

## Diversity and Structure of Bacterial Communities in Arctic versus Antarctic Pack Ice

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**A comprehensive assessment of bacterial diversity and community composition in arctic and antarctic pack ice was conducted through cultivation and cultivation-independent molecular techniques. We sequenced 16S rRNA genes from 115 and 87 pure cultures of bacteria isolated from arctic and antarctic pack ice, respectively. Most of the 33 arctic phylotypes were >97% identical to previously described antarctic species or to our own antarctic isolates. At both poles, the  $\alpha$ - and  $\gamma$ -proteobacteria and the *Cytophaga-Flavobacterium* group were the dominant taxonomic bacterial groups identified by cultivation as well as by molecular methods. The analysis of 16S rRNA gene clone libraries from multiple arctic and antarctic pack ice samples revealed a high incidence of closely overlapping 16S rRNA gene clone and isolate sequences. Simultaneous analysis of environmental samples with fluorescence in situ hybridization (FISH) showed that ~95% of 4',6'-diamidino-2-phenylindole (DAPI)-stained cells hybridized with the general bacterial probe EUB338. More than 90% of those were further assignable. Approximately 50 and 36% were identified as  $\gamma$ -proteobacteria in arctic and antarctic samples, respectively. Approximately 25% were identified as  $\alpha$ -proteobacteria, and 25% were identified as belonging to the *Cytophaga-Flavobacterium* group. For the quantification of specific members of the sea ice community, new oligonucleotide probes were developed which target the genera *Octadecabacter*, *Glaciecola*, *Psychrobacter*, *Marinobacter*, *Shewanella*, and *Polaribacter*. High FISH detection rates of these groups as well as high viable counts corroborated the overlap of clone and isolate sequences. A terrestrial influence on the arctic pack ice community was suggested by the presence of limnic phylotypes.**

Pack ice in the Arctic and Antarctic, with its vast extension and high biological productivity (3, 34, 35, 36, 56), constitutes one of the most significant polar ecosystems. Several similarities exist between the sea ice regimes in the north and the south; however, there are also fundamental differences in formation, development, thickness, maturity, and ice crystal structure (57). Moreover, the Arctic Ocean, in contrast to the Southern Ocean, is strongly influenced by warm Atlantic waters and has a high terrestrial input due to its nearly complete enclosure by landmasses. Whether these differences influence the colonization of sea ice and the development of microbial sea ice communities is still an open question.

Phylogenetic diversity studies of sea ice bacterial communities have focused mainly on the Antarctic (8, 18). In particular, land-fast ice surrounding the McMurdo base and pack ice between the Casey and Davis bases has been investigated. Sampling was limited to spring and summer seasons. The few arctic sea ice samples considered came from Baffin Bay (18), the Chukchi Sea (41), and Barrow, Alaska (31). Cultivation approaches provided the initial view of diversity of sea ice bacteria, mainly in the Antarctic, and revealed several novel genera and species that appear to be specific to sea ice (8, 9, 10, 11, 12, 13, 14, 15, 30, 32, 33, 40, 46). Only recently, the first phylogenetic analyses of environmental DNA from antarctic sea ice including a single arctic sample was reported, indicating

some overlap of antarctic and arctic sea ice 16S rRNA gene phylotypes (18).

The aim of this study was to compare the diversity and structure of bacterial communities in arctic versus antarctic pack ice by cultivation and cultivation-independent methods, making use of sea ice samples from yet-unconsidered geographical areas and seasons. We sequenced 16S rRNA genes from pure-culture isolates and from environmental samples derived from multiyear arctic pack ice north of Svalbard and the Fram Strait. The pack ice in this region is strongly influenced by terrestrial input from Siberian rivers (51). 16S rRNA genes from pure cultures isolated from first-year (annual) antarctic pack ice sampled in the Weddell Sea during midwinter (36) as well as environmental samples from the Lazarev Sea taken during autumn were also sequenced. To find out if community composition is affected by the starkly contrasting sea ice habitats at either pole, fluorescence in situ hybridization (FISH) was applied to the environmental samples. New oligonucleotide probes specific for several members of the sea ice bacterial community were developed and used in combination with previously published probes for the FISH analyses. We present a comprehensive survey of bacterial diversity in arctic and antarctic pack ice and report the first data for in situ distribution and abundance of bacteria in sea ice.

### MATERIALS AND METHODS

**Sampling, processing, total and viable counts, and isolation.** Multiyear arctic pack ice samples (thickness, 3 to 5 m) were collected during the R. V. *Polarstern* summer cruises ARKXIII/2 (June to July 1997), ARKXV/1 (June to July 1999), and ARKXVI/2 (July to August 2000) in the Fram Strait and northeast of

