Acta Oceanol. Sin., 2013, Vol. 32, No. 6, P. 66-74
DOI: 10.1007/s13131-013-0271-y
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Phylogenetic diversity of planktonic bacteria in the Chukchi Borderland region in summer

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Received 10 May 2012; accepted 28 September 2012

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Abstract
Planktonic bacteria are abundant in the Chukchi Borderland region. However, little is known about their diversity and the roles of various bacteria in the ocean. Seawater samples were collected from two stations K2S and K4S where sea ice was melting obviously. The analysis of water samples with fluorescence in situ hybridization (FISH) showed that DMSP-degrading bacteria accounted for 13% of the total bacteria at the station K2S. No aerobic anoxygenic phototrophic (AAP) bacteria were detected in both samples. The bacterial communities were characterized by two 16S rRNA gene clone libraries. Sequences fell into four major lineages of the domain *Bacteria*, including *Proteobacteria* (Alpha, Beta and Gamma subclasses), *Bacteroidetes, Actinobacteria* and *Firmicutes*. No significant difference was found between the two clone libraries. SAR11 and *Rhodobacteraceae* clades of *Alphaproteobacteria* and *Pseudoalteromonas* of *Gammaproteobacteria* constituted three dominant fractions in the clone libraries. A total of 191 heterotrophic bacterial strains were isolated and 76% showed extracellular proteolytic activity. Phylogenetic analysis reveals that the isolates fell into *Gammaproteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes*. The most common genus in both the bacterial isolates and protease-producing bacteria was *Pseudoalteromonas*. UniFrac data showed suggestive differences in bacterial communities between the Chukchi Borderland and the northern Bering Sea.
Key words: diversity, planktonic bacteria, 16S rRNA gene clone library, Chukchi Borderland

Citation: Zeng Yinxin, Yu Yong, Li Huirong, He Jianfeng, Lee Sang H, Sun Kun. 2013. Phylogenetic diversity of planktonic bacteria in the Chukchi Borderland region in summer. Acta Oceanologica Sinica, 32(6): 66–74, doi: 10.1007/s13131-013-0271-y

1 Introduction

About 600 miles north of the Bering Strait, the Chukchi Borderland is at the entrance to the Arctic Ocean and is known as a complex area of tortuous topography in the northern Chukchi Sea (Hall, 1990). Waters from the Pacific and the Atlantic meet and interact in this adjoining region of ridges and deep-sea plateaus. Nutrient-rich and low-salinity Pacific Ocean-derived waters flowing northward through the Bering Strait are an important component of the Arctic Ocean nutrient maximum (Cooper et al., 1997). Similar to the northern Bering Sea, sea ice cover is one of the several physico-chemical properties that vary dramatically with the seasons in this area. Seasonal ice melt could induce changes both in the community structure and physiological activity of bacterioplankton in the underlying seawater (Grzymski and Murrsay, 2007; Sala et al., 2010). Increasing global temperatures are having a profound impact in the Arctic, including the dramatic loss of multiyear sea ice in 2007 that has continued to the present (Comeau et al., 2011). Differences between the bacterial communities sampled in summers 2002–2004 and those sampled from summer 2007 in the western Arctic Ocean were observed (Kirchman et al., 2010).

Bacterioplankton are a significant component of the planktonic community in the ocean, and are responsible for modifying and decomposing organic matter (Azam et al., 1983), as well as mediating the transformation of dissolved organic matter (DOM) into particulate organic matter (POM; Azam and Hodson, 1977), which is the source of long food webs. High abundance (10^8–10^9 cells/L) and planktonic bacteria activity have been observed in the Chukchi Sea (Steward et al., 1996; Bano and Hollibaugh, 2002; Kirchman et al., 2007; Nikrad et al., 2012). However, information about the diversity of planktonic bacteria in this area is still scarce. It is important to know which phylogenetic groups of bacteria dominate marine bacterioplankton communities because abundant groups may have different roles in carbon cycling and other biogeochemical processes (Cottrell and Kirchman, 2000a).

Members of the *Gammaproteobacteria, Alphaproteobacteria* and *Bacteroidetes* generally dominate bacterioplankton communities in the Arctic Ocean (Bano and Hollibaugh, 2002; Garneau et al., 2006; Malmstrom et al., 2007; Alonso-Sáez et al., 2008; Kirchman et al., 2010). In spite of much variation in biogeochemical properties, Arctic bacterial communities are rem-

Foundation item: The National Natural Science Foundation of China under contract No. 41076131; the Youth Marine Science Foundation of State Oceanic Administration under contract No. 2011104; the Public Science and Technology Research Funds Projects of Ocean under contract No. 201105022; the Chinese Polar Environment Comprehensive Investigation and Assessment Program under contract No. CHINARE2012-02-01; the National High-Tech Research and Development Program of China under contract No. 2012AA021706; supports for SH Lee were provided by grants for the Polar Academic Program (PAP) and Korea Polar Research Institute (KOPRI) under contract No. PM11080.
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arkably stable over the temporal and spatial scales (Kirchman et al., 2010). Due to world distribution and availability of degradation of POM in the ocean, *Gammaproteobacteria* and *Bacteroidetes* play an important role in carbon cycling in marine environments (Cottrell and Kirchman, 2000b; Riemann et al., 2000; Kirchman, 2002; González et al., 2008). The *Roseobacter* lineage of the *Alphaproteobacteria* is broadly distributed across diverse marine environments, and constitutes up to 25% of the total bacterial community (Brinkhoff et al., 2008). Organisms of this group form a coherent cluster within the *Rhodobacteraceae*. Aerobic anoxygenic photosynthesis and dimethylsulphoniopropionate (DMSP) degradation are some of the important traits found in this clade (Brinkhoff et al., 2008; Slightom and Buchan, 2009). Roseobacter abundance is often highest near phytoplankton blooms or association with organic particles (Slightom and Buchan, 2009).

