Antarctic Science 19 (2), 245–252 (2007) © Antarctic Science Ltd Printed in the UK DOI: 10.1017/S0954102007000326

First evidence for a bipolar distribution of dominant freshwater lake bacterioplankton

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Abstract: As a result of the recent application of DNA based technology to the investigation of maritime Antarctic freshwater lakes, patterns have begun to emerge in the bacterioplankton communities that dominate these systems. In this study, the bacterioplankton communities of five Antarctic and five Arctic freshwater lakes were assessed and compared with existing data in the literature, to determine whether emerging patterns in Antarctic lakes also applied to Arctic systems. Such a bipolar comparison is particularly timely, given the current interest in biogeography, the global distribution of microorganisms and the controversy over the global ubiquity hypothesis. In addition, it has recently been discovered that commonly encountered bacterial sequences, often originating from uncultivated bacteria obtained on different continents, form coherent phylogenetic freshwater clusters. In this study we encountered both identical sequences and sequences with a high degree of similarity among the bacterioplankton in lake water from both poles. In addition, Arctic freshwater lakes appeared to be dominated by some of the same groups of bacterioplankton thought to be dominant in Antarctic lakes, the vast majority of which represented uncultivated groups.

Received 29 March 2006, accepted 16 August 2006

Key words: 16S rRNA gene, bacteria, biogeography, clone library, cosmopolitan, DGGE, freshwater cluster

Introduction

It has been suggested that geographic barriers are of limited importance for the speciation and biogeography of microbes (as compared to larger organisms) and that microbial species are ubiquitous (Finlay 2002). As the number of environments studied continues to grow, so patterns in the global diversity and distribution of different micro-organisms are beginning to emerge. Recently, for example, it has been observed that bacteria from a range of different freshwater environments sampled on different continents can be grouped into a number of relatively tight freshwater clusters (Zwart *et al.* 1998, Glöckner *et al*. 2000, Hahn 2003). However, whereas the global species ubiquity hypothesis has been tested for various groups of microbial eukaryotes, it remains inconclusive when applied to the prokaryotes. Indeed, there is currently no consensus regarding the geographic distribution and extent of endemism in Antarctic micro-organisms.

The location of the Antarctic and Arctic regions represents the most extreme geographic separation possible on earth, yet these two high latitude environments share similar selection pressures, and as such, offer the unique opportunity to test theories and investigate patterns of global distribution. In particular, the phenomenon of bipolarity has been investigated since the mid-nineteenth century (Crame 1993). Bipolar studies have been conducted on a wide range of organisms, including molluscs (Crame 1993), nematodes (Orecchia *et al.* 1994), lichens (Sochting & Olech 1995), dinoflagellates (Okolodkov & Dodge 1996, Okolodkov 1999, Montresor *et al.* 2003), foraminifera (Darling *et al.* 2000), cyanobacteria (Chevalier *et al.* 2000), mosses (Lud *et al.* 2002), freshwater ciliates (Petz 2003), hydrozoa (Stepanjants *et al.* 2003), Cidaroid sea urchins (Pearse & Lockhart 2004), diatoms (Van de Vijver *et al.* 2005) and phage (Short & Suttle 2005) yet the patterns of bipolar distribution vary significantly both with the species investigated and by study.

For the bacteria, a number of bipolar comparisons have been made for the marine system, where bipolar or cosmopolitan distribution appears to be common (Mergaert *et al.* 2001, Hollibaugh *et al.* 2002, Bano *et al.* 2004). In studies of the sea ice, bipolar (Brinkmeyer *et al.* 2003), endemic (Gosink *et al.* 1998) and heterogeneous populations (Brown & Bowman 2001) have all been found, and cosmopolitan bacteria have been isolated from polar deep-sea marine sediment (Ruger *et al.* 2000). To date, however, bipolar comparisons of freshwater lake bacterioplankton are lacking. These systems are particularly interesting, as the same environmental factors that determine which species come to dominance in Antarctic lakes should also be active in Arctic systems. Freshwater lakes, therefore, constitute confined ecosystems suitable for investigating the global distribution of bacterioplankton.

