two orders *Desulfobacterales* (80%) and *Myxococcales* (20%). The class α-*Proteobacteria* contained representatives of the orders *Sphingomonadales* (42%) *Rhodobacterales* (32%), *Rhizobiales* (21%) and *Caulobacterales* (5%).

Phylogenetic trees constructed to analyse groupings of sequences from the clone libraries and cultured representatives obtained in this study indicated some similarities in community structure between the two ice shelves. Many sequences from the two different libraries clustered within the *Proteobacteria* (Fig. 4) with remaining sequences clustering with other phyla (Fig. 5). The phylogenetic trees also reflect diversity in the clone libraries that was not observed in the cultured communities from either site. Although all of the cultured representatives grouped within the major phyla covered by the bacterial clone libraries, there were many sequences found within the libraries that do not group closely with cultured representatives of the communities. Conversely, 11/34 of the isolates recovered from the samples, shared greater than 99% sequence similarity with at least one clone found in the clone libraries. Comparison of sequences from both libraries to their closest BLAST match in the NCBI database revealed that many sequences were highly similar to previously described sequences. For example, 66% of the sequences in the Markham library and 85% in the Ward Hunt library showed greater than 97% sequence identity with their closest BLAST match. Sequences that shared less than 95% sequence identity with their closest match in the NCBI database represent 19% of the Markham library and 5% of the Ward Hunt library.

Archaeal 16S rRNA gene clone libraries

Restriction fragment length polymorphism (RFLP) analysis of the archaeal 16S rRNA gene inserts from 41 clones from the Markham Ice Shelf library and 39 clones from the Ward Hunt Ice Shelf library revealed the dominance of a single phylotype. Four inserts of the 80 analysed were found to have RFLPs differing from the dominant pattern, and sequencing of these inserts revealed either very slight sequence divergence (< 1%) from the dominant sequence, or did not provide reliable sequence reads. A total of 31 reliable sequence reads, 27 from the Markham library and 4 from the Ward Hunt library, were obtained by sequence analysis of inserts. A single sequence differing from the other 30 sequences had a closest BLAST match to a bacterial 16S rRNA sequence. A pairwise alignment of the other 30 sequences revealed that these sequences shared a minimum of 99% sequence identity (571/576 bp) with one another. The dominant archaeal sequence shared 74% sequence identity with its closest NCBI BLASTN match, a sulfidic surface water clone from white biomaterial (clone HOEHK17, Accession No. AJ631250), when aligned over the entire 714 base pair insert sequence read. Despite this low overall sequence identity, there were regions of higher sequence similarity observed from base pairs 1–68, 102–439 and 567–713 which share 94%, 97% and 91% sequence identity with clone HOEHK17 respectively. Phylogenetic grouping of this sequence reveals it grouped within the *Euryarchaeota*, but distantly from other known sequences (Fig. 6).

Microbial mat activity at subzero temperatures

Mineralization of glucose was observed in Markham and Ward Hunt ice shelf mat samples at 5°C, −5°C and −10°C, showing that these communities are metabolically active at subzero temperatures (Fig. 7). Glucose respiration rates were extrapolated from the glucose mineralization curves from days 56 to 141 (85 days) when mineralization curves were most linear after the initial burst (Fig. 7). Glucose respiration rates (mg of glucose per day per gram of mat with standard error shown in brackets) were as follows for Markham Ice Shelf: −10°C, 0.024 (±0.002); −5°C, 0.050 (0.002); 5°C, 0.101 (±0.009); and for Ward Hunt Ice Shelf: −10°C, 0.020 (±0.004); −5°C, 0.019 (0.001); 5°C, 0.133 (±0.023). Background respiration rates in corresponding sterile controls were 0.001 for 5°C, 0.0008 for −5°C and 0.0005 for −10°C. Both the rates and levels of mineralization decreased with temperature. Levels of mineralization were ~2.4-fold and 4.2-fold less at −5°C and −10°C, respectively, in comparison with mineralization levels observed at 5°C. Mineralization was observed to a greater extent in the Markham Ice Shelf microcosms compared with the Ward Hunt samples, perhaps reflective of the greater biomass observed in the Markham samples in 2006. Glucose mineralization in sterile controls remained less than 1% after 141 days of incubation.

Discussion

Cultured community characteristics

The microbial abundances of these communities were similar at each ice shelf and were similar to those reported in microbial mats from Antarctic lakes (Van Trappen *et al.*, 2002). Culture-dependent analyses indicated that the heterotrophic bacterial populations in the cultured communities of the two ice shelves were primarily cold-adapted. Heterotrophic plate counts were similar at 5°C and 25°C and no growth was observed in plates incubated at 37°C. All isolates characterized grew at 5°C and 4/30 isolates were capable of growth at −5°C. The number of isolates capable of subzero growth is probably underestimated as

Fig. 4. Phylogenetic analysis of bacterial 16S rRNA gene sequences from clone libraries and cultured representatives of the Markham (MIS) and Ward Hunt (WIS) grouping with the *Proteobacteria*. Sequences sharing >95% sequence identity to each other were grouped as a phylotype. Phylogenetic relationships were constructed by neighbour joining analysis of 430 positions of homologous sequence, and rooted to the outgroup *Aquifex pyrophilus* strain Kol5A (M83548). Bootstrap values above 50% are shown as a percentage of 1000 replicates. Sequences obtained in this study are in bold type, and parentheses denote the site from which each isolate was obtained for cultured representatives and the percentage in which each sequence was found in each clone library for cloned representatives. The scale represents the number of mutations per nucleotide position.

only 8/30 strains were capable of growth at 5°C on the subzero medium which appeared to contain an inhibitory combination of 5% NaCl and 5% glycerol. On the other hand, 19/30 strains tested were halotolerant to 5% salt but only four of these strains were capable of subzero growth indicating that halotolerance and subzero growth are not inclusive; many of these strains are most closely related to strains isolated from other cryo-environments (glaciers, sea ice, Antarctic soil). The adaptation to cold temperatures observed in the cultured communities from these ice shelves is not surprising given that air temperatures in this region drop to −50°C in the winter, and throughout all but a brief meltwater period the mats are encased in snow and ice. *In situ* thermal studies on these mats have shown that they remain below 0°C through most of the year (~300 days), experience an average annual mat temperature of −8.6°C, drop to a minimum mat temperature of −17°C and rise to a maximum of only 1.7°C in late summer (Mueller *et al.*, 2005). Many of the isolated strains are phylogenetically related to the genera *Cellulomonas*, *Algibacter*, *Pseudoxanthomonas* and *Lysobacter* that are often associated with plants and algae and are capable of metabolizing polysaccharides. These mat isolates are likely to have a similar role in utilizing organic carbon derived from the microbial phototrophs present in the mats.