The present study aimed at investigating the bacterioplankton community composition in summer in the Chukchi Borderland region using 16S rRNA gene clone library analysis. Fluorescence in situ hybridization (FISH) was used to compare the abundance of aerobic anoxygenic phototrophic (AAP) bacteria and DMSP-degrading bacteria to that of planktonic bacteria. Abundance data may provide insight into the biogeochemical importance of planktonic bacteria in this region. In addition, the diversity of extracellular protease-producing bacteria, which are of essential importance for nitrogen cycling in marine environments (Huston and Deming, 2002), was investigated using a combination of amplified ribosomal DNA restriction analysis (ARDRA) and 16S rRNA gene sequencing. Such studies are anticipated to improve our understanding of the planktonic bacterial communities in the Chukchi Borderland region.

2 Materials and methods

2.1 *Sample collection*

Seawater samples were collected from two locations (Table 1) in the Chukchi Borderland region in July 2010 during the first Korean Arctic cruise. A CTD-rosette system was used to collect water samples. For prokaryote enumeration, 50 ml of seawater fixed with formaldehyde (2%) were filtered onto a 0.2-μm-pore-size black polycarbonate filter (Whatman, UK), which was then stained with a solution containing 0.1 mg/ml 4', 6-diamidino-2-phenylindole (DAPI) as described by Schwalbach and Fuhrman (2005). Filter was stored at –80°C until further processing. For FISH, 50 ml of seawater fixed with formaldehyde (2%) were filtered onto a 0.2-μm-pore-size white polycarbonate filter (Whatman, UK), which was washed by 5 ml of distilled water and then stored at –80°C until further processing. Samples for bacterial community DNA extraction were collected by filtering 1.5 L to 2 L of seawater onto 0.2-μm-pore-size polycarbonate filters (Whatman, UK), which were then stored at –80°C until analysis.

Table 1. Summary of samples for community structure

Sample	Location	Depth/ m	Temperature/ °C	Salinity	δ_t/ kg·m^{-3}	Chlorophyll/ mg·m^{-3}	DO/ mg·L^{-1}	Prokaryotes by DAPI/ 10^8 cells·L^{-1}	Prokaryotes by FISH/ 10^8 cells·L^{-1}	DMSP-degrading bacteria/ 10^7 cells·L^{-1}
K2S	73°30.71′N, 166°59.25′W	10	–1.4	28.6	23.2	0.5	13.4	5.3	5.2	7.1
K4S	75°N, 158°30′W	6	–0.9	27.4	22.1	0.3	12.2	3.5	3.5	ND

Note: ND not detected.

2.2 *Media and cultivation*

Following serial dilutions, seawater samples were spread on pre-cooled marine agar 2216 plates (Difco) and incubated at 4–6°C for 30 d. Repeated transfers were performed until a pure culture of each strain was obtained. Bacterial strains were subsequently inoculated on marine agar 2216 plates fortified with 1% skimmed milk (skimmed milk 10 g, peptone 5 g, yeast extract 1 g, $FePO_4$ 0.01 g, agar 15 g, sterilized water 250 ml, aged sea water 750 ml, pH 7.2), and were incubated at 8–10°C for 5 d (Lee et al., 2005). The proteolytic activity of the bacteria was detected as clear zones of hydrolysis around the colony.

2.3 *Media and cultivation*

Prokaryote abundance was determined manually on a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) by exciting DAPI-stained cells with ultraviolet (UV) light (Excitation, 330-380 nm; Dichromatic mirror, 400 nm; Barrier filter emission, 420 nm). Images were captured using a Nikon Digital camera DXM 1200 (Nikon, Tokyo, Japan). Each filter was counted using the semi-automatic image analysis system Jeda 801D (Jeda, Nanjing, China).

2.4 *Fluorescence in situ hybridization*

The abundance of selected bacteria was determined by FISH using probe EUB338 for the domain *Bacteria* (Webster et al., 2001), PUFMR for AAP bacteria (Beja et al., 2002) and DDDL (5′-CTATGCCTATGCCGAACT-3′) for DMSP-degrading bacteria. Oligonucleotides labeled with the cyanin dye Cy3 were purchased from Sangon Biotech (Shanghai, China). A negative control probe (NonEUB338) with the antisense sequence of the domain-level probe EUB338 was used to check for nonspecific hybridization (Webster et al., 2001). A slice of the filter was placed on glass slide and covered with 40 μl of a hybridization buffer prewarmed to 46°C. This buffer (0.9 mol/L NaCl, 20 mmol/L Tris/HCl [pH=7.4], 0.01% sodium dodecyl sulfate [SDS]) was used with a formamide concentration of 35% plus 4 μl of Cy3-labeled oligonucleotide probe (50 ng/μl). Sample was then incubated for 3 h at 46°C. The filter was rinsed with 48°C prewarmed washing buffer (80 mmol/L NaCl, 20 mmol/L Tris/HCl [pH=7.4], 5 mmol/L EDTA, 0.01% SDS) and incubated for 15 min at 48°C. The filter was then rinsed with distilled water, dried, and mounted on slide with Citifluor. The slide was examined under an epifluorescence microscope (Nikon Eclipse 80i, Tokyo, Japan) fitted with a camera (Nikon Digital camera DXM 1200, Tokyo, Japan) under green light (Excitation, 510–560 nm; Dichromatic mirror, 575 nm; Barrier filter emission, 590 nm). Each filter was counted using the semi-automatic image analysis system Jeda 801D (Jeda, Nanjing, China).