In this study, we examined the bacterioplankton

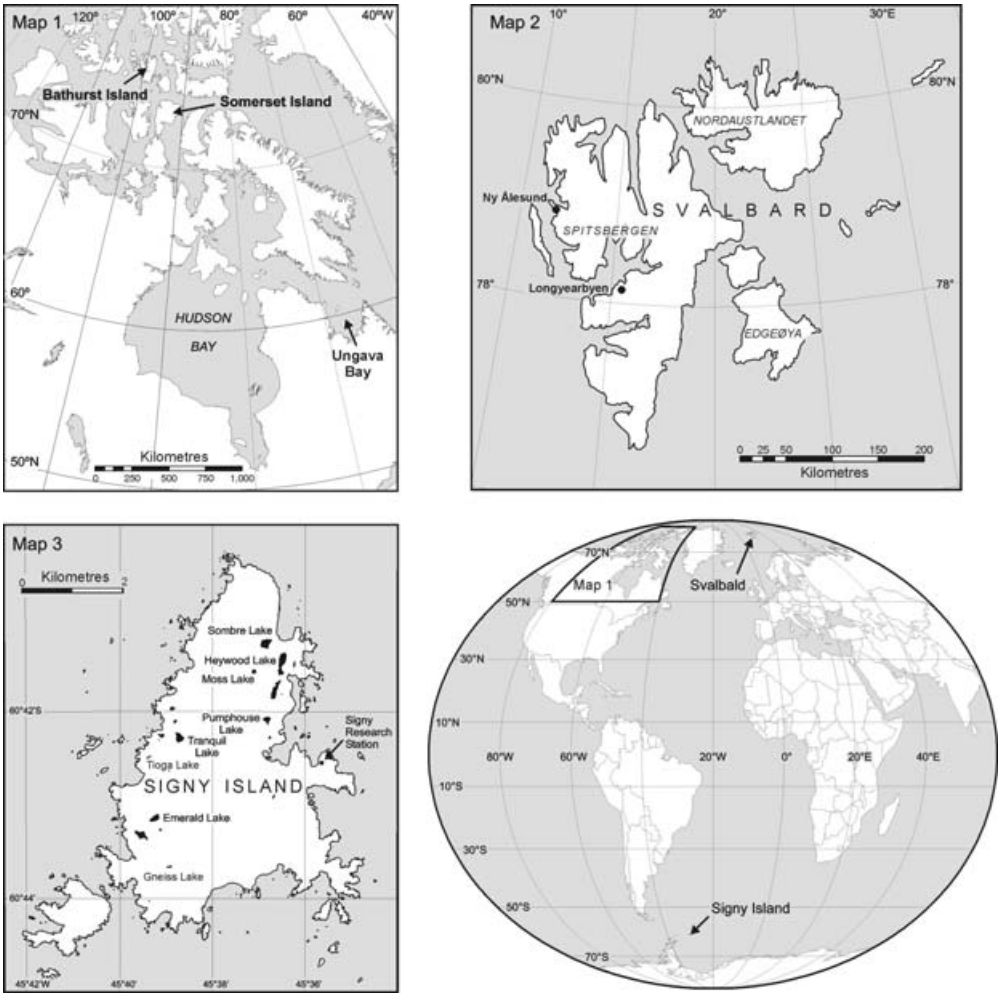

Fig. 1. Arctic and Antarctic lake sample locations.

community of five diverse Antarctic freshwater lakes by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments and compared the results to similar data obtained for five Arctic lakes from two distinct geographical locations, three from the Canadian Arctic and two from the Norwegian Arctic (Fig. 1). The nucleotide sequences of individual DGGE bands were determined by cloning and sequencing, and assumed to represent dominant populations within the lakes, as over 5000 cells ml^{-1} are necessary to generate a DGGE fragment (Kan *et al.* 2004). These sequences were then compared against 16S rRNA gene sequence databases, which include a dataset from Signy Island based upon earlier studies of oligotrophic Moss Lake (Pearce 2003), oligotrophic, but becoming progressively enriched Sombre Lake (Pearce *et al.* 2003) and nutrient enriched Heywood Lake (Pearce *et al.* 2005). Here we evaluate the similarity of sequences obtained, and determine the extent to which Arctic sequences match Antarctic sequences.

Materials and methods

Antarctic sample sites

Pumphouse Lake is in Three Lakes Valley on the north east coast of Signy Island. It is 160 x 147 m covering an area of 1.22 ha. The lake is 4 m deep, 20 m a.s.l., and it is 150 m from the sea. The catchment area covers 17 ha and contains skua nests. The inflow consists of streams running off an ice slope, which flow through moss, marble and schist. It is similar in character to Heywood Lake, with nutrient concentrations of nitrate 128 µg l^{-1}, ammonium 27 µg l^{-1}, total phosphate 15 µg l^{-1}, chloride 65 µg l^{-1}, a conductivity of 118 µS and a Chl *a* concentration of 12 µg l^{-1}.