Although a general downward trend in plate counts was observed when mat samples were cultured on medium supplemented with higher concentrations of NaCl, the cultured community was moderately salt tolerant. In experiments conducted at 5°C, the highest counts were observed on R2A medium that was not supplemented with additional NaCl; however, counts on plates supplemented with 5.0% NaCl were only decreased by one order of magnitude. Furthermore, 63% of the isolates tested were capable of growth on medium supplemented with 5.0% NaCl. Although the NaCl concentrations used to supplement the medium cover a relatively narrow range, medium containing 5% NaCl represented a 30 times greater concentration of Na^+ and Cl^- than has been reported for the meltwater pools on the Markham Ice Shelf in the summer melt season (Vincent *et al.*, 2004). The observation of a degree of halotolerance may be expected, as the mat communities must tolerate increased solute concentrations in the surrounding water as the meltwater ponds become frozen (Mueller *et al.*, 2005). Automated *in situ* measurements in the water overlying mats in a pond on the Ward Hunt Ice Shelf have

shown large increases in salinity during freeze-up, with conductivities rising from 5 to > 25 mS cm^{-1}, and water temperatures dropping below 0°C (Mueller and Vincent, 2005). In an analogous pond in Antarctica, the salinity of remaining water above the mats rose to more than five times that of seawater during winter freeze-up, with a minimum observed water temperature of −13°C (Schmidt *et al.*, 1991).

Arctic ice shelf microbial mat composition

The stratification found in some Antarctic mats of a highly pigmented aerobic surface layer overlying a black, anoxic layer (Vincent *et al.*, 1993) was not apparent from our visual inspection of these communities, consistent with the relatively homogeneous, aerobic oxygen conditions that have been previously measured throughout these mat profiles (Mueller and Vincent, 2005). Our DGGE analyses of the upper and lower halves of core samples from each ice shelf suggested similar community compositions between the two layers in the mats from both ice shelves, which implies habitat similarities between the portions of the mat analysed.

The homogeneity in vertical profile may be related to mat thickness. The Arctic mat samples analysed in this study were ~2.2 cm in thickness, compared with thicker Antarctic McMurdo ice shelf mat communities (~8 cm thick) which are highly stratified (Mountfort *et al.*, 1999) relative to the mats analysed here. It is also possible that variations in species distribution over a smaller scale were not observed because only the upper and lower halves of the mats were analysed and species in very thin layers may be present in too low a proportion to be discerned by DGGE analyses (Muyzer *et al.*, 1993). The results show very little difference in community structure within the sampled areas of the same ice shelf, although more extensive survey data would be required to verify this. The DGGE analysis also showed little difference in community composition between samples from the two ice shelves, although in the banding pattern analyses the two replicate samples from each ice shelf grouped more closely with one another than they did samples from different ice shelves.

Phylogenetic analyses and biodiversity

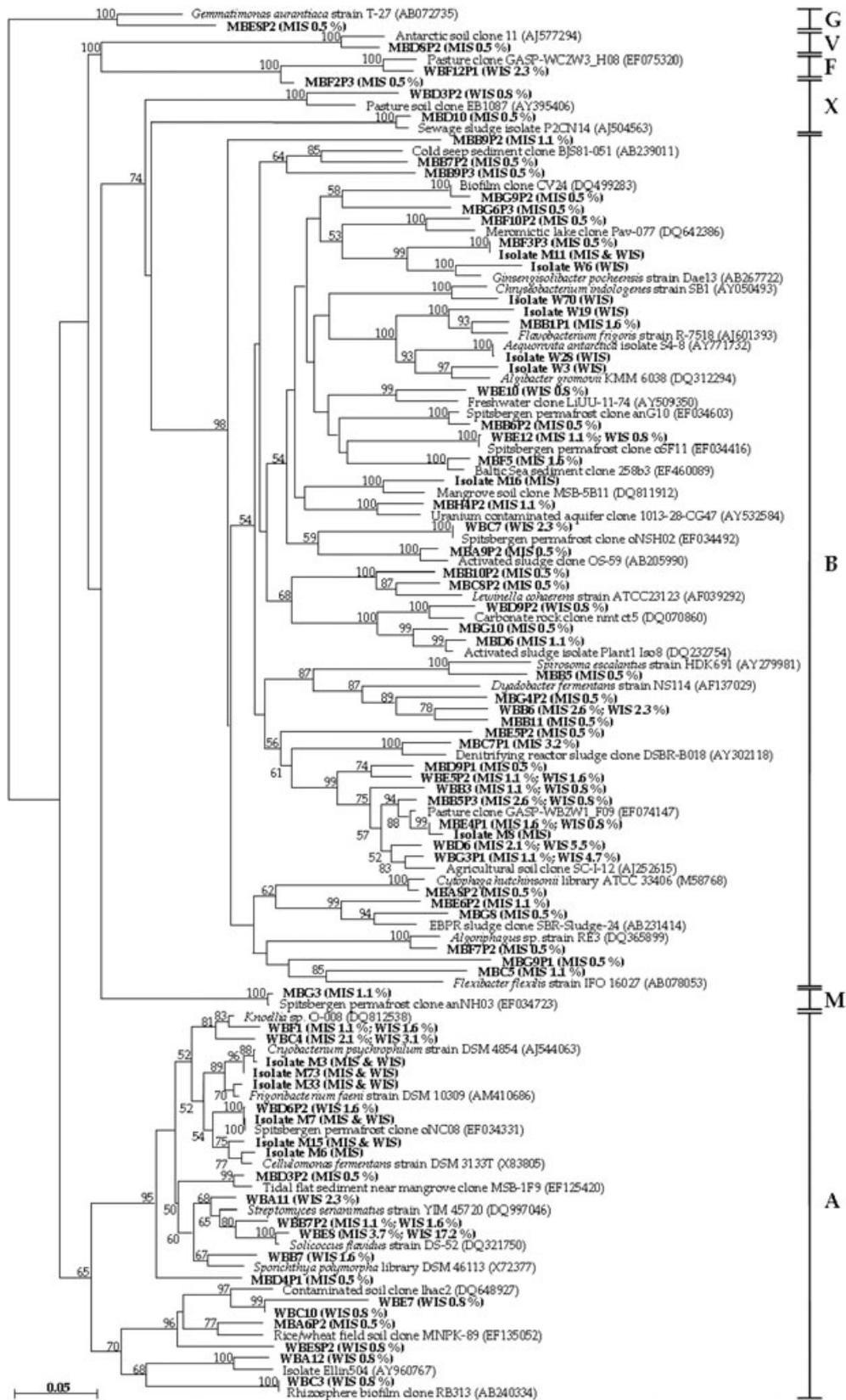Clone library analyses revealed that the mat communities from both the Markham and Ward Hunt ice shelves have