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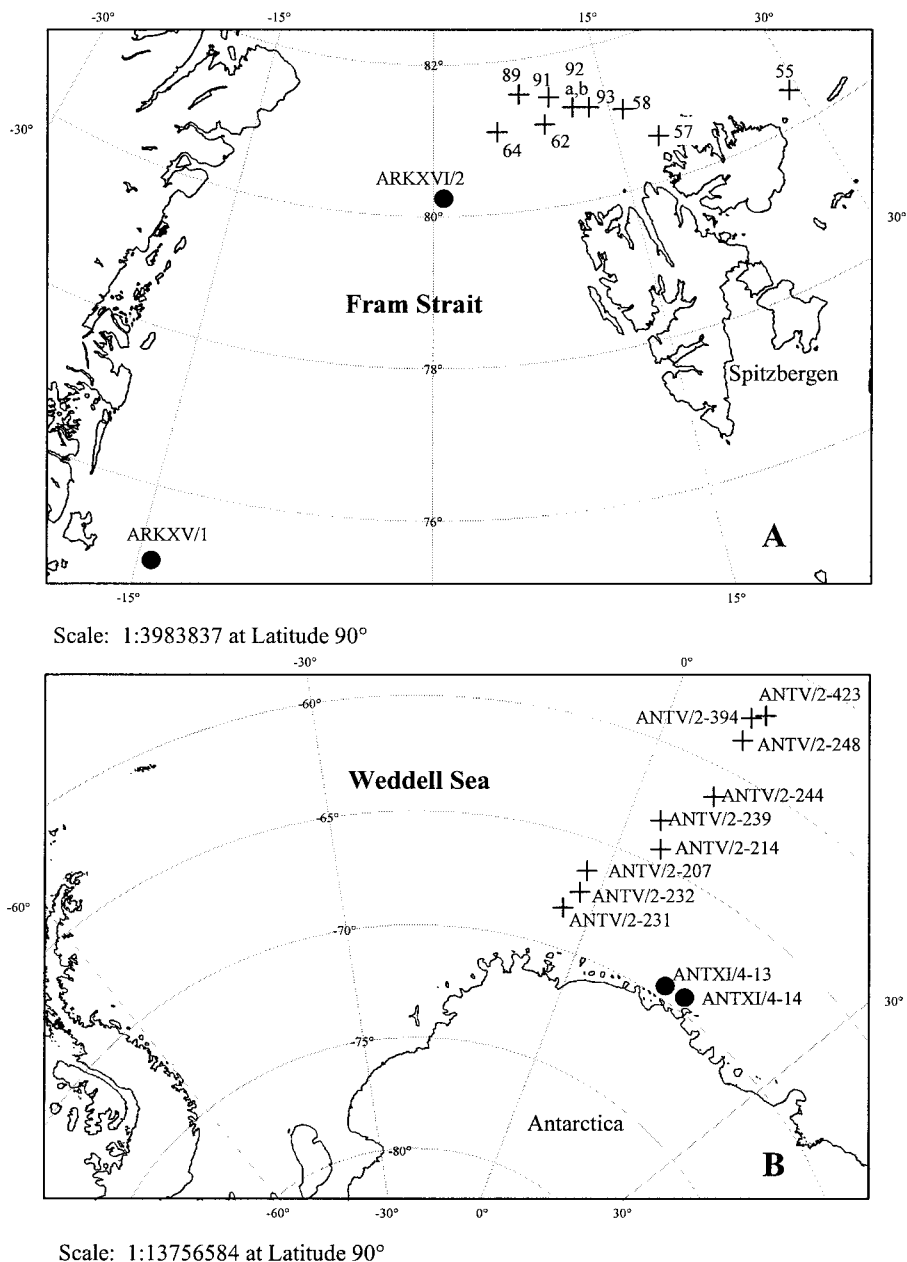


FIG. 1. Sampling stations in the Arctic Ocean and Fram Strait (A) and the Weddell and Lazarev Seas in the Southern Ocean (B). Crosses represent sampling stations for isolate cultures and closed circles represent sampling stations for environmental samples. Maps were generated with PanMap software (<http://www.pangaea.de>).

Svalbard in the Arctic Ocean. First-year antarctic pack ice was sampled during the R. V. *Polarstern* midwinter cruise ANTV/2 (July to September 1986) in the open Weddell Sea and during the autumnal cruise ANTXI/4 (March to April 1994) in the area of the Lazarev Sea. Positions of all stations are shown in Fig. 1. Sea ice samples with and without algal accumulation were present among the arctic and the antarctic samples. The ARKXXV/1 station ice core contained several large sediment inclusions. Samples were collected and processed as described previously by Helmke and Weyland (36). The arctic pack ice isolates were derived from the bottom sections of 10 different ice cores collected during cruise ARKXXIII/2. The antarctic strains were isolated from the upper, middle, and bottom sections of seven ice cores as well as from grease ice and freshly formed pancake ice sampled during cruise ANTV/2 (36). Pure cultures were obtained from chitin agar (36), ZoBell agar 2216E, and a nutrient-poor agar medium containing 200 mg of yeast extract and 1 g of potassium nitrate in 1 liter

of natural seawater. Total and viable counts were determined as previously described by Helmke and Weyland (36). Samples for clone libraries and FISH were collected during cruises ARKXXV/1, ARKXXVI/2, and ANTXI/4. Bacterioplankton of melted-ice samples (36) were collected onto polycarbonate filters (pore size, 0.2  $\mu\text{m}$ ) and stored at  $-80^\circ\text{C}$  for later extraction of nucleic acids. Parallel samples were fixed with paraformaldehyde (final concentration, 2 to 4% [vol/vol]), immobilized on polycarbonate filters (pore size, 0.2  $\mu\text{m}$ ), and then rinsed with 3 ml each of phosphate-buffered saline and distilled water. Air-dried filters were stored at  $-20^\circ\text{C}$  until analysis with FISH. Total count preservations were fixed with formalin (final concentration, 3% [vol/vol]) and stored at  $2^\circ\text{C}$  for up to 2 months before enumeration (38).

**Extraction of nucleic acids, amplification of 16S rRNA gene, and clone library construction.** Nucleic acids from pure cultures were extracted with a 3% cetyltrimethylammonium bromide procedure (22) For clone libraries, total commu-