Tranquil Lake is an oligotrophic lake north of Everson Ridge on the west coast of Signy Island. It is 206 x 138 m covering an area of 2.1 ha and is 8 m deep. It receives inflow from streams containing sediment and there are birds nesting nearby. It is 24 m a.s.l., 400 m from the sea and has a catchment area of 36 ha. Its water flows into a number of lakes downstream, with nutrient concentrations of nitrate 206 µg l^{-1}, ammonium 8 µg l^{-1}, total phosphate 8 µg l^{-1},

https://doi.org/10.1017/S0954102007000326 Published online by Cambridge University Press

chloride 68 µg l^{-1}, a conductivity of 87 µS and a Chl *a* concentration of 68 µg l^{-1}.

Tioga Lake, is another lake to the north of Everson Ridge on the west coast of Signy Island. It receives most of its inflow through seepage from waterlogged moss, both fur seals and birds inhabit the catchment of only 4 ha. It is 100 x 25 m with an area of 0.21 ha and a depth of 4m. It is 35 m a.s.l. and 200 m from the sea. Tioga is starting to experience nutrient enrichment with nutrient concentrations of nitrate 99 µg l^{-1}, ammonium 11 µg l^{-1}, total phosphate 51 µg l^{-1}, chloride 72 µg l^{-1}, a conductivity of 154 µS and a Chl *a* concentration of 14 µg l^{-1}.

Gneiss Lake is unusual in that it is ultra-oligotrophic, with nutrient concentrations of nitrate 181 µg l^{-1}, ammonium 5 µg l^{-1}, total phosphate 9 µg l^{-1}, chloride 69 µg l^{-1}, a conductivity of 131 µS and a Chl *a* concentration of 69 µg l^{-1}. It is on the top of the island 150 m a.s.l. and 640 m from the sea. It receives most of its water from adjacent snow banks and there are no animals in the area. Gneiss Lake lies to the south of Everson Ridge on the west coast of Signy Island, is 105 x 40 m, 7.4 m deep and has a catchment of only 9 ha.

Emerald Lake, is a second lake to the south of Everson Ridge on the west coast of Signy Island. It receives most of its water from ice streams, which bring glacial sediment. There are birds but no seals in the catchment of 35 ha. Emerald Lake is 45 m a.s.l. and 500 m from the sea. It is relatively deep at 15 m, has an area of 2.22 ha and dimensions of 190 m x 140 m. Typical nutrient concentrations are; nitrate 116 µg l^{-1}, ammonium 12 µg l^{-1}, total phosphate 24 µg l^{-1}, chloride 55 µg l^{-1}, a conductivity of 78 µS and a Chl *a* concentration of 2 µg l^{-1}.

Arctic sample sites

Lake water samples were taken from two Svalbard lakes (79ºN, 15ºE) between 14 and 21 July 1997 as described previously (Lindström & Leskinen 2002). Sarsvatnet Lake is a freshwater lake with a total phosphorus concentration of 3 µg l^{-1}. The surface area of the lake is 0.22 km^2. Øvretjørna Lake had a phosphorus concentration of 7 µg l^{-1} and its surface area is 0.01 km^2. This lake had a considerable bird population. The characteristics of the Svalbard lakes are further described in Lindström & Leskinen (2002).

Lakes in the Canadian Arctic were sampled during the Tundra Northwest Expedition arranged by the Swedish Polar Research Secretariat in 1999. Three lakes are included in this study, situated at the Ungava Peninsula, Ungava Bay (62.23ºN 73.42ºW, sampled 2 July), North Bathurst Island (76.26ºN 97.43ºW, sampled 16 July), and Somerset Island (72.55ºN 93.30ºW, sampled 10 July). Total phosphorus in all lakes was 8–9 µg l^{-1}, and DOC ranged between 0.9 and 2.7 mg l^{-1}. All lakes were roughly 10 ha in size, and had ≤ 0.4 µg Chl *a* l^{-1}. Thus all Arctic lakes studied here were small, oligotrophic clearwater lakes.

Sampling regime

Antarctic lake water samples were taken as described in Pearce (2000). Composite samples of the Svalbard lakes were collected using a Ruttner water sampler. Physical and

Table I. Common Antarctic lake DGGE band clones.