Fig. 5. Phylogenetic analysis of bacterial 16S rRNA gene sequences from clone libraries and cultured representatives of the Markham (MIS) and Ward Hunt (WIS) not grouping with the *Proteobacteria*. Sequences sharing > 95% sequence identity to each other were grouped as a phylotype. Sequences were either unclassified (X) or grouped within the phyla *Gemmatimonadetes* (G), *Verrucomicrobia* (V), *Fibrobacteres* (F), *Bacteroidetes* (B), *Firmicutes* (M) or *Actinobacteria* (A). Phylogenetic relationships were constructed by neighbour joining analysis of 414 positions of homologous sequence, and rooted to the outgroup *Aquifex pyrophilus* strain Kol5A (M83548). Bootstrap values above 50% are shown as a percentage of 1000 replicates. Sequences obtained in this study are in bold type, and parentheses denote the site from which each isolate was obtained for cultured representatives and the percentage in which each sequence was found in each clone library for cloned representatives. The scale represents the number of mutations per nucleotide position.

diverse bacterial populations, with statistical analyses suggesting that the diversity at the Markham Ice Shelf exceeds that of the Ward Hunt Ice Shelf. The diversity of the two ice shelves ($H' = 3.5, 4.4$) was higher in comparison with bacterial diversity examined in other polar regions, including cold saline springs in the Canadian

Arctic ($H' = 2.2–3.2$) (Perreault *et al.*, 2007), high Arctic permafrost ($H' = 1.8–3.6$) (B. Steven and L.G. Whyte, unpubl. data), Antarctic mineral soils ($H' = 1.2–1.6$) (Smith *et al.*, 2006), and Arctic and Antarctic sea ice ($H' = 0.8–1.3$) (Brinkmeyer *et al.*, 2003). The relatively complex DGGE banding patterns of the Arctic microbial
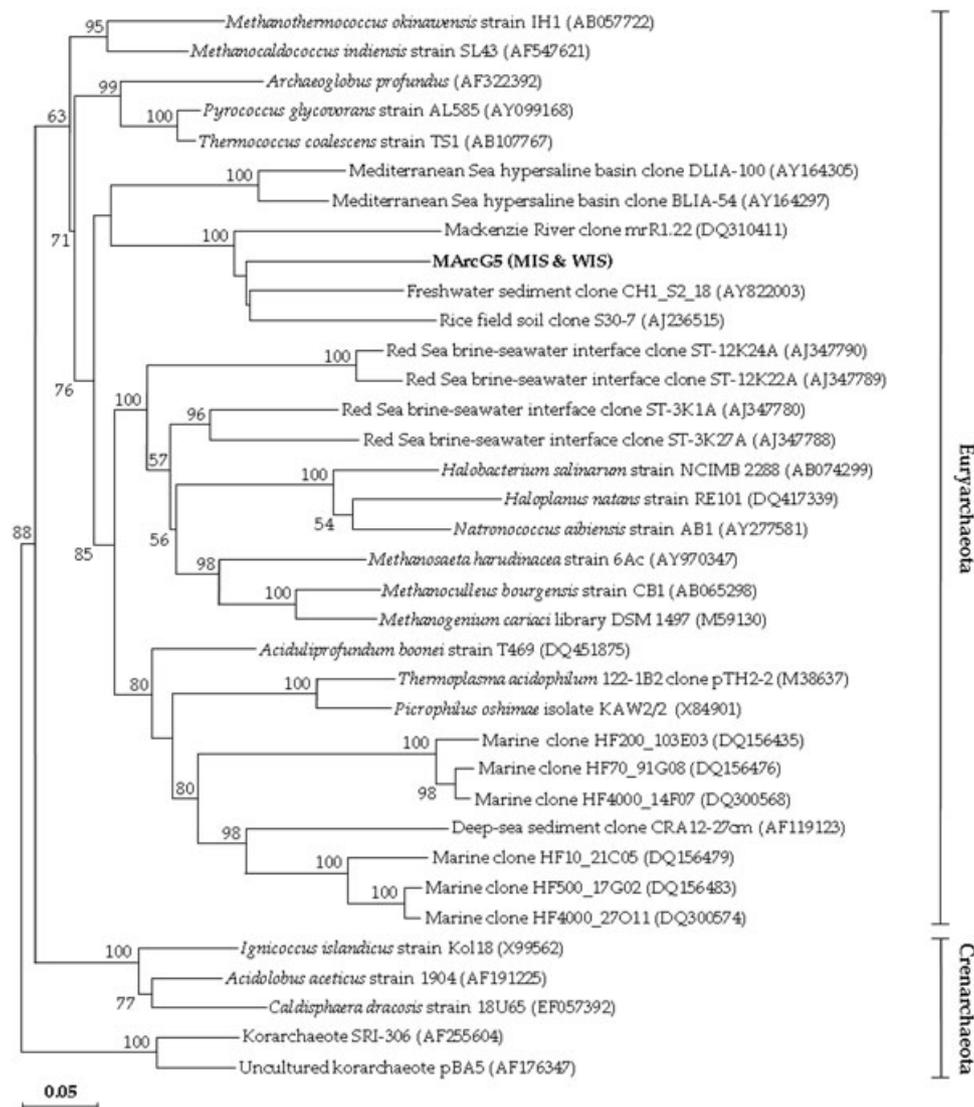

Fig. 6. Phylogenetic grouping of an archaeal 16S rRNA gene sequence obtained from clone libraries of the Markham and Ward Hunt ice shelves analysed in this study. A single archaeal clone sequence, identified by bold type, was found in analysis of the two libraries, and this sequence was found to be common to both libraries. Phylogenetic relationships were constructed by neighbour joining analysis of 680 positions of homologous sequence, and rooted to the outgroup *Aquifex pyrophilus* strain Kol5A (M83548). Bootstrap values above 50% are shown as a percentage of 1000 replicates. The scale represents the number of mutations per nucleotide position.
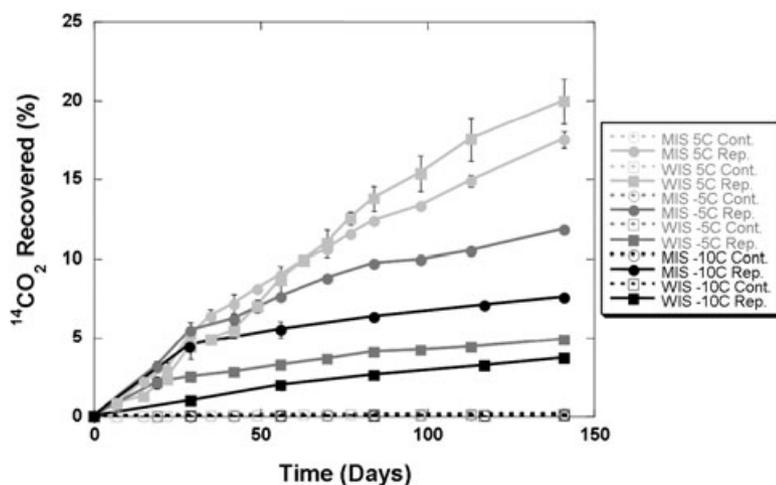
Fig. 7. Metabolic activity of microbial mat communities from the Markham (MIS) and Ward Hunt (WIS) ice shelves. Abiotic control (Cont) and experimental replicate (Rep) microcosms (5 g of mat material) supplemented with 14-C-labelled glucose were incubated at 5°C, −5°C and −10°C. The amount of radioactive CO_2 recovered in a 1 M KOH trap was recorded as a percentage of the total activity initially added to the microcosm. Each point represents the mean cumulative mineralization from triplicate mat microcosms with the error bars representing standard deviation of the mean.

mats also indicate a greater diversity than we have observed in other high Arctic samples including cold saline springs (Perreault *et al.*, 2007) and permafrost/ground ice (B. Steven and L.G. Whyte, unpubl. data). The higher diversity observed in the Markham library compared with the Ward Hunt library was not unexpected, as macroscopically, the mat communities of the Markham Ice Shelf appear to be more extensive and highly developed than those found on the Ward Hunt Ice Shelf (Vincent *et al.*, 2004).