TABLE 1. Oligonucleotide probes used in this study

Probe	Specificity	Probe sequence (5'-3')	Target site 16S or 23S rRNA position (nucleotide)	% FA <sup>a</sup>	Source or reference
NON338		ACTCCTACGGGAGGCAGC	16S (338–355)	0	64
EUB338	<i>Bacteria</i>	GCTGCCTCCCGTAGGAGT	16S (338–355)	35	2
ALF968	$\alpha$ -Proteobacteria	GGTAAGGTTCTGCGCGTT	16S (968–985)	20	49
SPH120	<i>Sphingomonas</i> spp.	GGGACGATCCCACGCGT	16S (120–137)	30	49
ROSEO536	<i>Roseobacter</i> clade	CAACGCTAACCCCTCCG	16S (536–553)	20	16
ODB1021	<i>Octadecabacter</i> spp.	GCGTCCCCTAAGGGAAGT	16S (1021–1038)	15	This study
RSHP995	<i>Roseobacter</i> sp. strain Shippagan group	CTCGGATTGTCCAGGCAT	16S (995–1012)	10	This study
BET42a	$\beta$ -Proteobacteria	GCCTTCCCCTTCGTTT	23S (1027–1043)	35	44
GAM42a	$\gamma$ -Proteobacteria	GCCTTCCCACATCGTTT	23S (1027–1043)	35	44
ALT1413	<i>Alteromonas-Cobwellia</i>	TTTGCATCCCCTCCCAT	16S (1413–1430)	40	23
PSA184	<i>Pseudoalteromonas-Cobwellia</i>	CCCCTTTGGTCCGTAGAC	16S (184–210)	30	23
GPU622	<i>Glaciicola punicea</i>	CTAAAAGGCCTTCCCACG	16S (622–639)	17	This study
GVstr214 <sup>b</sup>	<i>Glaciicola</i> sp. strain GVstr214.6	CTAAATGCTATTTCCACG	16S (622–639)	12	This study
GVstr214c	Competitor for GVstr214	CCAAATGCTATTTCCACG	16S (622–639)	12	This study
NOR2-1453	<i>Psychromonas</i> spp.	GGTCATCGCCATCCCC	16S (1453–1468)	30	23
SF825	<i>Shewanella frigidimarina</i>	AAGTCACCAAACCTCCGAG	16S (825–842)	10	This study
PSYB476	<i>Psychrobacter</i> spp.	CTGCAGCTAATGTCATCG	16S (476–493)	10	This study
MB-IC022 <sup>b</sup>	<i>Marinobacter</i> sp. strain IC022 group	GTTTCCGCTCGACTTGCA	16S (55–72)	25	This study
MB-IC022c	Competitor for MB-IC022	GTTTCCGCTCGACTTGCA	16S (55–72)	25	This study
G V	<i>Vibrio</i> spp.	AGGCCACAACCTCCAAGTAG	16S (822–841)	30	26
OCE232	<i>Oceanospirillum</i> spp.	AGCTAATCTACGCAGGC	16S (232–249)	40	23
PS56a	True <i>Pseudomonas</i> spp.	GCTGGCCTAGCCTTC	23S (1432–1446)	0	55
CF319a	CF group	TGGTCCGTTGCTCAGTAC	16S (319–336)	35	43
PB223 <sup>b</sup>	<i>Polaribacter</i> spp.	GGACGCATAGCCATCTTT	16S (223–240)	15	This study
PB223c	Competitor for PB223	GAACGCATAGCCATCTTT	16S (223–240)	15	This study
HGC69a	Gram positive ( <i>Actinobacteria</i> )	TATAGTTACCACCGCGT	23S (1901–1918)	25	53
PLA46	<i>Planctomycetales</i>	GACTTGCATGCCTAATCC	16S (46–63)	30	50
ARCH915	<i>Archaea</i>	GTGCTCCCCGCCAATTCCT	16S (915–934)	20	59

<sup>a</sup> Values represent percent formamide in the hybridization buffer.

<sup>b</sup> Probes GVstr214, MB-IC022, and PB223 were used with competitors GVstr214c, MBIC022c, and PB223c, respectively.

nity nucleic acids from filtered samples were extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany) with additional lysozyme and lysostaphin preincubations. Efficiency of the preincubation was controlled by microscopic examination of filters before and after processing. Between 95 and 97% of observable 4',6'-diamidino-2-phenylindole (DAPI)-stained bacterial cells were effectively lysed. An additional purification step carried out with the WIZARD DNA Cleanup System (Promega Corp., Madison, Wis.) was necessary to remove potential PCR inhibitors that were coextracted from samples.

Nearly full-length 16S rRNA gene sequences were amplified from isolate and environmental sample nucleic acid extracts (approximately 100 ng) by hot-start PCR with an automated thermal cycler (Eppendorf, Hamburg, Germany) by using the bacterium-specific primers 8f and 1542r (for isolates) or 1492r (for environmental samples) under the conditions described previously by Kopp et al. (42). PCR products were purified with the QIAquick purification kit (Qiagen). 16S rRNA gene clone libraries were constructed with the pGEM-T-Easy vector system (Promega Corp.).

**ARDRA, sequencing, and phylogenetic analysis.** Amplified rDNA restriction analysis (ARDRA) (45) was used to characterize the 16S rRNA gene diversity within the culture collections and clone libraries. One, or in some cases, several representatives of the ARDRA pattern groups from each culture collection and clone library were selected for sequencing. Sequence data were analyzed with the ARB software package (<http://www.mikro.biologie.tu-muenchen.de>). Dendrograms (viewable at [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice1-dohtml](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice1-dohtml)) were reconstructed for the phylogenetic analysis. The frequencies of 16S rRNA gene phylotypes determined by ARDRA and subsequent sequencing (i.e., those sharing >97% identity) were used for analysis of diversity. Shannon's index for diversity ( $H'$ ) was calculated according to the method of Zar (65). Rarefaction curves were interpolated with the freeware program Analytic Rarefaction 1.3 (<http://www.u-ga.edu/~strata/software/index.html>). Coverage of the clone libraries was estimated as described previously by Mullins et al. (48).

**Probe design and FISH.** Oligonucleotide probes ranging in specificity from domain to species level (Table 1) were used with FISH to examine community structure of bacteria in arctic and antarctic pack ice. Probes for several bacterial groups characteristic of the sea ice community were developed and tested for

hybridization specificity according to the method of Stahl and Amann (59) (Table 1). All of the probes have at least one strong mismatch (1.1 to 2.8 weighted mismatches); however, in some cases, a competitor was designed to enhance probe specificity (Table 1). FISH analysis with CY3-labeled oligonucleotide probes (final concentration, 5 ng/ $\mu$ l; Interactiva, Ulm, Germany) was conducted according to the previously described method of Glöckner et al. (27).

**Nucleotide sequence accession numbers.** The almost full-length (>1,400 bases) 16S rRNA gene sequences from 196 isolates and 84 clones generated in this study were deposited in GenBank under the accession numbers AF468274 to AF468299, AF468301 to AF468321, AF468343 to AF468447, AY167251 to AY167341, AY165563 to AY165581, and AY165583 to AY165598.