Sample site: Closest BLAST match of new Antarctic lake DGGE band clone sequences with the 16S rRNA database	fragment match length (bp)	 (%)	Equivalent match from published Antarctic freshwater lake DGGE band clone sequences
Emmerald Lake (oligotrophic, filled with rock debris):			
Uncultured bacterium FukuN33 (*P. necessarius*) AJ289997[1]	184	100	AJ520091 & AJ548781
Uncultured Crater Lake bacterium CL500-95 AF316665[2]	188	98	AJ520092
Gneiss Lake (ultra-oligotrophic):			
Alcaligenes sp. AY131212 / *Streptomyces* sp. AY232829	211	99	AJ520093
Pumphouse Lake (high nutrient enrichment):			
Nostoc sp. DQ185253	69	100	AJ520095
Flavobacterium xinjiangense AS1.2749 AF433173[3]	179	98	AJ520090 AJ548783
Tranquil Lake (oligotrophic):			
Uncultured bacterium FukuN33 (*P. necessarius*) AJ289997[1]	194	100	AJ548781 & AJ520091
Flavobacterium xinjiangense AS1.2749 AF433173[3]	202	100	AJ520090 & AJ548783
Uncultured Crater Lake bacterium CL500-95 AF316665[2]	171	97	AJ520092
Tioga Lake (medium nutrient enrichment):			
Uncultured Crater Lake bacterium CL500-95 AF316665[2]	178	98	AJ520092
Leptothrix cholodnii X97070	190	97	AJ520098
Nostoc sp. DQ185253	189	96	AJ520095
Uncultured bacterium FukuN33 (*P. necessarius*) AJ289997[1]	192	96	AJ548781 & AJ520091

AJ289997[1], AF316665[2] and AF433173[3] each occur in more than one lake in this study.

https://doi.org/10.1017/S0954102007000326 Published online by Cambridge University Press

Table II. Arctic lake DGGE band clones.

Sample site: DGGE band closest BLAST match in the 16SrRNA gene sequence database	Closest phylogentic affiliation of this match to a specific genus or species	Origin of this matched sequence	sequence length (bp)	match (%)
North Bathurst Island (Canadian Arctic):				
Aquatic bacterium R1-B19 AB195751	*Aquaspirillum* sp. GOBB3-215 AF321032	Lake Inba	198	98
Bacterium H22 AF234697[2]	*Flectobacillus speluncae* AY065626	Activated sludge/ bofilm in a spring cave	191	98
Bacterium isolate AH57 AJ289964	*Flectobacillus* sp. EP293 AF493693	Freshwater/River Taff water	178	98
Uncultured earthworm cast clone C042 AY037695	*Flavobacterium* sp. EP131 AF493665	Agricultural soil/River Taff epilithon	169	97
Marine bacterium SIMO-IS152 AF460933	*Sphingomonas* sp. SW54 U85838[1]	Salt marsh/Antarctic sea ice	105	95
Somerset Island (Canadian Arctic):				
Uncultured eubacterium WR878 AJ292846	*Sphingomonas* sp. SW54 U85838[1]	Polluted soil/Antarctic sea ice	163	98
Uncultured Bacteroidetes CF39 AY274854	*Flavobacterium* sp. GOBB3-209 AF321038	Delaware Estuary/Estuary N. Baltic sea	184	97
Comamonadaceae bacterium BP-8 AY145570[3]	*Aquaspirillum* sp. GOBB3-215 AF321032	Weser Estuary /Estuary N. Baltic sea	182	97
Flavobacterium columnare AJ491824	*Cytophaga* sp. X85210	Fish pathogen/marine sediment	183	96
Antarctic bacterium R-9478 AJ441013	*Agrobacterium sanguineum* AB 021493	Antarctic lake mat/Arctic sea ice	164	95
Ungava Bay (Canadian Arctic):				
Janthinobacterium agaricidamnosum AY167838	*Ultramicrobacterium* str. Um1 AY367012	Factory/soil	195	100
Bacterium H22 AF234697 AF234697[2]	*Flectobacillus speluncae* AY065625	Activated sludge/biofilm in a spring cave	189	100
Arctic sea ice bacterium ARK10164	*Flavobacterium* sp. PDD-146-7 DQ512791	Arctic sea-ice	189	100
Uncultured alpha prot JG37-AG-130 AJ518779	*Caulobacter vibrioides* AB008531	Uranium mine waste	170	100
Rhodoferax sp. Fon05 AY788954	*Rhodoferax* sp. 7M1 AB206450	Estuary	192	97
Antarctic bacterium R-8287 AJ440992	*Cryobacterium* sp. 0549 DQ155963	Antarctic lake	179	97
Beta proteobacterium LI1-63 AJ964890	*Collimonas fungivorans* CTE118 AJ496444	Melt ponds on Arctic sea ice floes/soil	195	96
Arctic sea ice bacterium ARK1072 AF468348	*Alcaligenes* sp. LMG 5906 AY131213	Alpine freshwater lake/Weser estuary	182	96
Curtobacterium flaccumfaciens AM231279	*Plantibacter agrosticola* NSF34 AM048800	Psychrotolerant sub-Antarctic region	179	96
Unidentified bacterium clone LWSR-14 AY345543	*Erythromonas* sp. QSSC5-6 AF170744	Lake Hawaii/Antarctic sublithic	170	96
Arctic sea-ice bacterium ARK10172	*Collimonas* sp. AJ496444	Arctic sea-ice	194	95
Lake Øvretjørna, Svalbard (Norwegian Arctic):				
Uncultured gamma prot GOBB3-B07-4-2 AF494505	*Burkholderi*a sp. LB400 U86373	Estuary	184	100
Beta proteobacterium zj53 AF530961	*Comamonadaceae* bacterium AY145570[3]	Planktonic bacteria /Weser estuary	188	99
Lake Sarsvatnet, Svalbard (Norwegian Arctic):				
Flavobacterium johnsoniae DSM425 AM230488	*Flavobacterium* sp. E300 AF493662	Freshwater	177	99
Antarctic bacterium R-7579	*Flavobacterium* sp. AS1.3801 DQ021903	Antarctic lake	186	97
Rhodoferax antarcticus strain Fryx1 AY609198	*Variovorax* sp. DQ432053	Antarctic lake	192	96
Rhodoferax fermentans DSM 13 235 AJ289107	*Rhodoferax* sp. DQ227795	Temperate microbial mat	185	96
Unidentified bacterium Mul01-10 AJ518107	*Variovorax* sp. P16G933 AF214128	Reservoir sediment/Rhizosphere	188	95