2.5 *DNA extraction and PCR amplification of 16S rRNA genes*

Total community DNA extraction from seawater was car-

ried out as described by Bosshard et al. (2000) and Bano and Hollibaugh (2000). Bacterial cells were grown at 8–10°C for 3 d in marine broth 2216 medium (Difco). DNA was extracted from broth cultures using a bacterial genomic DNA extraction kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The molecular weight and concentration of DNA in extracts were determined using ethidium bromide-UV detection on 1% (w/v) agarose gel. Nucleic acid extracts from each sample were also analyzed spectrophotometrically at 260 nm and 280 nm using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA, USA).

Almost full-length 16S rRNA genes (~1.5 kb) were amplified from the extracted DNA by PCR with a universal bacterial primer pair S-D-Bact-0008-a-S-20 (5′-AGAGTTTGATCCTGGCTCAG-3′) and S-D-Bact-1492-a-A-19 (5′-GGTTACCTTGTTACGACTT-3′) (Bosshard et al., 2000). Amplification was performed in 50-μl reactions with an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) as described by Zeng et al. (2007). The success of PCRs was determined by electrophoresis of 4 μl of the reaction mixture in 1% (w/v) agarose gel.

2.6 *Clone library construction*

The 16S rRNA gene-PCR products of the total community were ligated to the vector pMD-18T (TaKaRa, Dalian, China), and used to transform *Escherichia coli* DH5α competent host cells. About 250 colonies were randomly selected for each library. Clone inserts were PCR amplified with vector-specific standard M13 primers (M13forGT: 5′-TGTAAAACGACGGCCAGT and M13rev-C: 5′-CAGGAAACAGCTATGACC) to determine the presence and size of the inserts as described by Zeng et al. (2009). Clones that produced a band with an approximate size of 1.5 kb, which corresponded with the expected size of the cloned 16S rRNA gene fragment, were chosen for ARDRA.

2.7 *ARDRA*

To analyze the bacterial diversity in the clone libraries, ARDRA of the PCR products of 16S rRNA gene clones was performed using *Alu*I as described by Zeng et al. (2007, 2009). ARDRA of the 16S rRNA gene PCR products was also used to group bacterial isolates (Zeng et al., 2007). Clones or strains with an identical ARDRA pattern were considered to be members of the same Operational Taxonomic Unit (OTU). Representative clones or strains of each OTU were subjected to 16S rRNA gene sequence analysis to determine their phylogenetic affiliation with the described species.

2.8 *Sequencing and phylogenetic analysis*

Clones of each representative OTU were sequenced directly using M13 primers with an ABI Prism 3730 DNA analyzer (PE Applied Biosystems, Foster City, USA). Near full-length 16S rRNA genes of strains of each representative OTU were cloned and sequenced as described by Zeng et al. (2007). The obtained 16S rRNA gene sequences were checked for chimeras using the Chimera Check Software included in the Ribosomal Database Project (RDP) II (Michigan State University, USA). Approximately 1.5-kb-long sequences obtained for all the clones were subjected to a BLAST sequence similarity search in GenBank (http://www.ncbi.nlm.nih.gov) and EzTaxon (http://www.eztaxon.org/; Chun et al., 2007) to identify the nearest taxa. Sequence alignment and phylogenetic tree building were completed using the MEGA 5.05 program (Tamura et al., 2011) with a Kimura 2-parameter model. Neighbor-joining bootstrap tests of phylogeny were run with 1 000 replicates.

2.9 *Statistical analyses*

In order to compare the bacterial diversity between the two samples, 16S rRNA gene sequences showing sequence similarity not less than 97% were grouped into the same phylotype. The estimation of species richness, diversity indices, and the percentage of coverage in each library were determined using the SPADE software program (http://chao.stat.nthu.edu.tw). Statistical comparison of clone libraries was accomplished using the UniFrac program (http://128.138.212.43/unifrac) to assess their similarity. The two clone libraries (K2S and K4S) were also compared with earlier published data of clone libraries from the northern Bering Sea (Zeng et al., 2012).

2.10 *Nucleotide sequence accession number*

Sequences reported in this study have been deposited in the GenBank database and assigned GenBank accession numbers: JF697294-697303, JQ069958-069960 and JN177585-177708.

3 Results

3.1 *Abundance of bacteria in seawater samples*

The bacterial probe EUB338-positive cells accounted for 98% to 100% of the total DAPI-stained cells in the two samples. Higher abundance of bacteria was detected in surface water of station K2S than that of station K4S (Table 1). The percentage of cells that were scored as DMSP-degrading positive was 13% of the total FISH-detected bacteria at station K2S. However, no AAP bacteria were observed at both stations.

3.2 *Analysis of 16S rRNA gene clone libraries*

Two 16S rRNA gene clone libraries were constructed from the environmental DNA of the surface water from stations K2S and K4S. In order to avoid sequencing several identical 16S rRNA genes, PCR products were digested with restriction endonuclease *Alu*I and grouped into different ARDRA patterns. Analysis of ARDRA patterns of 16S rRNA genes allowed grouping of 191 and 222 clones from libraries K2S and K4S into 60 and 68 different OTUs, respectively.