## RESULTS

**Total counts and culturability.** Total counts of bacteria in arctic pack ice from bottom sections at 10 stations, collected during the R.V. *Polarstern* ARKXIII/2 cruise, ranged from 0.98  $\times 10^5$  to 14.90  $\times 10^5$  cells/ml (Table 2). Viable counts determined by CFU on chitin agar (36) at 1°C varied from 4.1 to as high as 26.5% (Table 2), which was within the range of viable/total count ratios for the middle and bottom sections of the diverse ANTV/2 winter pack ice samples (36). Total counts of bacteria in sea ice-adjacent water were comparable to densities in the ice floes; however, similar to the antarctic results (36), less than 1% of the bacteria were cultivatable (Table 2).

Total counts of samples used for FISH and cloning were comparable to those used for isolation. Samples from arctic pack ice contained  $(3.61 \pm 0.25) \times 10^5$  (ARKXV/1) and  $(4.56 \pm 0.26) \times 10^5$  (ARKXVI/2) cells/ml. Cell counts for the autumnal antarctic ice samples with very high algae accumula-

TABLE 2. Cell counts and culturability of bacteria in pack ice and seawater sampled during cruise ARKXIII/2

Station	Pack ice		Seawater (adjacent to ice floe)	
	Total counts <sup>a</sup> (10 <sup>5</sup> cells ml <sup>-1</sup> )	CFU/total counts (%)	Total counts <sup>a</sup> (10 <sup>5</sup> cells ml <sup>-1</sup> )	CFU/total counts (%)
44/055	4.18 ± 2.03	24.4		
44/057	0.98 ± 0.48	4.1		
44/058	1.06 ± 0.49	4.3		
44/062	3.58 ± 0.67	14.0	2.26 ± 0.19	0.8
44/064	1.35 ± 0.88	26.5	17.9 ± 0.48	0.02
44/089	1.76 ± 0.89	5.6		
44/091	1.35 ± 0.54	12.6	3.04 ± 1.28	0.2
44/092a	13.50 ± 0.65	11.2		
44/092b	14.90 ± 0.44	13.0		
44/093	3.76 ± 1.77	11.8	4.46 ± 1.35	0.2

<sup>a</sup> Mean ± standard deviation.

tions were almost an order of magnitude higher than those for the arctic samples, with  $(2.11 \pm 0.51) \times 10^6$  (ANTXI/4\_13) and  $(1.12 \pm 0.72) \times 10^6$  (ANTXI/4\_14) cells/ml.

**Phylogenetic diversity of arctic isolates and 16S rRNA gene clones.** ARDRA screening of pure cultures of arctic pack ice and subsequent sequencing of representatives from each ARDRA pattern group revealed 33 phylotypes distributed among the  $\gamma$ - and  $\alpha$ -proteobacteria, the *Cytophaga-Flavobacterium* (CF) group, and the *Actinobacteria* (Table 3). Thirteen phylotypes had >97% 16S rRNA gene sequence identity with species previously isolated from polar sea ice (8, 30, 32, 33) and other polar environments. Most of those sea ice isolates are recognizable in Table 3 and in the figures accessible online at [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice1-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice1-d.html), [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice2-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice2-d.html), and [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice3-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice3-d.html) by strain names beginning with ACAM or IC. Seven phylotypes shared <97% 16S rRNA gene sequence identity with previously described bacteria and can be considered new species (58). The remaining 13 phylotypes were related to isolates or 16S rRNA gene clones from nonpolar habitats and are therefore suspected to be allochthonous.

The highest diversity of phylotypes was found within the  $\gamma$ -proteobacteria (see supplemental figure at [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice1-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice1-d.html)) with *Marinobacter* spp. as the dominant phylotype. Most of the phylotypes identified as  $\alpha$ -proteobacteria were affiliated to the *Roseobacter* clade, with *Octadecabacter* spp. being the most frequently isolated phylotype (see supplemental figure at [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice2-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice2-d.html)). Additional phylotypes within the  $\alpha$ -proteobacteria were associated with *Sphingomonas* spp. Seven phylotypes were detected within the CF group (see supplemental figure at [http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke\\_seaice3-d.html](http://www.awi-bremerhaven.de/Pelagic/Sections/MarineChemistry/Helmke_seaice3-d.html)) with the numerically abundant *Salegentibacter* spp. and *Psychroserpens* spp. Four highly diverse gram-positive phylotypes representing potentially four genera (*Clavibacter*, *Microbacterium*, *Brachybacterium*, and *Nesterenkonia*) within the phylum *Actinobacteria* were also identified (Table 3).

PCR and cloning analysis of 16S rRNA genes from two

discrete arctic samples revealed 28 different phylotypes (Table 3). Ten clone phylotypes were 95 to 100% identical to our arctic isolates, and seven were identical to previously cultivated antarctic bacteria. Only four clones clustered with sequences from isolates of nonpolar origin, and six clones clustered with sequences from not-yet-cultivated bacteria. Although clones and isolates derived from different sea ice samples, the same phylotypes (the *Colwellia* spp. and the *Glaciecola* spp. within the *Colwellia* assemblage [61], the *Marinobacter* spp., and the *Octadecabacter* spp.) were found to dominate. Within the CF group, only three different clone phylotypes were obtained. The most abundant CF clone phylotype had no counterpart among the sea ice isolates.