Despite the apparent differences in the two communities at the species level, as indicated by the statistical analyses of the clone libraries, the sequences of both bacterial clone libraries and the sequences from all isolates were found to group predominantly within three phyla: *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. Although the abundances in which individual sequences are observed in clone library analyses do not necessarily reflect their abundances in an environmental sample (Achenbach and Coates, 2000), the finding that the dominant sequences in the clone libraries grouped within the same three phyla that were uncovered in the cultured communities suggests the importance of the members of these phyla to the mat ecosystems. Members of these three phyla also represent a substantial proportion of the phyla detected in diversity studies of microbial mats present in Antarctic lakes (Brambilla *et al.*, 2001; Van Trappen *et al.*, 2002). The presence of similar phyla in both the Markham Ice Shelf and Ward Hunt Ice Shelf communities was expected, as these two systems appear to face many of the same environmental conditions and selective pressures. Furthermore, the similar surrounding habitats may act as a source of inoculation of the two ice shelves, due to the close proximity of these two ice shelves to one another.

The closest cultured BLAST matches to many of the isolates recovered in this study were bacteria first char-

acterized from other cold temperature ecosystems, including sea ice, glaciers, snow cover and Antarctic microbial mats. While many of the isolates from these mats showed high sequence identity to previously cultured bacteria, ~33% of the isolates showed less than 97% sequence identity to their closest cultured BLAST match indicating that a considerable proportion of the bacteria isolated may represent novel species.

In contrast to the BLAST matches of isolates recovered in this study, few common trends were noticed in the environmental origins of the closest BLAST matches in the NCBI database for sequences obtained in analyses of either bacterial clone library. Environmental origins of BLAST matches sharing greater than 97% identity with sequences in the clone libraries included marine and freshwater environments, polar sea ice, agricultural soils, legume nodules and uranium mining waste. Interestingly, there was nearly equal representation of the α- and β-*Proteobacteria* within both libraries. α-*Proteobacteria* are of widespread occurrence in the marine plankton, including within Arctic communities, while β-*Proteobacteria* are generally more important in freshwater environments (Garneau *et al.*, 2005). The marine influence of the basal sea ice in these meltwater pools was previously noted (Vincent *et al.*, 2004), and the distribution of these two proteobacterial classes in the bacterial clone libraries further underscores the unique nature of these ecosystems.

While many of the sequences in the two bacterial libraries grouped closely to previously reported sequences, most did not group to previously characterized cultured bacteria. As such, it is difficult to infer the characteristics or metabolic activity of the bacteria represented in the clone libraries. With this in mind, the phyla observed in this study could provide a number of metabolic activities important to the mat communities as a whole. The *Proteobacteria* represented may be responsible for a diverse

suite of aerobic and anaerobic metabolic activities (Madigan *et al.*, 2000). Of particular interest to sulfur cycling processes in these microbial systems is the presence of δ-*Proteobacteria* sequences, found to group within the orders *Desulfobacterales* in both libraries and *Desulfuromonales* in the Markham Ice Shelf library; these are orders characterized by members of sulfate-reducing or sulfur-reducing bacteria (SRBs) (Madigan *et al.*, 2000). The presence of SRBs in the Arctic mats would be consistent with the high sulfate concentrations (177–169 mg l⁻¹) previously reported in the pond water overlaying the mats (Mueller *et al.*, 2005), although we detected no strong smell of H_2S during sampling.

That no cyanobacterial sequences were detected in the clone libraries was surprising, considering that cyanobacteria are reported to dominate the algal communities in mats from the Markham Ice Shelf (Vincent *et al.*, 2004), as in many other polar habitats (Vincent, 2007). The lack of cyanobacterial sequences in the bacterial clone libraries and the similar DGGE patterns observed between the two layers may be explained by primer bias as it is well documented that cyanobacterial 16S rRNA genes are not efficiently amplified by domain-specific polymerase chain reaction (PCR) primers (Taton *et al.*, 2003; Waleron *et al.*, 2006). It is also possible that the proportion of cyanobacterial cells in these systems represents a small proportion of the total prokaryotic community. For example, 62–73% of the cyanobacteria/algal phototrophs in Markham Ice Shelf mats exist in the top 100–500 μm of the mats that can be several centimetres in total depth (Vincent *et al.*, 2004). Much of the rest of the mat may be composed of decaying phototroph-derived organic matter (including fluorescing degradation pigments), and the associated bacterial communities responsible for its decomposition.

An interesting finding of this study was the apparent lack of archaeal diversity, and a novel archaeal phylotype in these two mat ecosystems. *Archaea* can be abundant and diverse in cold ecosystems, where they play an important role in biogeochemical cycling (DeLong *et al.*, 2002; Cavicchioli, 2006; Steven *et al.*, 2006; Perreault *et al.*, 2007). However, microbial mats in Lake Fryxell in Antarctica also showed low archaeal diversity, with only two archaeal phylotypes detected in analyses of archaeal 16S rRNA gene clone libraries: one being distantly related to the methanogen *Methanoculleus palmolei* and the other sequence closely related to an Antarctic clone sequence (Brambilla *et al.*, 2001). The archaeal sequence uncovered in this study does not group closely with any other reported archaeal sequences and groups very distantly from any cultured archaea, and it will be of great interest in the future to determine the biogeochemical role of the species represented by this sequence.

Microbial mat activity at subzero temperatures

The detection of metabolic activity at subzero temperatures in these microbial mats augments recent reports of similar activity from a number of other cryo-ecosystems (e.g. Carpenter *et al.*, 2000; Bakermans *et al.*, 2003; Junge *et al.*, 2004) and is also consistent with the observation that a number of isolates obtained in this study were capable of growth at −5°C. The subzero activity potential demonstrated in the microcosm analyses suggests that these communities are metabolically active during both the summer melt season and during the ~10 months of the year when the ambient temperatures are < 0°C.