Nucleotide sequences of OTUs representing distinct ARDRA patterns in each library were then determined. Seven and twelve of the clones sequenced successfully from libraries K2S and K4S, respectively, were identified as the most likely chimeras, and were thus excluded from further analysis. Unique phylotypes in each library were distinguished on the basis of a conservative definition (clones with 97% and greater sequence similarity were considered to be the same phylotype). The analysis revealed 53 and 66 distinct phylotypes in clone libraries K2S and K4S, respectively (Table 2), and 15 phylotypes

Table 2. Estimation of phylotype coverage and sequence diversity of 16S rRNA gene clone libraries using the SPADE software

Library	No. of clones	No. of phylotypes	Species richness	Coverage	Shannon's index	Simpson's index
K2S	184	53	32.6	85.1	3.713	0.047 46
K4S	210	66	38.4	80.0	3.769	0.075 51

were observed in both clone libraries. Using SPADE software, the coverage values of the two clone libraries were calculated to be higher than 80%, indicating that most of the bacterial diversity in each clone library was detected. At the same time, no significant differences in community composition ($P > 0.1$) were observed between the two clone libraries based on the UniFrac program.

3.3 *Phylogenetic diversity of 16S rRNA gene clone libraries*

The cloned sequences from the two libraries were similar to their closely related GenBank sequences (with similarity values ranging from 86.0% to 100%), with more than 92% originating from marine environments. Sequences shared by both clone libraries fell into three bacterial divisions (Fig. 1): *Proteobacteria* (Alpha, Beta and Gamma subclasses), *Bacteroidetes* and *Actinobacteria*. However, sequences affiliated with *Firmicutes* (2 clones) and chloroplasts of algae (2 clones) were only detected in library K4S.

Compared with the bacterioplankton community composition in the northern Bering Sea (Zeng et al., 2012), lower diversity at the phylum level was observed in the Chukchi Borderland. Sequences affiliated with *Deltaproteobacteria, Verrucomicrobia, Planctomycetes, Acidobacteria, Fusobacteria* and TM7 detected in surface water of the northern Bering Sea were not observed in the Chukchi Borderland. At the same time, UniFrac analysis revealed marginally significant differences ($P < 0.06$) in bacterial communities between the Chukchi Borderland and the northern Bering Sea.

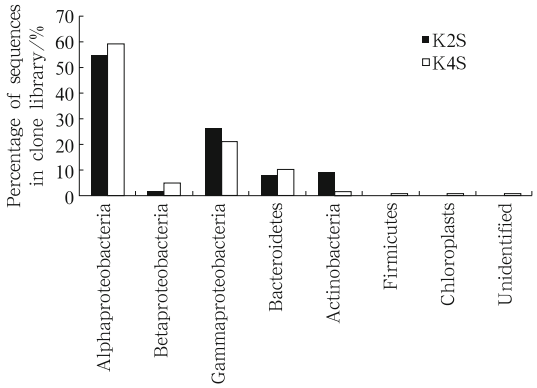

Fig.1. Clone library composition for surface water collected from stations K2S and K4S in the Chukchi Borderland region. Prokaryotes were displayed at the phylum level (and also subphylum level for *Proteobacteria*).

The two clone libraries were dominated by representatives of the phylum *Proteobacteria* (84.1% of the total clones), and the most abundant organisms within this phylum were in the alpha class (67.8% of the *Proteobacteria*-related clones). Clones affiliated with the *Rhodobacterales* and SAR11 clades accounted for 66.5% and 26.3% of the *Alphaproteobacteria*, respectively. A total of 37 and 63 clones in libraries K2S and K4S (Fig. 2a), accounting for 17.7% and 30.1%, respectively, of the *Alphaproteobacteria*, showed significant sequence similarities to *Planktomarina temperata* 01-003883 (EU819141) isolated from coastal marine water in a Norwegian fjord. In addition, phylotype K2S92 (JN177604), containing 12 and 26 clones in libraries K2S and K4S, respectively, showed 99.8% similarity to the cosmopolitan oceanic bacterium *Pelagibacter ubique* HTC-C1062 (CP000084).

Another dominant group within the phylum *Proteobacteria* was *Gammaproteobacteria* (27.9% of the *Proteobacteria*-related clones) containing cultured representatives of the genera *Alteromonas, Pseudoalteromonas, Pseudomonas* and *Vibrio*. Phylotype K2S15 (JN177588), containing 13 and 15 clones in libraries K2S and K4S, respectively, showed 98.9% similarity to *Pseudoalteromonas marina* Mano4 (AY563031) isolated from the tidal flats of the Huanghai Sea (Yellow Sea). In addition, a total of 17 clones belonging to phylotype K2S137 (JN177613) were detected in two libraries. The phylotype K2S137 showed only 85.9% similarity to *Marinimicrobium locisalis* ISL-43 (EU874388) isolated from marine solar saltern of the Huanghai Sea.

Clones affiliated with *Bacteroidetes* accounted for 9.2% of the total clones. Represented by phylotype K2S105 (JN177606), more clones clustered in the genus *Polaribacter* were detected in library K2S than in library K4S (Fig. 2b). On the contrary, *Flavobacterium*-related clones were only detected in library K4S.

Actinobacteria were more frequently encountered in library K2S than in library K4S. Compared to only one clone detected in library K4S, a total of four clones belonging to phylotype K2S133 (JN177612) were observed in library K2S. The phylotype K2S133 shared 96% sequence similarity to *Ilumatobacter fluminis* YM22-133 (AB360343) isolated from estuary sediment. In addition, showing 97.4% similarity to *Aquiluna rubra* MWH-Dar4 (AJ565416) from freshwater, phylotype K2S84 (JN177602) contained 11 and two clones in libraries K2S and K4S, respectively.