**Phylogenetic diversity of antarctic isolates and 16S rRNA gene clones.** With only 20 phylotypes, the diversity of the antarctic sea ice isolates was less than that among our arctic isolates (Table 3). Most of the antarctic isolates were >98% identical to species previously isolated from the antarctic and/or arctic sea ice or other polar habitats (supplemental figures). Similar to the arctic isolates, the antarctic phylotypes were distributed among the  $\gamma$ - and  $\alpha$ -proteobacteria as well as the CF group. The *Actinobacteria* were represented by only one isolate. *Colwellia* spp. and *Glaciecola* spp. were the most abundant  $\gamma$ -proteobacteria. In contrast to the arctic isolates, the *Marinobacter* spp. were rare. Similar to arctic sea ice, the  $\alpha$ -proteobacteria were dominated by members of the *Roseobacter* clade, but *Octadecabacter* was not the prevalent phylotype in antarctic pack ice. Instead, three phylotypes that clustered with *Roseobacter* sp. strain Shippagan and other yet-undescribed isolates were quite numerous. In comparison to the arctic isolates, the diversity of the CF group was clearly reduced and concentrated within the *Polaribacter* group.

Like our arctic clone libraries, cloned 16S rRNA genes from antarctic pack ice samples (ANTXI/4\_13 and ANTXI/4\_14) revealed a strong overlap of phylotypes with cultivated isolates (Table 3), although these samples were derived from sea ice of a different season. However, unlike our arctic libraries, diversity of the antarctic clones was limited to typical sea ice phylotypes. Again, the *Colwellia* assemblage, the *Roseobacter* clade, and the CF group (in particular, the *Polaribacter* spp.) were the dominant phylogenetic groups.

**Analysis of diversity.** Using a two-tailed *t* test ( $\alpha = 0.05$ ) (65), we tested Shannon's index ( $H'$ ) of diversity for arctic versus antarctic isolates and clone libraries for significant differences. These results are included in Table 3 and show that diversity of arctic isolate phylotypes was significantly higher than that of antarctic isolates. Similarly, diversity in the arctic clone libraries was significantly higher than in the antarctic clone libraries. Coverage indicated that most of the actual diversity of antarctic pack ice (82 and 85%) had been revealed by cloning, whereas only 50 to 75% of the actual diversity in arctic pack ice had been detected. For rarefaction analysis, the expected number of ARDRA patterns was plotted at one-knot intervals (63) against the number of individuals (clones or isolates) screened. Curves calculated for the isolates and clones indicated that the number of 16S rRNA phylotypes in arctic and antarctic pack ice had reached saturation (data not shown).

**Community structure.** Percent distributions of bacteria in samples from arctic and antarctic pack ice that hybridized with domains to species-specific probes are listed in Table 4. Target



TABLE 3. Phylogenetic affiliations and frequency of isolates and clone libraries

Phylogenetic grouping	Arctic			Antarctic			Nearest phylogenetic relative <sup>a</sup>	% Identical 16S rRNA <sup>b</sup>
	No. of isolates	No. of clones	No. of isolates	No. of clones	No. of clones			
	ARKXIII/2	ARKXV/1	ARKXXVI/2	ANTV/2	ANTXI/4_13	ANTXI/4_14		
<i>Bacteria</i>	115	86	106	87	103	95		
<i>α-Proteobacteria</i>								
<i>Roseobacter</i>	1	1	3	4	6	4	Antarctic quartz stone sublithic isolate OSSC9-5 (AF170750)	93.0–97.8
	2			5			Adriatic Sea isolate AS-26 (AJ391187)	97.2–99.7
	2						<i>Roseobacter</i> sp. strain Shippagan (AF100168)	98.1–98.9
	3		1	4			<i>Roseobacter litoralis</i> (X78312)	98.0
	29	7	5	8	1		North Atlantic continental slope isolate EI-2 (AF254103)	96.9–98.0
	7		5				<i>Octadecabacter arcticus</i> (U73725)	97.0–99.0
	1						Ligurian Sea isolate SRF3 (AJ002565)	98.1
	2						Mediterranean Sea clone MED-6 (AF025549)	97.0–98.3
	1						Nankai Trough clone NKB7 (AB013259)	95.1
	2						Antarctic seawater isolate SW54 (U85838)	96.5
	1						<i>Erythrobacter longus</i> (M59062)	97.8
<i>β-Proteobacteria</i>							Soil ultramicrobacterium strain ND5 (AB008506)	97.8
<i>γ-Proteobacteria</i>								
<i>Shewanella</i>	1		8				<i>Shewanella frigidimarina</i> ACAM584 (U85902)	99.3–99.7
<i>Psychromonas</i>	5	15	21	6	9	1	<i>Psychromonas antarctica</i> (U14697)	97.7–99.5
<i>Colwellia</i>				9	37	40	<i>Colwellia psychroerythraea</i> IC064 (U85842)	98.2–98.9
				4			<i>Colwellia</i> sp. strain ANT5-9 (AF19350)	97.4–99.6
				5			<i>Colwellia rossensis</i> ACAM608 (U14589)	99.3–99.7
				23	7	8	<i>Colwellia</i> -like sp. strain IC169 (AF001376)	98.0–98.2
				10	25	15	Gas vacuolate sea ice bacterium strain 214.6 (GVstr214.6 [U73724])	97.6–98.7
<i>Glaciecola</i>				2	2	4	<i>Glaciecola punicea</i> ACAM 611 <sup>T</sup> (U85853)	97.0–98.3
				4	5	4	<i>Glaciecola pallidula</i> ACAM 615 <sup>T</sup> (U85854)	98.6–98.7
<i>Pseudoalteromonas</i>				2			<i>Pseudoalteromonas antarctica</i> (X98336)	99.7–100
<i>Methylophaga</i>				1			<i>Methylophaga marina</i> (X95459)	94.5
<i>Psychrobacter</i>	4	1	2	3	1	3	<i>Psychrobacter glacincola</i> IC084 (U85876)	98.1–99.7
	1						<i>Psychrobacter marincola</i> (PMA309941)	99.6
	3						<i>Psychrobacter</i> sp. strain S11 (AF260715)	98.0–99.5
<i>Acinetobacter</i>	1						<i>Acinetobacter johnsonii</i> (X89775)	99.5
				1			<i>Acinetobacter hwoffii</i> (U10875)	98.8
<i>Pseudomonas</i>	4						<i>Pseudomonas</i> sp. strain ACAM213 (U85868)	98.3–100
	2						Arctic seawater bacterium R7366 (UBA293826)	99.4
<i>Marinobacter</i>	8	13	1	1			<i>Marinobacter</i> sp. strain IC022 (U85863)	97.1–99.0
		18					<i>Marinobacter</i> sp. strain DS40M8 (AF199440)	95.0–99.7
		1					<i>Marinobacter hydrocarboniclasticus</i> (AB021372)	91.2
<i>Oceanospirillum</i>		2			2		<i>Oleispira antarctica</i> (AJ426420)	98.4–99.3
<i>Halomonas</i>	1	1	11	2			<i>Halomonas protea</i> (AJ238597)	96.0–99.4
				2			<i>Halomonas variabilis</i> strain SW04 (U85873)	98.6–99.8
	1						Gamma <i>Proteobacterium</i> MBIC3958 (AB020600)	95.1
							Antarctic shelf clone MERTZ_2CM_20 (AF424059)	97.0