U85838[1], AF234697[2] and AY145570[3] each occur in more than one lake in this study.

chemical analyses, collection of bacterioplankton cells and DNA-extraction procedures were described previously (Lindström & Leskinen 2002). For the Canadian lakes, water was sampled at *c.* 0.5 m depth with a Ruttner sampler. All samples for bacterial analysis were retrieved on sterile 0.2 µm cellulose nitrate membrane filters (47 mm diameter), and frozen (-70°C) until further analysis.

Analysis of 16S rRNA gene fragments from lake samples

Enzymatic amplification of the 16S rRNA gene was performed on DNA extracted directly from filters using the method described previously (Pearce 2000). For these amplifications, each PCR mixture (50 µl) contained 10 ng of extracted DNA as a template, 20 pmol of each primer and 46 µl ReddyMix PCR master mix (ABgene, Epsom, UK). Primers used were: primer 2 and primer 3 (Muyzer *et al.* 1993). Amplification reactions were performed with a Genius thermocycler (Techne, Minneapolis, USA) using the following conditions: an initial denaturation step consisting of 94ºC for 5 min, 30 cycles consisting of 94°C for 45 sec, 55°C for 45 sec, 72°C for 70 sec, and a final elongation step consisting of 72°C for 5 min. Controls containing no DNA were also used to ensure that contaminants were not being amplified.

Denaturing gradient gel electrophoresis (DGGE)

25 µl of PCR product was separated by DGGE as described by Pearce (2000). The gel was run for 80 min at 60°C and 10 V cm^{-1}, before staining for 45 min in 0.5 µg ml^{-1} ethidium bromide solution. This was visualized on a UV transilluminator (UVP, Cambridge, UK). Bands were removed from the gel using a sterile scalpel blade and each placed in a separate sterile 1.5 ml Eppendorf tube. The gel fragments were incubated with 200 µl DNA/RNA free

Table III. Comparison of Arctic lake DGGE band clones ≥ 95% sequence similarity to Antarctic lake DGGE band clones.