In summary, this work underscores the importance of the Canadian High Arctic ice shelves as rich sites of polar microbial diversity. These ecosystems are vulnerable to the ongoing effects of global warming, but may have served as refugia and sites of microbial growth and evolution during periods of extensive ice cover, such as glaciations during the Proterozoic. The genetic diversity discovered in the present study suggests that such communities may also provide an inoculum of taxonomically and functionally diverse microbiota to colonize other habitats during warmer periods.

Experimental procedures

Study sites and sampling

Microbial mat samples were collected aseptically from the Ward Hunt Ice Shelf and Markham Ice Shelf during two field seasons in August 2005 and June 2006. In 2005, mat samples and core samples were collected from two independent meltwater pools on the Ward Hunt Ice Shelf (83°04.93′N, 74°25.70′W) and two sites within the same meltwater pool on the Markham Ice Shelf (83°01.90′N, 71°27.15′W and 83°01.93′N, 71°29.72′W). In 2006, frozen mat samples were collected from beneath the snow at approximately the same locations as were sampled in 2005 on both the Ward Hunt Ice Shelf (83°04.87′N, 74°25.84′W) and the Markham Ice Shelf (83°01.92′N, 71°29.27′W). In both seasons, mat samples were placed on ice immediately after collection and transported to the Ward Hunt Island field camp. Samples were maintained on ice from the time of sampling for up to 9 days before being frozen at −20°C and maintained frozen until further analyses. In 2005, temperature, pH and conductivity measurements were recorded for the meltwater pools from which the samples were collected using an Oakton pH/CON 10 multiparameter meter equipped with a multisensor probe (Oakton Instruments, Vernon Hills, IL).

Microscopy

Direct microscopic counts were performed on 2005 and 2006 samples collected from both ice shelves after being stained with the LIVE/DEAD *Bac*Light Bacterial Viability Kit (Invitro-

gen Corporation, Carlsbad, CA). Mat samples 2.0–5.0 g were transferred to 20 ml dilution tubes containing 2.5 g of sterile 3-mm-diameter glass beads (Fisher Scientific, Waltham, MA) and three times the sample weight of cold, sterile, dilution buffer [0.1% w/v $Na_4P_2O_710H_2O$ (pH 7.0 ± 0.2)]. Samples were vortexed at maximum speed for 2 min and subsequently used to prepare serial dilutions used for microscopic analyses. An aliquot of 0.5 ml of each diluted sample was stained for 15 min, as described by the manufacturer, and filtered through 25-mm-diameter, 0.2-µm-poresize, black polycarbonate membrane filters (Poretics, Livermore, CA), backed with 25-mm-diameter, 0.2-µm-poresize, white polycarbonate membrane filters (Poretics, Livermore, CA). Filters were dried, mounted on glass microscope slides with one drop of *Bac*Light mounting oil and covered with a glass coverslip. Filters were viewed using a Nikon Eclipse E600 microscope, equipped with a super high-pressure mercury lamp at 1000× magnification through a Texas Red filter. Cells were counted in a minimum of 10 fields of view for each sample and results are reported as mean counts with standard errors calculated from counts of individual samples.

Bacterial cultivation and characterization

Heterotrophic plate counts were prepared in the field in 2005 within 12 h of sample collection by spread plating 100 µl aliquots of 10^{-3}, 10^{-4} and 10^{-5} serial dilutions of samples on R2A agar (Becton, Dickson and Company, Sparks, MD) Plates were incubated at ambient temperatures (~5–10°C) for up to 10 days before incubation at 5°C for 2 months upon return to Montreal, Canada. Heterotrophic plate counts were prepared in the laboratory from the same samples on R2A agar plates supplemented with 0%, 1.0%, 2.5% or 5.0% w/v NaCl and incubated at 5°C, 25°C and 37°C. Subzero medium (R2A agar supplemented with 50 g l^{-1} NaCl and 50 ml l^{-1} glycerol) was also spread plated and incubated at −10°C, −5°C and 5°C. All plating was performed in triplicate or quadruplicate and results are reported as mean counts with standard error. Colonies with different macroscopic morphologies were isolated from plates prepared in the field and the laboratory from each ice shelf after 2 months of incubation at 5°C. Colonies were streaked for isolation on fresh medium of the same type from which they were isolated and incubated at 5°C. Isolates were re-streaked a minimum of two times before being subcultured with sterile toothpicks on to medium to determine salt and temperature tolerances and before genetic analyses of isolates were performed. DNA was extracted from isolates by boiling lysis (Sambrooke and Russell, 2001) or using the DNeasy Tissue Kit (Qiagen, Mississauga, ON) according to the manufacturer's method for purification of genomic DNA from Gram-positive bacteria.

Community DNA extractions and 16S rRNA gene amplification

All community DNA extractions were completed using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Solana Beach, CA), as described in the alternative protocol of the manufacturer's instructions. Total community DNA extractions were completed within 12 h of sample collection at the Ward Hunt Island field camp. Extractions from layers of core samples were completed in the laboratory in Montreal, Canada. The cores from the Markham and Ward Hunt ice shelves were each 2.2 cm in length and were split equally into 1.1 cm top and bottom halves using a sterile hot razor to cut through the frozen mat material. All community DNA extractions were purified using polyvinylpolypyrrolidone (PVPP) columns as previously described (Berthelet *et al.*, 1996).

Bacterial isolate 16S rRNA genes were amplified by PCR using the primer pair 27F (AGA GTT TGA TCC TGG CTC AG) (Lane, 1991) and 758R (CTA CCA GGG TAT CTA ATC C) (Woese, 1987; Lee *et al.*, 1993): thermocycling conditions consisted of 3 min at 95°C; 30 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C; and a final extension of 7 min at 72°C. The primer pair 341F (CCT ACG GGA GGC AGC AG) (Muyzer *et al.*, 1996) with a 5′-GC clamp (GCG GGC GGG GCG GGG GCA CGC GGG GCG CGG CGG GCG) and 758R was used for amplification of bacterial 16S rRNA genes for DGGE analyses: thermocycling conditions consisted of 5 min at 96°C, followed by 10 touchdown cycles (68–59°C), and a further 15 cycles at 59°C for 45 s, followed by 72°C for 1 min 30 s and a final extension of 5 min at 72°C. The primer pair 341F and 758R was also used for amplification of bacterial 16S rRNA genes for clone library production: thermocycling conditions consisted of 5 min at 96°C, followed by 15 touchdown cycles (65–59°C), and a further 15 cycles at 59°C for 30 s, followed by 72°C for 1 min 30 s, and a final extension of 5 min at 72°C. Archaeal 16S rRNA genes were amplified using the primers 109F (ACK GCT CAG TAA CAC GT) (Whitehead and Cotta, 1999) and 915R (GTG CTC CCC CGC CAA TTC CT) (DasSarma and Fleischmann, 1995): thermocycling conditions consisted of 5 min at 94°C; 20 touchdown cycles (62–52°C), and a further 15 cycles at 52°C for 30 s, followed by 72°C for 1 min 30 s; and a final extension of 5 min at 72°C.