3.4 *Isolation and phylogenetic diversity of strains*

A total of 191 bacterial strains were isolated from seawater in the Chukchi Borderland region using aerobic heterotrophic conditions. Isolates that were able to degrade skimmed milk accounted for 76% of the planktonic strains. The isolates were compared applying *Alu*I -ARDRA fingerprinting. Strains with identical ARDRA profiles were grouped in clusters. Representative strains of each cluster were subjected to 16S rRNA gene sequence analysis. Nearly full-length 16S rRNA gene sequences were obtained for a total of 13 strains. Overall, the bacterial isolates fell into four phylogenetic groups: *Gammaproteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes* (Table 3). The diversity of bacterial strains was not evenly distributed among the major groups. Over 95% of the isolates were affiliated with the *Gammaproteobacteria*. Furthermore, the genus *Pseudoalteromonas* within the *Gammaproteobacteria* was the dominant fraction of the overall diversity in both the bacterial isolates and protease-producing bacteria.

4 Discussion

The abundant, large phylogenetic groups represented in our clone libraries were similar to the previous studies in Arctic and sub-arctic areas (Bano and Hollibaugh, 2002; Malmstrom et al., 2007; Zeng et al., 2009, 2012; Kirchman et al., 2010; Comeau et al., 2011; Teske et al., 2011), indicating that these Arctic waters are usually dominated by *Alphaproteobacteria, Gammaproteobacteria* and *Bacteroidetes*. At the same time, the relative abundance of major phylogenetic groups in the libraries in this study differed from the previous marine library examined in the Chukchi Sea. The library of 16S rRNA sequences from seawater collected from 20 m in 2004 were dominated by *Gammaproteobacteria*, while *Alphaproteobacteria* sequences were the third

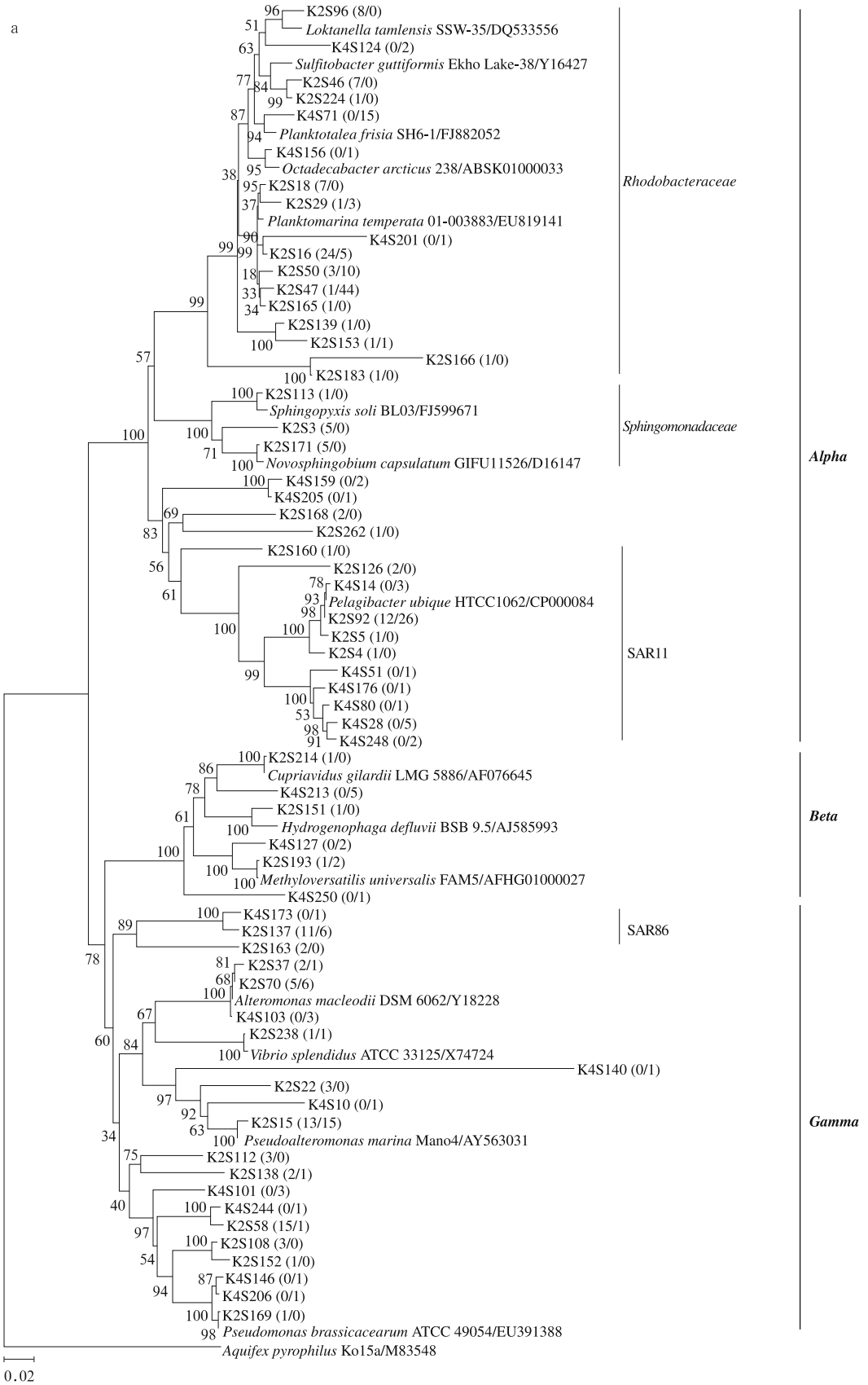
a
K2S96 (8/0)
Loktanella tamlensis SSW-35/DQ533556
K4S124 (0/2)
Sulfitobacter guttiformis Ekho Lake-38/Y16427
K2S46 (7/0)
K2S224 (1/0)
K4S71 (0/15)
Planktotalea frisia SH6-1/FJ882052
K4S156 (0/1)
Octadecabacter arcticus 238/ABSK01000033
K2S18 (7/0)
K2S29 (1/3)
Planktomarina temperata 01-003883/EU819141
K4S201 (0/1)
K2S16 (24/5)
K2S50 (3/10)
K2S47 (1/44)
K2S165 (1/0)
K2S139 (1/0)
K2S153 (1/1)
K2S166 (1/0)
K2S183 (1/0)
Rhodobacteraceae
K2S113 (1/0)
Sphingopyxis soli BL03/FJ599671
K2S3 (5/0)
K2S171 (5/0)
Novosphingobium capsulatum GIFU11526/D16147
Sphingomonadaceae
K4S159 (0/2)
K4S205 (0/1)
K2S168 (2/0)
K2S262 (1/0)
K2S160 (1/0)
K2S126 (2/0)
K4S14 (0/3)
Pelagibacter ubique HTCC1062/CP000084
K2S92 (12/26)
K2S5 (1/0)
K2S4 (1/0)
K4S51 (0/1)
K4S176 (0/1)
K4S80 (0/1)
K4S28 (0/5)
K4S248 (0/2)
SAR11
Alpha
K2S214 (1/0)
Cupriavidus gilardii LMG 5886/AF076645
K4S213 (0/5)
K2S151 (1/0)
Hydrogenophaga defluvii BSB 9.5/AJ585993
K4S127 (0/2)
K2S193 (1/2)
Methyloversatilis universalis FAM5/AFHG01000027
K4S250 (0/1)
Beta
K4S173 (0/1)
K2S137 (11/6)
K2S163 (2/0)
K2S37 (2/1)
K2S70 (5/6)
Alteromonas macleodii DSM 6062/Y18228
K4S103 (0/3)
K2S238 (1/1)
Vibrio splendidus ATCC 33125/X74724
K4S140 (0/1)
K2S22 (3/0)
K4S10 (0/1)
K2S15 (13/15)
Pseudoalteromonas marina Mano4/AY563031
K2S112 (3/0)
K2S138 (2/1)
K4S101 (0/3)
K4S244 (0/1)
K2S58 (15/1)
K2S108 (3/0)
K2S152 (1/0)
K4S146 (0/1)
K4S206 (0/1)
K2S169 (1/0)
Pseudomonas brassicacearum ATCC 49054/EU391388
SAR86
Gamma
Aquifex pyrophilus Ko15a/M83548
0.02