<i>Terredinibacter</i>	2					Arctic sea ice clone ARCTIC_ICE-12 (AF277547)	98.1
NORS <sup>c</sup>	1					North Sea clone KTc1119 (AF235120)	95.1
CF							
<i>Polaribacter</i>	2	1	1			<i>Polaribacter filamentus</i> (U73726)	98.9–99.5
						Arctic Ocean clone Arctic 96B-11 (AF354621)	93.7
<i>Cellulophaga</i>	1	3	8			<i>Polaribacter franzmannii</i> (U14586)	97.9–98.1
<i>Flavobacterium</i>	3	2	7			<i>Polaribacter irgensii</i> (M61002)	99.1–99.3
<i>Salegentibacter</i>	7	3				Mesocosm diatom bloom isolate 5.3.10 (AF367849)	98.0–98.3
<i>Psychroflexus</i>	2					<i>Flavobacterium xanthum</i> IC001 (U85889)	98.7–99.1
	16					<i>Salegentibacter salegens</i> (M92279)	97.9–99.1
<i>Psychroserpens</i>	6	2				<i>Psychroflexus torquis</i> ACAM623 (U85881)	98.1–98.7
<i>Cytophaga</i>	2	5				North Atlantic abyssal isolate AIII4 (AF254114)	92.9–96.2
		1				<i>Psychroserpens burtonensis</i> ACAM188 (U62913)	94.0–95.8
		1				Arctic Ocean clone Arctic96AD-4 (AF354619)	96.8–98.1
		1				Japan Trench clone JTBI32 (AB015260)	98.8
<i>Green nonsulfur bacteria</i>	1					Antarctic sea ice clone SIC.42370 (AF277483)	93.8
						Arctic Ocean clone Arctic 95A (AF355054)	87.3
<i>Actinobacteria</i>	2						
	1	1				<i>Nesterenkonia halobia</i> (X80847)	97.9
	1					Soil ultramicrobacterium str. 121 (AB008511)	97.8–98.0
	1					<i>Brachybacterium faecium</i> (X91032)	91.8
	1	3				<i>Microbacterium</i> sp. VKM Ac-2047 (AB042082)	99.4
						Freshwater clone HTG6 (AF418963)	91.7–92.0

Total 16S rRNA phylotypes	33	15	19	20	13	11	
Shannon index ± variance	1.291 ± 0.002	1.065 ± 0.002	1.091 ± 0.001	1.127 ± 0.002	0.844 ± 0.002	0.812 ± 0.001	
Coverage (%)	53.3	73.6	84.6	81.8			

<sup>a</sup> In ARB database.  
<sup>b</sup> Estimated in ARB.  
<sup>c</sup> Reference 24.

TABLE 4. Frequency of bacterial phylotypes in Arctic and Antarctic pack ice observed by FISH in comparison to clone and isolate libraries

Probe target group	Probe	Results for samples collected in the <sup>a</sup> :											
		Arctic						Antarctic					
		FISH <sup>b</sup>		Clone library <sup>c</sup>		Isolates <sup>d</sup>		FISH <sup>b</sup>		Clone library <sup>c</sup>		Isolates <sup>d</sup>	
ARKXV/1	ARKXVI/2	ARKXV/1	ARKXVI/2	ARKXIII/2	ARKXIV/2	ANTXI/4_13	ANTXI/4_14	ANTXI/4_13	ANTXI/4_14	ANTXI/4_13	ANTXI/4_14	ANTV/2	
<i>Bacteria</i>	EUB338	94 ± 5	95 ± 5	N/A	N/A	N/A	93 ± 6	96 ± 4	N/A	N/A	N/A	N/A	N/A
α-Proteobacteria	ALF968	28 ± 6	33 ± 7	9	13	42	26 ± 5	24 ± 4	7	7	4	24	24
Roseobacter clade	ROSEO536	27 ± 8	28 ± 5	9	13	39	16 ± 5	7 ± 2	7	7	4	24	24
<i>Octadecabacter</i> spp.	ODBI021	19 ± 5	23 ± 5	8	5	25	<1	<1	1	1	0	9	9
Roseobacter sp. strain Shippagan group	RSHIP995	<1	<1	0	0	1	3 ± 3	2 ± 1	6	6	15	5	5
<i>Spirogonomonas</i> spp.	SPHI20	<1	<1	0	0	3	4 ± 3	<1	0	0	0	0	0
β-Proteobacteria	BET42a	7 ± 6	5 ± 4	1	0	0	2 ± 2	2 ± 3	0	0	0	0	0
γ-Proteobacteria	GAM42a	49 ± 5	51 ± 9	71	78	30	34 ± 11	40 ± 9	88	88	80	65	65
<i>Alteromonas-Cobwellia</i>	ALT1413	30 ± 7	33 ± 9	22	20	4	19 ± 4	22 ± 4	42	42	46	17	17
<i>Pseudoalteromonas-Cobwellia</i>	PSA184	32 ± 11	37 ± 14	5	32	9	16 ± 5	19 ± 5	27	27	22	41	41
<i>Glaciecola</i> sp. strain GVstr214.6	GVstr214	4 ± 5	<1	0	18	4	3 ± 4	<1	25	25	18	28	28
<i>Glaciecola punicea</i>	GPU622	5 ± 5	3 ± 4	0	10	0	3 ± 2	3 ± 3	2	2	4	1	1
<i>Marinobacter</i> sp. strain IC022 group	MB-IC022	23 ± 13	22 ± 8	14	1	7	<1	7 ± 3	0	0	0	1	1
<i>Psychrobacter</i> spp.	PSYB476	4 ± 3	9 ± 3	1	2	7	5 ± 1	7 ± 3	1	1	3	3	3
<i>Shewanella frigidimarina</i>	SF825	3 ± 2	7 ± 4	0	5	1	<1	<1	0	0	0	0	0
<i>Psychromonas</i> spp.	NOR2-1453	<1	1 ± 1	0	3	0	<1	<1	0	0	0	3	3
<i>Vibrio</i> spp.	GV	<1	<1	0	0	0	<1	3 ± 3	0	0	0	0	0
<i>Oceanospirillum</i> spp.	OCE232	3 ± 5	1 ± 1	2	11	1	1 ± 1	5 ± 4	2	2	0	0	0
<i>Pseudomonas</i> spp.	PS56a	<1	<1	1	0	5	<1	<1	0	0	0	0	0
CF group	CF319a	22 ± 4	28 ± 6	18	6	23	31 ± 5	34 ± 4	8	8	17	9	9
<i>Polaribacter</i> spp.	PB223	2 ± 1	<1	0	0	3	28 ± 6	35 ± 7	5	5	17	6	6
<i>Actinobacteria</i>	HGC69a	<1	<1	0	3	4	<1	<1	0	0	0	1	1
<i>Planctomycetales</i>	PLA46	<1	<1	0	0	0	<1	<1	0	0	0	0	0
<i>Archaea</i>	ARCH915	<1	<1	0	0	0	<1	<1	0	0	0	0	0