Polymerase chain reaction reagents and their final concentrations in a 50 µl reaction mixture were as follows: 1.5 U of *Taq* polymerase (Invitrogen Canada, Burlington ON), 1× PCR buffer, 3.5 mM $MgCl_2$ (both supplied with the *Taq* polymerase), 0.2 mM of each deoxynucleotide triphosphate, 1 µM of each primer and 5 µl of DNA extract. All PCR mixtures for the amplification of community DNA were prepared as stated, but additionally contained 12.5 µg of BSA.

DGGE analyses

Denaturing gradient gel electrophoresis was used to compare the bacterial communities present between the two ice shelves, between different samples collected from within the same ice shelf, and between different layers in the vertical profile of individual mat samples. DGGE was performed using a DCode Universal Mutation Detection System as described in the manufacturer's instructions (Bio-Rad Laboratories, Hercules, CA). An 8% acrylamide gel with a gradient of urea and formamide from 35% to 65% was prepared and each lane was loaded with approximately 800 ng of PCR product. Samples were run for 16 h at 80 V in 1× TAE buffer at 60°C. Gels were stained for 30 min in a solution of 0.01% v/v Vistra Green (Amersham Biosciences, Fairfield, CT) prepared in 1× TAE buffer, and destained for 30 min in 1× TAE

buffer. Gels were viewed on a Bio-Rad Molecular Imager FX equipped with an External Laser Imager FX (Bio-Rad Laboratories, Hercules, CA). DGGE runs were completed in replicate to ensure reproducibility of results. DGGE results were analysed using Quantity One imaging software version 4.2.1 (Bio-Rad Laboratories, Hercules, CA) to compare banding patterns between samples. Bands in each lane were compared based on position and a neighbour joining distance-based tree was constructed.

16S rRNA gene clone library construction

Total community DNA from total mat samples collected in 2005 from the Markham and Ward Hunt ice shelves were used for clone library construction. A total of four clone libraries were produced to analyse the bacterial and archaeal communities at the two ice shelves. Polymerase chain reaction-amplified community 16S rRNA gene fragments of ~417 bp for bacterial libraries and ~806 bp for archaeal libraries were cloned into the pGEM-T Easy Vector System I using the manufacturer's instructions (Promega Corporation, Madison, WI) and transformed into *Escherichia coli* strain DH5α by a standard protocol (Sambrooke and Russell, 2001). Clones of interest were selected by blue/white screening on LB/amp plates spread with 100 µl of 40 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and 100 µl of 2% w/v 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) prepared in N,N-dimethyl formamide 30 min prior to inoculation. DNA was extracted from clones by boiling lysis (Sambrooke and Russell, 2001) and cloned inserts were amplified from the pGEM-T Easy vector by PCR using the pGEM-T-targeted primers T7 and SP6 (Promega Corporation, Madison, WI). The PCR reaction mixtures were prepared with reagent concentrations as described above. Thermocycling conditions consisted of 5 min at 95°C; 30 cycles of 45 s at 94°C, 30 s at 57°C and 1 min at 72°C; and a final extension of 5 min at 72°C. RFLP analyses, essentially as described by Steven and colleagues (2007a), were used to group clones within individual clone libraries and to group isolates with similar morphologies. Sequences with identical RFLP patterns were grouped together as similar sequences and representatives of each group were chosen for sequence analyses. For clone libraries that showed high diversity in RFLP results, the DNA from each clone was sequenced. All sequencing was undertaken by Genome Quebec, based at McGill University, Montreal, Quebec.

Nucleotide accession numbers

These sequence data have been submitted to the GenBank database under Accession No. EU044919–EU045213.

Phylogenetic and statistical analyses

16S rRNA sequences of clones and isolates were compared with known sequences in the NCBI database using the BLASTN algorithm (Altscul *et al.*, 1990) and grouped for phylogenetic analyses using the RDP Classifier function of the Ribosomal Database Project-II release 9 with a confidence threshold of 80% (Cole *et al.*, 2005). Potential chimeras iden-

tified at 99.9% in Mallard version 1.02 (Ashelford *et al.*, 2006) were analysed in Pintail version 1.1 (Ashelford *et al.*, 2005), and those identified as chimeras were deleted from the clone libraries. Distance matrices of clone sequences were constructed from CLUSTALW alignments using the DNADIST function, with the Jukes Cantor correction, in the PHYLIP program version 3.65 (Felsenstein, 2005). CLUSTALW alignments compared 430 bp of homologous sequence in analyses of bacterial sequences grouping most closely with the phylum *Proteobacteria*, 414 bp of homologous sequence in analyses of bacterial sequences not grouping within the phylum *Proteobacteria*, and 680 bp of homologous sequence in analyses of archaeal sequences. Distance matrices were used in order to complete clone library analyses in the programs DOTUR (Schloss and Handelsman, 2005) and WEBLIBSHUFF version 0.96 (Singleton *et al.*, 2001). WEBLIBSHUFF was used to compare the two libraries, in order to determine if their compositions were statistically different from one another. The DOTUR output files were used as a basis for comparisons of richness, diversity and library composition, as well as to assess library coverage. Two indices were used to measure phylotype richness, the Chao1 richness estimator (Chao, 1984) and the ACE richness estimator (Chao *et al.*, 1993), and two indices were used to measure species diversity, the Shannon's diversity index (*H'*) (Shannon and Weaver, 1949) and the reciprocal of the Simpson's diversity index (1/*D*) (Simpson, 1949), which were calculated directly by the DOTUR program for both bacterial libraries. Phylotype composition in each of the libraries was calculated by the Sorenson index (Magurran, 1988) and library coverage was calculated according to Good (1953). Phylogenetic trees were constructed from the CLUSTALW alignments of clone and isolate 16S rRNA gene sequences in the program MACVECTOR 7.0. Neighbour joining best trees were constructed using the Jukes Cantor correction and the reliability of the tree branch points was assessed by bootstrap analysis of 1000 replicates.