Arctic lake clone accession no.	Closest BLAST match to this Arctic sequence with accession numbers	Division	fragment length (bp)	match (%)	Equivalent match from Antarctic freshwater lake DGGE bands
North Bathurst Island (Canadian Arctic)					
AJ865446	Uncultured bacterium S9F-17 AB154306	Unidentified	158	100	AJ520092
AJ865447	Moss Lake DGGE band AJ520091	β-Proteobacteria	175	98	AJ520091
AJ865448	Uncultured Crater Lake bacterium AF3116665	Actinobacteria	158	98	AJ520097
AJ865449	*Flavobacterium xinjiangense* AF433172	Bacteroidetes	173	95	AJ520090
Somerset Island (Canadian Arctic)					
AJ865450	Uncultured bacterium S9F-17 AB154306	Unidentified	158	100	AJ520092
AJ865451	Uncultured bacterium PRD01a006B AF289154	β-Proteobacteria	177	97	AJ520091
Ungava Bay (Canadian Arctic)					
AJ865454	Glacier bacterium FJS11 AY315169	Unidentified	156	96	Antarctic bacterium R-8287 AJ440992
AJ865455	Sombre Lake DGGE band AJ520083	Bacteroidetes	202	96	AJ520083
Lake Øvretjørna, Svalbard (Norwegian Arctic)					
AJ865457	Unidentified bacterium oxSCC-25 AJ387870	α-Proteobacteria	136	100	*Sphingomonas* sp. Ant17 AF184222
AJ865458	Unidentified bacterium ACK-C4 U85124	β-Proteobacteria	160	100	AJ548781
Lake Sarsvatnet, Svalbard (Norwegian Arctic)					
AJ865460	*Sphingomonas yanoikuyae* Q1 U37525	α-Proteobacteria	153	100	Sphingomonas sp. G296-3 AF395036
AJ865461	Unidentified bacterium ACK-C4 U85124	β-Proteobacteria	176	99	AJ548781
AJ865462	Uncultured *Flavobacterium* sp. AF493667	Bacteroidetes	176	99	*Flavobacterium frigidarium* AF162266

water (Sigma, St. Louis, USA) for one hour at 4ºC to extract the ethidium bromide. The resultant solution was then removed and discarded and replaced by 50 µl water. The samples were left for 24 hours at 4ºC and 1 µl of the resultant solution used as a template in a second PCR reaction under the same reaction conditions. The products of this amplification were run in a second DGGE gel to check the purity of the DNA in the band extraction.

Cloning and sequencing PCR products

The PCR products from excised bands were cleaned using GFX PCR clean up columns (Pharmacia, New Jersey, USA). An attempt was made to sequence all bands present. Cleaned products were ligated into the pGEMT-Easy vector (Promega, Wisconsin, USA) and ligation mixtures were transformed into competent JM109 cells as recommended by the manufacturer. Transformants were screened using black/white selection on Luria agar containing S-gal/IPTG and 50 µg ml^{-1} ampicillin (Sigma, St Louis, USA). Putative positive colonies were transferred to individual tubes containing 50 µl of sterile water. The cell suspensions were subjected to two freeze/thaw cycles and 1 µl aliquots were used as templates in a PCR reaction containing the M13F/M13R universal primers: M13F 5'- CGC CAG GGT TTT CCC AGT CAC GAC -3' and M13R 5'- GAG CGG ATA ACA ATT TCA CAC AGG-3'. PCR conditions were as above, except that the annealing temperature was raised to 58.5°C. Each clone obtained was sequenced with the M13F primer using the DYEnamic ET Dye Terminator Kit (Amersham Biocience, Buckinhamshire, UK). Sequence reactions were carried out at the British Antarctic Survey (BAS) using a Megabace 500 capillary sequencer. Clone sequences were compared with the Genbank nucleotide data library using GAPPED-BLAST (Basic Local Alignment Search Tool) searches (at http://www.ncbi.nlm.nih.gov/blast/blast.cgi) (Altschul *et al.* 1997) to determine their closest phylogenetic neighbours.

Results

Twelve clone sequences from the five Antarctic lakes tested had ≥ 95% similarity to sequences obtained from Moss Lake (Pearce 2003) or Sombre Lake (Pearce *et al.* 2003) and four of these sequences were identical (Table I). Of the 82 DGGE band clone sequences in total from the five Arctic lakes studied, 41 clone sequences had ≥ 95% similarity to sequences in the BLAST database (Tables II & III). Of these Arctic sequences, 13 (~16%) had ≥ 95% similarity to Antarctic sequences and five of these sequences were identical (Table III). Ten of these sequences showed a high degree (≥ 97%) of similarity to DGGE product sequences obtained from Antarctic freshwater lake samples. In addition to the Arctic sequences that matched Signy Island DGGE clones, eight further sequences matched sequences derived from other polar studies. The origin of sequences which show best matches to the Arctic DGGE clones were; 33.3% brackish, estuarine or marine, 28.5% freshwater (non-polar), 19% human activity/industry, 9.5% soil and 9.5% Antarctic (Table II). Unsurprisingly, as slightly over 6200 prokaryotes have been named (Oren 2004) from a global prokaryote diversity with an estimated 2 million species in the marine system alone (Curtis *et al.* 2002), all lake systems studied appeared to be dominated by as yet