Fig.2.

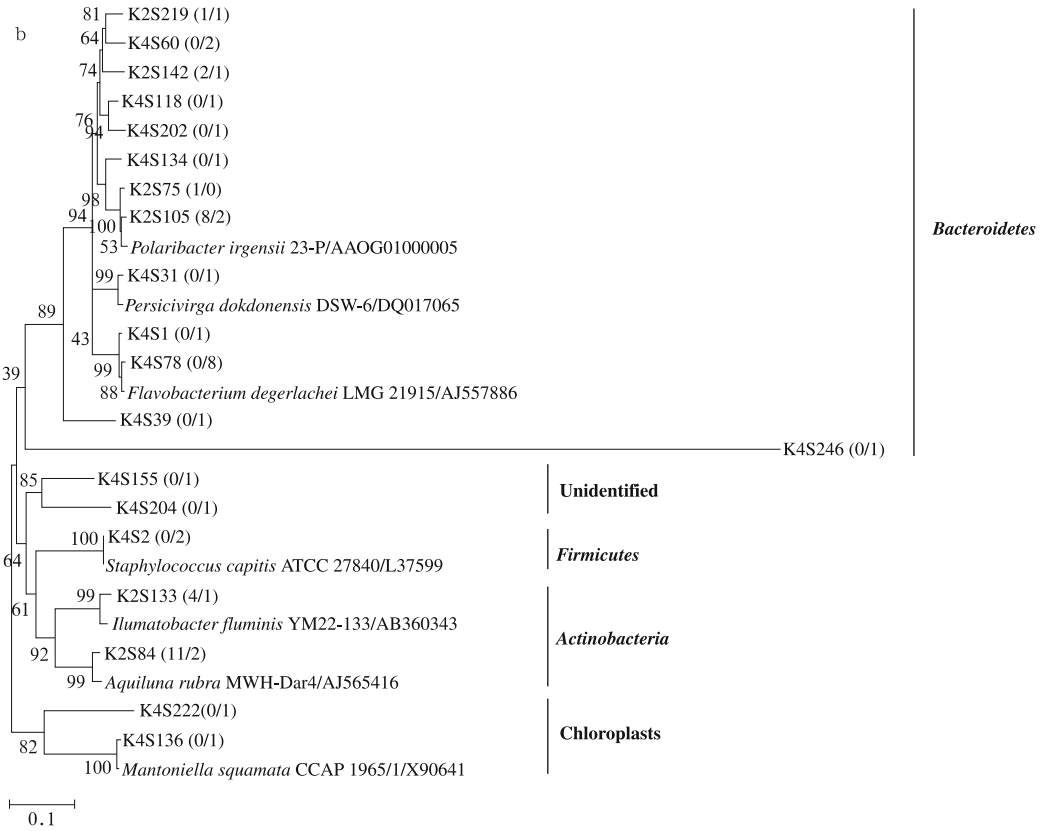

Fig.2. Neighbor-joining trees showing phylogenetic relationships of 16S rRNA gene sequences obtained from clone libraries of seawater at stations K2S and K4S from the Chukchi Borderland region. a. *Proteobacteria* and b. all others. Numbers in parentheses following clone names indicate the number of times found in clone libraries K2S and K4S, respectively. The scale bar indicates evolutionary distance.

most abundant (Malmstrom et al., 2007). In contrast, *Alphaproteobacteria* sequences were the most abundant in our libraries, and *Gammaproteobacteria* sequences were the second most abundant. Differences between the bacterioplankton community sampled in summer 2004 and those sampled in summer 2010 probably reflect the large-scale, climate-induced changes in the Arctic over these years. Comeau et al. (2011) examined bacterial communities over time by using high-throughput sequencing of microbial DNA collected between 2003 and 2010 from the subsurface chlorophyll maximum layer of the Beaufort Sea, and found that bacterial communities overall are less diverse after 2007 following the massive loss of summer sea ice, with a significant decrease of the *Bacteroidetes*.