Microbial mat community activity at subzero temperatures

Microbial activity at 5°C and subzero temperatures was detected and quantified by measuring $^{14}CO_2$ evolved during mineralization of [2-^{14}C] glucose in microbial mat microcosms employing a specialized $^{14}CO_2$ trap solution that would not freeze, maintained sufficient CO_2 trapping efficiency and did not interfere with liquid scintillation counting at subzero temperatures (Steven *et al.*, 2007b). Mat samples collected in spring 2006 from the Markham and Ward Hunt ice shelves were used to perform these analyses essentially as described (Steven *et al.*, 2007b). All microcosms were prepared in triplicate by aseptically dispensing 5.0 g of mat material into sterile 30 ml serum bottles on ice. A 20 µl aliquot of ^{14}C-labelled glucose solution (Dupont, Boston, MA), containing 0.5 mg of glucose and with an activity of 115 940 disintegrations per minute (dpm), was dispensed into each microcosm with a Hamilton syringe, yielding a final glucose concentration of 0.1 mg per gram of mat in each microcosm. Microcosms were sealed, vortexed briefly and immediately placed at their final incubation temperature for 1 h. Microcosms were then briefly removed from their incubators on ice,

and a sterile 8 mm × 40 mm culture tube (Fisher Scientific, Waltham, MA) containing 0.5 ml of trap solution (1 M KOH and 20% ethylene glycol) was aseptically placed inside the serum bottle. Microcosms were resealed and incubated at 5°C, −5°C or −10°C without shaking in Fisherbrand Isotemp Refrigerated Incubators (Fisher Scientific). Sterile negative control microcosms were prepared in triplicate by the same methods used for preparation of the experimental microcosms; however, prior to the addition of the radioactive glucose, the serum bottles containing sample were autoclaved twice, 24 h apart, at 121°C and 15 pounds per square inch (103 kPa) for 30 min. The amount of radioactivity trapped in the microcosms over a 12-week period was determined by liquid scintillation spectrometry using a LS 6500 multipurpose scintillation counter and supplied software (Beckman Coulter, Fullerton, CA) (Steven *et al.*, 2007b). All manipulations of the microcosms were performed on ice to maintain subzero temperatures and the microcosms were outside of the incubator for less than 5 min; thawing of the samples during $^{14}CO_2$ sampling was never observed.

Acknowledgements

References

Achenbach, L.A., and Coates, A.J. (2000) Disparity between bacterial phylogeny and physiology. *ASM News* **66:** 714–715.

Altscul, S., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215:** 403–410.

Ashelford, K.E., Chuzhanova, N.A., Fry, J.C., Jones, A.J., and Weightman, A.J. (2005) At least 1 in 20, 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. *Appl Environ Microbiol* **71:** 7724–7736.

Ashelford, K.E., Chuzhanova, N.A., Fry, J.C., Jones, A.J., and Weightman, A.J. (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. *Appl Environ Microbiol* **72:** 5734–5741.

Bakermans, C., Tsapin, A.I., Souza-Egipsy, V., Gilichinsky, D.A., and Nealson, K.H. (2003) Reproduction and metabolism at −10 degrees C of bacteria isolated from Siberian permafrost. *Environ Microbiol* **5:** 321–326.

Berthelet, M., Whyte, L.G., and Greer, C.W. (1996) Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns. *FEMS Microbiol Lett* **138:** 17–22.

Brambilla, E., Hippe, H., Hagelstein, A., Tindall, B.J., and Stackebrandt, E. (2001) 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Extremophiles* **5:** 23–33.

Brinkmeyer, R., Knittel, K., Jurgens, J., Weyland, H., Amann, R., and Helmke, E. (2003) Diversity and structure of bacterial communities in Arctic versus Antarctic pack ice. *Appl Environ Microbiol* **69:** 6610–6619.

Brown, M.V., and Bowman, J.P. (2001) A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microbiol Ecol* **35:** 267–275.

Carpenter, E.J., Lin, S., and Capone, D.G. (2000) Bacterial activity in South Pole snow. *Appl Environ Microbiol* **66:** 4514–4517.

Cavicchioli, R. (2006) Cold-adapted archaea. *Nat Rev Microbiol* **4:** 331–343.

Chao, A. (1984) Non-parametric estimation of the number of classes in a population. *Scand J Stat* **11:** 265–270.

Chao, A., Ma, M.C., and Yang, M.C.K. (1993) Stopping rules and estimation for recapture debugging with unequal failure rates. *Biometrika* **80:** 193–201.

Christner, B.C., Kvitko, B.H., 2nd, and Reeve, J.N. (2003) Molecular identification of bacteria and Eukarya inhabiting an Antarctic cryoconite hole. *Extremophiles* **7:** 177–183.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., *et al.* (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res* **33:** D294–D296.

DasSarma, S., and Fleischmann, E.F. (1995) *Archaea: A Laboratory Manual – Halophiles*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

DeLong, E.F., Wu, K.Y., Prezelin, B.B., and Jovine, R.V.M. (2002) High abundance of Archaea in Antarctic marine picoplankton. *Nature* **371:** 695–697.

Deming, J.W. (2002) Psychrophiles and polar regions. *Curr Opin Microbiol* **5:** 301–309.

Felsenstein, J. (2005) PHYLIP *(Phylogeny Inference Package)*, Version 3.65. Seattle, WA, USA: Department of Genome Sciences, University of Washington.

Garneau, M.-E., Vincent, W.F., Alonso-Saez, L., Gratton, Y., and Lovejoy, C. (2005) Prokaryotic community structure and heterotrophic production in a river-influenced coastal arctic ecosystem. *Aquat Microb Ecol* **42:** 27–40.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J., Claverie, P., Collins, T., *et al.* (2000) Cold-adapted enzymes: from fundamentals to biotechnology. *Tibtech* **18:** 103–107.

Good, I. (1953) The population frequencies of species and the estimation of population parameters. *Biometrika* **40:** 237–264.

Jakosky, B.M., Nealson, K.H., Bakermans, C., Ley, R.E., and Mellon, M.T. (2003) Subfreezing activity of microorganisms and the potential habitability of Mars' polar regions. *Astrobiology* **3:** 343–350.

Jungblut, A.D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D.R., Burns, B.P., and Neilan, B.A. (2005) Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environ Microbiol* **7:** 519–529.

Junge, K., Eicken, H., and Deming, J.W. (2004) Bacterial activity at −2 to −20 degrees C in Arctic wintertime sea ice. *Appl Environ Microbiol* **70:** 550–557.

Kivelson, M.G., Khurana, K.K., Russell, C.T., Volwerk, M.,

Walker, R.J., and Zimmer, C. (2000) Galileo magnetometer measurements: a stronger case for a subsurface ocean at Europa. *Science* **289:** 1340–1343.

Lane, D.J. (1991) 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*. Stackebrandt, E., and Goodfellow, M. (eds). Chichester, UK: John Wiley & Sons, pp. 115–176.

Lee, S., Malone, C., and Kemp, P.F. (1993) Use of multiple 16S rRNA-targeted fluorescent probes to increase signal strength and measure cellular RNA from natural planktonic bacteria. *Mar Ecol Prog Ser* **101:** 193–201.

Lorenz, R. (2003) Planetary science. The glitter of distant seas. *Science* **302:** 403–404.

Madigan, M.T., Martinko, J.M., and Parker, J. (2000) *Brock Biology of Microorganisms*. Upper-Saddle River, NJ, USA: Prentice Hall.

Magurran, A.E. (1988) *Ecological Diversity and Its Measurement*. Princeton, NJ, USA: Princeton University Press.

Malin, M.C., and Edgett, K.S. (2000) Evidence for recent groundwater seepage and surface runoff on Mars. *Science* **288:** 2330–2335.