<sup>a</sup> Data present results obtained during the indicated research cruises; for more information, please refer to the text.<sup>b</sup> Values represent the mean percent total cells observed with DAPI staining ± standard deviation.<sup>c</sup> Values represent the percent total 16S rRNA gene clones. N/A, not applicable.<sup>d</sup> Values represent the percent total isolates. N/A, not applicable.

groups for most of the probes used in this study are bracketed in the online dendrograms. The target groups for probes and their percent frequencies (identified from ARDRA patterns) in the clone libraries and cultures are included in Table 4 for comparison. In most cases, phylogenetic groups detected by isolation and cloning were also detectable with FISH. Most of the bacteria visualized with DAPI staining (~95%) were detectable with the EUB338 probe specific for *Bacteria*. The background signal of samples, observed with the probe NON338, was negligible (0 to 0.1%). For all samples, most of the DAPI-stained cells (>95%) could be assigned with probes targeting the larger phylogenetic groups within the domain *Bacteria*. In arctic samples, the highest percentage of bacterial cells (~50%) was detected with the  $\gamma$ -proteobacteria-specific probe. The  $\alpha$ -proteobacteria and the CF group accounted for ~30% and ~25% of the total bacteria, respectively.  $\beta$ -Proteobacteria were also detected in samples, making up ~6% of the total bacteria. Abundances of gram-positive bacteria, *Planctomycetales*, and *Archaea* were below the detection limit of FISH. Similar to arctic samples, the  $\gamma$ -proteobacteria accounted for the highest fraction (~36%) of the total in antarctic samples. The CF group made up the second-highest distribution of cells, with a fraction of ~32%, followed by the  $\alpha$ -proteobacteria, with a fraction of ~25%. A few  $\beta$ -proteobacteria (~2%) were detectable. Gram-positive bacteria, *Planctomycetales*, and *Archaea* were not observable in either antarctic sample.

We took a closer look at the community composition using probes more specific for the most abundant group, the  $\gamma$ -proteobacteria. Application of two partially overlapping probes specific for the *Alteromonas-Colwellia* (ALT1413) and *Pseudoalteromonas-Colwellia* (PSA184) groups within the *Colwellia* assemblage resulted in very similar numbers. Probes ALT1413 and PSA184 hybridized with ~31 and ~34% of DAPI-stained cells in arctic samples, and each accounted for ~20% in antarctic samples. The newly designed probes GVstr214 and GPU622, nesting within the *Pseudoalteromonas-Colwellia* group, detected only up to 5% of the total bacteria, respectively, although they were quite redundant in the clone libraries. Most of the remaining  $\gamma$ -proteobacteria could be assigned with probes targeting groups outside of the *Colwellia* assemblage. *Marinobacter* sp. strain IC022, which was frequent in our ARKXV/1 clone library and among our arctic isolates, was also found in situ with the probe MB-IC022 to make up ~22% of the total bacteria in the arctic samples. Concurring with the clone library results, we detected no bacteria with this probe in the antarctic samples. *Psychrobacter* spp. were detectable in all samples with probe PSYB476 (~6%). *Shewanella frigidimarina* (probe SF825) appeared to occur mainly in arctic pack ice (3 to 7%), and *Oceanospirillum* spp., *Vibrio* spp., *Psychromonas* spp., and *Pseudomonas* spp. were either low in abundance (1 to 3%) or not detectable.

The  $\alpha$ -proteobacterial sequences in clone libraries and cultures were mostly, if not entirely, associated with members of the *Roseobacter* clade. Our probing results corroborated that this clade accounted for most of the  $\alpha$ -proteobacteria in the samples. *Octadecabacter* spp., which predominated in number among *Roseobacter* clade isolates and clone sequences, also dominated the in situ community of arctic samples. In antarctic samples, similar to the clone results, very few cells of *Octadecabacter* spp. were detected. Probe RSHP995, which was specific for a group of sequences that were >97% identical to

*Roseobacter* sp. strain Shippagan, detected only a small fraction (<1%) in arctic samples but was more abundant (~2 to 3%) in antarctic samples. Probe PB223, which was specific for *Polaribacter* spp., detected only ~1% of cells in arctic samples while, similar to our cultural and cloning results, *Polaribacter* spp. dominated the CF fraction of the antarctic samples.