Alphaproteobacteria typically dominate Arctic surface water (Bano and Hollibaugh, 2002; Alonso-Sáez et al., 2008; Galand et al., 2009; Bowman et al., 2012), though in one coastal study a greater abundance of *Gammaproteobacteria* was detected (Kirchman et al., 2010). Suggestive differences in bacterial communities were observed between the Chukchi Borderland and the sub-arctic northern Bering Sea, where *Actinobacteria* are the most abundant member of the bacterioplankton community (Zeng et al., 2012). It is common for all the sea ice to melt in summer in the two regions. In addition, the upper waters of the Chukchi Borderland are primarily influenced by nutrient-rich Pacific waters entering through the Bering Strait (Rella and Uchida, 2011). Compared to the northern Bering Sea during sea-ice melting season (Zeng et al., 2012), lower salinity and chlorophyll values, as well as lower proportion of chloroplast /Cynobacteria clones in clone libraries, were observed in the Chukchi Borderland, indicating the influence of sea ice melting on phytoplankton growth which can subsequently affect the community structure and dynamics of bacteria in surface water

Table 3. Diversity of cultivable bacterioplankton in the Chukchi Borderland region

Phylum	Genus	Bacteria /%	Protease-producing bacteria /%
Gammaproteobacteria	*Colwellia*	0.5	0
	Pseudoalteromonas	88.5	91.7
	Psychrobacter	1.0	0
	Vibrio	5.7	6.2
Bacteroidetes	*Polaribacter*	0.5	0
	Flavobacterium	2.6	2.1
Actinobacteria	*Kocuria*	2.9	0
Firmicutes	*Planococcus*	2.9	0

by changing the availability of nutrients, algal exudates and biological surfaces (Rösel and Grossart, 2012). *Actinobacteria* are widely distributed in both terrestrial and marine ecosystems, where they play a crucial role in the recycling of refractory biomaterials by decomposition and humus formation (Goodfellow and Williams, 1983; Ventura et al., 2007). It is worth mentioning that the proportion of *Actinobacteria* in clone libraries decreases dramatically with increasing latitude (Table 1) in this study (Fig. 1). In addition, phylotypes K2S84 and K2S133 show 99.8% and 98.9% similarity to actinobacterium clones s13a (GQ452883) and s80 (GQ452877) from the surface water in the northern Bering Sea, respectively. The results suggest that the bacterioplankton community composition in the Chukchi Borderland is related to the northern Bering Sea-derived waters, and is more influenced by sea ice melting during the summer season.

The SAR11 clade and the *Rhodobacterales* of *Alphaproteobacteria* account for as much as 30%–50% of all bacteria in the ocean surface waters (Giovannoni et al., 2005). The SAR11 clade is abundant throughout the oceans (Giovannoni and Rappé, 2000). In the surface waters of the Chukchi Sea, a well-known cosmopolitan group SAR11 clade can make up 25% of the prokaryotic communities (Malmstrom et al., 2007). A large number of clones that were clustered with *Pelagibacter ubique* HTCC1062 of the SAR11 clade (Fig. 2a) was observed in this study. Members of the ubiquitous and abundant SAR11 clade are deficient in assimilatory sulphate reduction genes. Tripp et al. (2008) reported that SAR11 requires exogenous sources of reduced sulphur compounds such as algal osmolyte dimethylsulphoniopropionate (DMSP) for growth. A DMSP dependent demethylase (DmdA) has been purified from *Pelagibacter ubique* HTCC1062 (Reisch et al., 2008). At the same time, members of the genera *Loktanella, Octadecabacter* and *Sulfitobacter* in the *Rhodobacteraceae* were also able to convert DMSP produced by marine phytoplankton to dimethylsulfide (DMS), a climate-relevant gas (Buchan et al., 2005; Curson et al., 2008). Accounting for 38% of the total clones, *Rhodobacteraceae*-related sequences dominated the clone libraries in this study. The results suggest that the SAR11 and *Rhodobacteraceae* clades may play an important role during decaying phytoplankton blooms in the Chukchi Borderland region in summer. In addition, members of the *Rhodobacteraceae* have the trait of aerobic anoxygenic photosynthesis (Yoon et al., 2007). A total of 15 clones in the library K4S (Fig. 2a), represented by K4S71 (JN177665), showed 96.9% similarity to *Planktotalea frisia* SH6-1 (FJ882052), which contains the *pufLM* genes coding for the bacterial photosynthesis reaction center (Hahnke et al., 2012). Represented by clone K2S16 (JN177589), a total of 100 clones (27.3% of the total clones) in the two libraries showed 92.9%–99.0% similarity to *Planktomarina temperata* 01-003883. A population of the *Roseobacteria* clade affiliated (RCA) cluster, dominated by a ribotype with a 16S rRNA gene sequence identical to strain RCA23 (Cand. *Planktomarina temperate*), constituted up to 21% of the total 16S rRNA genes of the bacterioplankton in the North Sea (Giebel et al., 2011). The *pufM* gene was also detected in strain RCA23, indicating the potential of the isolate for aerobic anoxygenic photosynthesis (Giebel et al., 2011). It is known that the abundance of the RCA cluster is inversely correlated with salinity, and the cluster only occurs in temperate and polar oceans with salinities below 33 (Giebel et al., 2011). The salinity in this study was from 27.4 to 28.6. The correlation we found is consistent with the global distribution patterns of the RCA cluster with respect to the salinity distribution. Our results support the previous findings that the SAR11 and *Rhodobacteraceae* clades are two abundant components of the marine bacterioplankton in polar seas, responding to hydrographic and plankton dynamics in the summer (Giebel et al., 2011).