Mosier, A.C., Murray, A.E., and Fritsen, C.H. (2007) Microbiota within the perennial ice cover of Lake Vida, Antarctica. *FEMS Microbiol Ecol* **59:** 274–288.

Mountfort, D.O., Kaspar, H.F., Downes, M., and Asher, R.A. (1999) Partitioning effects during terminal carbon and electron flow in sediments of a low-salinity meltwater pond near Bratina Island, McMurdo Ice Shelf, Antarctica. *Appl Environ Microbiol* **65:** 5493–5499.

Mueller, D.R., and Vincent, W.F. (2005) Microbial habitat dynamics and ablation control on the Ward Hunt Ice Shelf. *Hydrol Process* **20:** 857–876.

Mueller, D.R., Vincent, W.F., Bonilla, S., and Laurion, I. (2005) Extremotrophs, extremophiles and broadband pigmentation strategies in a high arctic ice shelf ecosystem. *FEMS Microbiol Ecol* **53:** 73–87.

Mueller, D.R., Vincent, W.F., and Jeffries, M.O. (2006) Environmental gradients, fragmented habitats, and microbiota of a northern ice shelf cryoecosystem, Ellesmere Island, Canada. *Arct Antarct Alp Res* **38:** 593–607.

Muyzer, G., de Waal, E.C., and Uitterlinden, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* **59:** 695–700.

Muyzer, G., Hottentrager, S., Teske, A., and Wawer, C. (1996) Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA. A new molecular approach to analyze the genetic diversity of mixed microbial communities. In *Molecular Microbial Ecology Manual*. Akkermans, A.D.L., van Elsas, J.D., and de Bruijn, F.J. (eds). Dordrecht, the Netherlands: Kluwer Academic Publishing, pp. 3.4.4.1–3. 4.4.22.

Perreault, N.N., Andersen, D.T., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007) Characterization of the prokaryotic diversity in cold saline perennial springs from the Canadian High Arctic. *Appl Environ Microbiol* **73:** 1532–1543.

Sambrooke, J., and Russell, D.W. (2001) *Molecular Cloning*. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Schloss, P.D., and Handelsman, J. (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microbiol* **71:** 1501–1506.

Schmidt, S., Moskall, W., De Mora, S.J.D., Howard-Williams, C., and Vincent, W.F. (1991) Limnological properties of Antarctic ponds during winter freezing. *Antarct Sci* **3:** 379–388.

Schulze-Makuch, D., Dohm, J.M., Fairen, A.G., Baker, V.R., Fink, W., and Strom, R.G. (2005) Venus, Mars, and the ices on Mercury and the moon: astrobiological implications and proposed mission designs. *Astrobiology* **5:** 778–795.

Shannon, C.E., and Weaver, W. (1949) *The Mathematical Theory of Communication*. Urbana, IL, USA: Illinois Press.

Simpson, E.H. (1949) Measurement of diversity. *Nature* **163:** 688.

Singleton, D.R., Furlong, M.A., Rathbun, S.L., and Whitman, W.B. (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. *Appl Environ Microbiol* **67:** 4374–4376.

Skidmore, M., Anderson, S.P., Sharp, M., Foght, J., and Lanoil, B.D. (2005) Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl Environ Microbiol* **71:** 6986–6997.

Smith, J.J., Tow, L.A., Stafford, W., Cary, C., and Cowan, D.A. (2006) Bacterial diversity in three different Antarctic Cold Desert mineral soils. *Microb Ecol* **51:** 413–421.

Steven, B., Leveille, R., Pollard, W.H., and Whyte, L.G. (2006) Microbial ecology and biodiversity of permafrost. *Extremophiles* **10:** 259–267.

Steven, B., Briggs, G., McKay, C.P., Pollard, W.H., Greer, C.W., and Whyte, L.G. (2007a) Characterization of the microbial diversity in a permafrost sample from the Canadian high arctic using culture-dependent and culture-independent methods. *FEMS Microbiol Ecol* **9:** 513–523.

Steven, B., Niederberger, T.D., Bottos, E.M., Dyen, M.R., and Whyte, L.G. (2007b) Development of a sensitive radiorespiration method for detecting microbial activity at subzero temperatures. *J Micro Methods* **71:** 275–280.

Stibal, M., Sabacka, M., and Kastovska, K. (2006) Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microb Ecol* **52:** 644–654.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R., and Wilmotte, A. (2003) Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. *Appl Environ Microbiol* **69:** 5157–5169.

Van Trappen, S., Mergaert, J., Van Eygen, S., Dawyndt, P., Cnockaert, M.C., and Swings, J. (2002) Diversity of 746 heterotrophic bacteria isolated from microbial mats from ten Antarctic lakes. *Syst Appl Microbiol* **25:** 603–610.

Vincent, W.F. (2007) Cold tolerance in cyanobacteria and life in the cryosphere. In *Algae and Cyanobacteria in Extreme Environments*. Seckbach, J. (ed.). Heidelberg: Springer, Heidelberg, pp. 287–301.

Vincent, W.F., Castenholz, R.W., Downes, M.T., and Howard-Williams, C. (1993) Antarctic cyanobacteria: light, nutrients and photosynthesis in the microbial mat environment. *J Phycol* **29:** 745–755.

Vincent, W.F., Gibson, J.A., Pienitz, R., Villeneuve, V., Broady, P.A., Hamilton, P.B., and Howard-Williams, C. (2000) Ice shelf microbial ecosystems in the high arctic and implications for life on snowball earth. *Naturwissenschaften* **87:** 137–141.

Vincent, W.F., Mueller, D.R., and Bonilla, S. (2004) Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology* **48:** 103–112.

Vishnivetskaya, T.A., Petrova, M.A., Urbance, J., Ponder, M., Moyer, C.L., Gilichinsky, D.A., and Tiedje, J.M. (2006) Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods. *Astrobiology* **6:** 400–414.

Waleron, M., Waleron, K., Vincent, W.F., and Wilmotte, A. (2006) Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. *FEMS Microbiol Ecol* **59:** 356–365.

Whitehead, T.R., and Cotta, M.A. (1999) Phylogenetic diversity of methanogenic *Archaea* in swine waste storage pits. *FEMS Microbiol Lett* **179:** 223–226.

Woese, C.R. (1987) Bacterial evolution. *Microbiol Rev* **51:** 221–271.

Zhang, X.F., Yao, T.D., Tian, L.D., Xu, S.J., and An, L.Z. (2007) Phylogenetic and physiological diversity of bacteria isolated from Puruogangri Ice Core. *Microb Ecol*. 18 September 2007 [Epub ahead of print] MID: 17876656.

Supplementary material

The following supplementary material is available for this article online:

Fig. S1. Rarefaction analysis of 16S rRNA gene clone libraries constructed from samples from the Markham Ice Shelf (MIS) and Ward Hunt Ice Shelf (WIS). Rarefaction curves were generated using the program DOTUR grouping operational taxonomic units as sequences with greater than 97% sequence identity.

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