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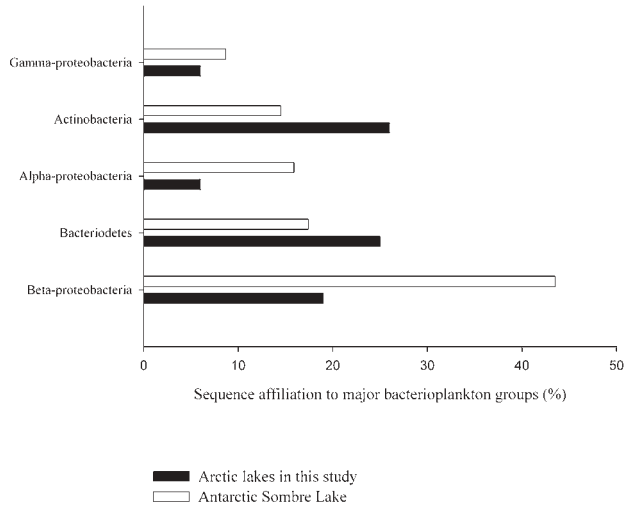

Fig. 2. The affiliation to major bacterioplankton groups of our sequences from the Arctic lakes and Antarctic Sombre Lake.

uncultivated bacteria. BLAST-matches from sequences obtained in this study showed that most of the Arctic sequences could be affiliated with β-Proteobacteria, Bacteroidetes, α-Proteobacteria or Actinobacteria (Fig. 2). In common with Antarctic studies close matches were found between the Arctic sequences and *Sphingomonas* sp. (98%), *Cryobacterium* sp. (97%), *Erythromonas* sp. (96%) and *Agrobacterium* sp. (95%). Cloned sequences of 16S rRNA genes from Sombre Lake in the Antarctic (Pearce *et al.* 2003), were dominated by the Actinobacteria, Bacteroidetes and β-Proteobacteria (Fig. 2). Thus the dominant major groupings of bacterioplankton were similar in the freshwater lakes at both poles.

Discussion

The lakes selected for this study were of very different origin and subject to a variety of different external influences, such as bird colonies or fur seals. Each of the Antarctic lakes was dominated by relatively low numbers of bacterioplankton groups and the majority of these dominant groups remain uncultivated. However, three sequences identified from Antarctic lakes on Signy Island were common to more than one lake studied. The uncultured bacterium FukuN33 (*P. necessarius*) sequence (AJ289997) occurred in Moss, Sombre, Emerald, Tranquil and Tioga Lakes, the uncultured Crater Lake bacterium CL500-95 (AF316665) occurred in Moss, Emerald, Tranquil and Tioga lakes, and *Flavobacterium xinjiangense* AS1.2749 (AF433173) occurred in Moss, Sombre, Pumphouse and Tranquil lakes, suggesting that a number of dominant Antarctic freshwater lake bacteria could dominate a range of different habitat types.

In the Arctic, DNA sequences similar to *Sphingomonas* sp. SW54 (U85838) occurred at both North Bathurst Island and Somerset Island in the Canadian Arctic. In addition, sequences similar to *Flectobacillus speluncae* (AY065626) occurred at both North Bathurst Island and Ungava Bay, suggesting that some dominant groups of freshwater Arctic bacteria could have a wide geographic distribution. The *Comamonadaceae* bacterium (AY145570) sequence occurred in both the Canadian Arctic and at Svalbard, suggesting that some dominant groups might also have a pan-Arctic distribution.

Table IV. The proportion of DGGE bands identified from Arctic lakes that also occur in Antarctic lakes.

	% DGGE bands also found in Antarctic lakes
Canadian Arctic	
North Bathurst Island	36.4
Somerset Island	20.0
Ungava Bay	11.8
Norwegian Arctic	
Lake Øvretjørna	33.3
Lake Sarsvatnet	30.0

The five Arctic lakes studied had between two and four DGGE band sequences in common with Antarctic freshwater lakes (Table III). This represented between 11.8 and 36.4% of the DGGE bands sequenced (Table IV). Again, most of the dominant groups represented uncultivated bacteria and the lakes were dominated by a relatively small number of groups. Common groups identified from lakes at both poles were; uncultivated Actinobacteria, *Polynucleobacter necessarius*, *Flavobacterium xinjiangensis*, *Sphingomonas* sp. and *Flavobacterium frigidarium*, so a number of dominant bacterioplankton groups from Antarctic lakes also appear to be dominant in Arctic lakes.