Abundance discrepancy of AAP bacteria and DMSP-degrading bacteria were observed between FISH and clone library results. Using FISH approach, no AAP bacteria were detected at both stations. However, high percentage of clones affiliated with *Planktomarina temperata* (an AAP bacterium) was observed in libraries of the two stations. It is not readily explicable because many factors, including the probe sequence, the target gene for FISH, the copy number of rRNA operons per bacterial genome and the PCR bias, can cause such discrepancy. DMSP-degrading bacteria were successfully detected at one of the stations by DDDL probe, indicating the existence of bacteria with *dddL* gene (Howard et al., 2008). High percentage of clones affiliated with *Pelagibacter ubique* (a DMSP-degrading bacterium) was observed in both libraries. However, instead of *dddL* gene, *dmdA* gene was found in *Pelagibacter ubique* (Reisch et al., 2008).

Represented by clone K2S137 (JN177613) showing only 85.9% similarity to *Marinimicrobium locisalis* ISL-43 (EU874388), a total of 20 clones (5.4% of the total clones) in the libraries were affiliated with the SAR86 cluster of *Gammaproteobacteria* (Fig. 2a). The result is similar to a previous report in that the well-known cosmopolitan group SAR86 clade makes up 8% of the prokaryotic communities in surface waters of the Chukchi Sea (Malmstrom et al., 2007). In addition, a high proportion of *Pseudoalteromonas*-related clones (9% of the total clones) was observed in the clone libraries. At the same time, in agreement with the previous studies in Arctic and sub-Arctic waters (Groudieva et al., 2004; Zeng et al., 2012), a conspicuous presence of the genus *Pseudoalteromonas* was found both in bacterial isolates and protease-producing bacteria (Table 3) in the Chukchi Sea, indicating the contribution of planktonic *Pseudoalteromonas* to carbon and nitrogen cycling in summer. Species of *Pseudoalteromonas* are generally found in association with marine eukaryotes and display algicidal activities (Holmström and Kjelleberg, 1999), indicating the competitive advantages for *Pseudoalteromonas* in nutrient acquisition and colonization against phytoplankton blooms (Kato et al., 1999). It is worth mentioning that isolate BSw21650 (JF697294) showed 98% similarity to phylotype K2S15 and *Pseudoalteromonas* marina Mano4, which can produce caseinase, amylase and alginase (Nam et al., 2007).

Sequences affiliated with the genera *Polaribacter* and *Flavobacterium* dominated the *Bacteroidetes*-related clones, and showed high sequence similarities to polar isolates like *Polaribacter irgensii* 23-P (AAOG01000005) or *Flavobacterium degerlachei* LMG 21915 (AJ557886). It suggests that the study of these bacteria may provide insight into the biogeographic distribution of prokaryotes. All *Actinobacteria* phylotypes were observed in both libraries. Phylotypes K2S84 (JN177602) and K2S133 (JN177612) showed 97.4% and 96% similarity to *Aquiluna rubra* MWH-Dar4 (AJ565416) from freshwater habitats, and *Ilumatobacter fluminis* YM22-133 (AB360343) from estuary sediment, respectively. However, seawater is needed for the growth of *Ilumatobacter fluminis* YM22-133 (Matsumoto et al., 2009). The results indicated a wide distribution of some *Actinobacteria* phylotypes in both marine and freshwater habitats.

Compared with the previous studies of bacterial communities in the coastal Chukchi Sea surface water by pyrosequencing of 16S rRNA genes (Kirchman et al., 2010), lower bacterial diversity in our clone libraries was observed at the phylum level by Sanger sequencing. Traditional Sanger sequencing-based approaches have suffered from high cost and low throughput that rendered many taxa undetectable. The recently developed massively parallel pyrosequencing enables metagenomic and metagenetic analyses in a manner that exceeds the capacity of traditional culture-independent molecular identification methods by several orders of magnitude (Margulies et al., 2005; Sogin et al., 2006; Tedersoo et al., 2010). On the other hand, the size of the recovered DNA fragments remains between 100 bp and 400 bp, which may reduce the taxonomic resolution and complicate the comparability of the results among different studies and identification methods (Dethlefsen et al., 2008; Nilsson et al., 2009; Tedersoo et al., 2010). Therefore, large-scale pyrosequencing-based analyses of 16S rRNA genes to assess the bacterial community composition in samples used in this study will be performed in the near future, and the results will be reported in another paper.

Our study reports an analysis of bacterioplankton community composition in the Chukchi Borderland using a combination of molecular and cultivation-based methods. The *Rhodobacteraceae* and SAR11 clades of *Alphaproteobacteria* and *Pseudoalteromonas* of *Gammaproteobacteria* dominated ocean surface bacterioplankton communities. In addition, *Pseudoalteromonas* isolates made up a significant fraction of both bacterial isolates and protease producers, suggesting their role in carbon and nitrogen cycling during summer seasons in the Chukchi Borderland.

Acknowledgements

The authors appreciate the assistance of the Korean icebreaker Araon crew and the scientists who collected samples for us during the first Korean Arctic cruise. We are also grateful to Dr. Ho Kyung Ha of Korea Polar Research Institute for kindly providing CTD data.

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