## DISCUSSION

**Congruence of cultivation, 16S rRNA gene cloning, and FISH.** The strong agreement of the results obtained with the described methods emphasizes the exceptional nature of sea ice bacterial communities, which have highly active members despite the extreme conditions in sea ice. The high percentages of cultivatable bacteria observed in the viable/total count ratio data from arctic pack ice in this study and former studies (36, 41) indicated that the composition of phylotypes observed among our cultures potentially represent much of the natural community composition. Although the original samples used for cultivation were not available for molecular analyses, cloning of 16S rRNA genes recovered from sea ice samples collected during subsequent cruises detected many of the same phylotypes found among cultivated sea ice bacteria. During the course of our clone library analyses, Brown and Bowman (18) presented the first 16S rRNA environmental sequence data from sea ice, mainly in the antarctic, which showed that previously cultivated phylotypes (see the sequences designated SIC, McMurdo, or ARCTIC\_ICE in the online dendrograms) are detectable with PCR. Their data agreed with our findings that the cultivatable fraction and the PCR-detected fraction of bacteria in sea ice are strongly overlapping. These findings are in strong contrast to those for most marine environments, for which cultivation and cloning analyses rarely agree.

The high FISH detection yield of the CY3-monolabeled probe EUB338, which was specific for the domain *Bacteria* with ~95% of DAPI-stained cells and the bright probe signals (54), indicated that the sea ice microbial community, as a whole, was highly active at the time of sampling. With the exception of activated sludge (2), there are very few examples of microbial habitats with FISH detection rates as high as those in sea ice. Typically, only ~50% of the bacterioplankton community can be detected with domain-specific probes (28), and much less (~60%, i.e., sum of probes) of the bacterial fraction can usually be assigned to specific phylogenetic groups. Some trends in the marine environment are emerging. Glöckner et al. (28) reported 96% detectability of DAPI-stained cells with EUB338 and >87% assignability with phylogenetic group probes in a sample collected from a *Phaeocystis* sp. bloom in the Southern Ocean. Similarly, Eilers et al. (24) observed that FISH detection rates of bacterial communities increased dramatically from 30 to >90% as phytoplankton biomass peaked over the course of a seasonal bloom in the German Bight. Unlike these studies that showed highest assignability for the CF group, the  $\gamma$ -proteobacteria was the predominating group in our sea ice samples, and this finding appears to be a unique characteristic for sea ice bacterial communities (7, 28). FISH analysis of sea ice sampled throughout several seasons is needed to confirm this.

A reason for the different FISH detection yields in sea ice and in the water column could be the different substrate quan-



tity and quality found in the two environments. High concentrations of dissolved organic matter (DOM) in sea ice have been reported for the Arctic Ocean (62) and the Southern Ocean (37), exceeding surface seawater concentration by a factor of 2 to 30. Additionally, DOM in sea ice appears to be very labile, providing a favorable growth substrate for bacteria (3). The high substrate quality leads to increased microbial activity in sea ice relative to surface seawater, which usually contains less fresh and labile (<10%) DOM (3, 4). The higher microbial activity in sea ice seems to be reflected in elevated FISH detection yields.

**Arctic versus antarctic communities.** The higher diversity of phylotypes indicated by the Shannon's index for our arctic sea ice isolates and clone libraries as well as the detection of typical limnic phylotypes, i.e.,  $\beta$ -proteobacteria and *Actinobacteria* (7, 29, 47), suggest a terrestrial influence on the sea ice bacterial communities found in the pack ice of the Fram Strait region. Seasonality also appears to influence the diversity of antarctic pack ice communities. Fewer taxonomic groups were detected in our winter and autumnal samples than in previous surveys of summer sea ice (8, 18).

Temperature plays an important role in shaping the composition of sea ice bacterial communities (18, 20, 36); however, the dominance of the *Colwellia* assemblage, the *Roseobacter* clade, and the CF group in both arctic as well as antarctic pack ice may also be explained by the strong association of these groups with surfaces (1, 19, 21, 24, 52). Studies have shown that the recruitment of bacteria into sea ice is primarily facilitated through their attachment to microalgae or particles (34, 35). The dominant groups in sea ice are also characteristic of the cultivatable fraction of bacteria in seawater (6, 23, 24). Although less than 1% of the bacteria in seawater adjacent to ice floes (Table 2) were cultivatable, most of these in the arctic were similar to phylotypes found in the sea ice (included in the online dendrograms).

The general similarity of bacterial phylotypes in arctic and antarctic pack ice implies that the same selective mechanisms occur at both poles. However, this alone cannot explain the extensive overlap of almost identical 16S rRNA phylotypes (25 out of 33) between the arctic and antarctic sea ice communities and makes a simple speciation model based on geographical isolation difficult to support, especially since phylogenetic surveys of the world's oceans and lakes (5, 17, 25, 29, 39, 48, 66) have strongly suggested the mixing of bacterial populations on a global scale. The physiological (i.e., psychrophily or high hydrostatic pressure intolerance) and geographic distance barriers (60) appear to be permeable. However, analysis at the conservative gene level of 16S rRNA is not sufficient to determine if the same species occur at both poles. Other analytical methods, e.g., DNA-DNA hybridization, could elucidate diversity that is not detectable by 16S rRNA gene sequencing.

**Conclusions.** The strong overlap of the cultivatable, PCR-, and FISH-detected bacterial fractions in arctic as well as antarctic sea ice indicated exceptional bacterial communities whose major parts are metabolically active and cultivatable. Similar to nutrient-enriched samples,  $\gamma$ -proteobacteria were dominant and  $\alpha$ -proteobacteria and members of the CF group were highly abundant. Although almost the same phylotypes were detected at both poles, differences in their quantitative

contribution to the arctic and antarctic pack ice communities were revealed by FISH.

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