Common freshwater clusters of cosmopolitan distribution have already been suggested (Glöckner *et al.* 2000), and this might be expected given the colonization of these lake systems by birds and mammals, some of which might act as common sources of inoculation. In 2002, Zwart *et al.* compared 689 bacterial 16S rRNA gene sequences from available freshwater datasets using BLAST. They found that the majority of the sequences (54%) were similar to other sequences found in freshwater habitats. In this study, the closest BLAST matches to our Arctic sequences, which did not appear to have a bipolar distribution, were also predominantly from the marine ecosystem or from non-polar freshwater lakes. Thus, both the Antarctic and Arctic bacterioplankton communities appear to show some similarity to freshwater lake communities elsewhere. Further results obtained by Zwart *et al.* (2003), who applied 15 probes specific for freshwater bacterioplankton groups to 80 lakes in Europe (including our Svalbard lakes) also support this conclusion.

In other systems, a bipolar or cosmopolitan distribution appears to be far more common. In the polar marine system, which has been more intensively studied, most of the

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bacterioplankton species appear to be either cosmopolitan or bipolar (with specific exceptions). Mergaert *et al.* (2001) characterized 98 Arctic and 75 Antarctic marine bacterial strains by gas-liquid chromatographic analysis of their fatty acid compositions and found eight clusters - five clusters contained strains from both poles, two minor clusters were confined to Arctic isolates and one cluster consisted of Arctic isolates only. In a DGGE based study, Bano *et al.* (2004) compared samples from both polar oceans. Although they found that most of the DGGE bands were common to both Arctic and Antarctic samples, a dominant band in the Arctic Ocean surface mixed layer was not detected in Antarctic samples and a dominant band present at all depths in the Antarctic was not found in the Arctic. Elsewhere, Hollibaugh *et al.* (2002) analysed the phylogenetic composition of ammonia-oxidizing bacteria of the β-subclass of the Proteobacteria from 42 Southern Ocean samples. They found a *Nitrosospira*-like 16S rRNA gene sequence in all 20 samples that gave PCR products. They also found this sequence in Arctic Ocean samples, and suggest a transpolar, if not global distribution. However, they cite slight differences between Arctic and Antarctic sequences as potential evidence of polar endemism.

For sea ice, it is possible to identify species that do not have a bipolar or cosmopolitan distribution (Gosink *et al.* 1998). Although even in this system, Brown & Bowman (2001) have found certain sea ice genera that were common to both poles, and Brinkmeyer *et al.* (2003) sequenced 16S rRNA genes from Arctic and Antarctic cultures derived from pack ice and found that most of the Arctic phylotypes were > 97% identical to previously determined Antarctic species or to their own Antarctic isolates.

There is, however, growing evidence that polar endemism can occur. Taton *et al.* (2003) used molecular methods to study the diversity of cyanobacteria in a field microbial mat sample from Lake Fryxell, Antarctica. They found that the 16S rRNA gene sequences were distributed in 11 phylogenetic lineages, three of which were exclusively Antarctic. They suggest that Antarctic endemic species are more abundant than has been estimated on the basis of morphological features alone.

We can therefore conclude, based on DGGE band analysis, that dominant freshwater lake bacterioplankton with a bipolar distribution do occur, and that most of the bacterioplankton groups encountered in both Arctic and Antarctic freshwater lake systems might fit into emerging freshwater groups described elsewhere (Zwart *et al.* 1998, 2002, Glöckner *et al.* 2000, Hahn 2003). However, although some similarities exist, individual lake differences in microbial populations are evident (Lindström & Leskinen 2002, Pearce 2003, Pearce *et al.* 2003, 2005), and the presence of distinct sequences at each pole does suggest some degree of geographical isolation for freshwater bacterioplankton.

Acknowledgements

This research was supported by the Natural Environment Research Council through the British Antarctic Survey. The sampling at Svalbard was supported by a grant from the YMER-80 foundation and from the training and mobility of researchers (TMR) programme of the commission of the European communities (CEC). Canadian sampling was supported by the Swedish Polar Research Secretariat and the Swedish Research Council. Peter Fretwell (BASMAGIC) produced Fig. 1.

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