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Archaeal and bacterial community structures in the anoxic sediment of Antarctic meromictic lake Nurume-Ike

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Abstract

Prokaryotic community structures in the anoxic sediment of the Antarctic meromictic Lake Nurume-Ike were revealed by sequence analysis of 16S rRNA gene clones. The archaeal clones obtained (205 total) consisted of only three phylotypes, and were dominantly affiliated with uncultured euryarchaeotes. Specifically, 93% of the clones were identified as marine benthic group-D archaeal phylotype. In contrast to the limited archaeal diversity, 53 phylotypes were detected within 312 bacterial clones. Major bacterial phylotypes were affiliated with α-Proteobacteria (20% of clones), d-Proteobacteria (9%), Planctmycetales (7%), and Cyanobacteria (7%). A small numbers of clones belonging to γ-Proteobacteria, Actinobacteria, Spirochaetes, Flavobacteria, and Verrucomicrobia were also found. A total of 53% of the bacterial clones, consisting of 13 phylotypes, could not be classified into any known group. These results indicated that the bacterial community of Lake Nurume-Ike sediment consisted of numerous phylogenetic groups and had a diversity comparable to the diversity of other Antarctic lakes communities previously reported. Interestingly, however, there were very few phylotypes shared between the communities of lakes Nurume-Ike and five other lakes located in the Vestfold Hills area. This is the first comprehensive study to analyze more than 500 16S rDNA clones for microbial community analysis of an Antarctic lake sediment sample, and the results significantly expand current views of bacterial diversity in Antarctic lakes.

Keywords: Antarctica; Meromictic lake; Archaea; Bacteria; Community structure

1. Introduction

Historically, the main targets of biological research in the Antarctic Continent have been mammals, birds, and some plants and invertebrates. These studies have generated considerable interest in the ecology and life histories of these organisms and revealed impacts of recent climate changes. However, the pioneering studies of Franzmann (1996), showed Antarctic prokaryotes also to be remarkably diverse and to represent most major evolutionary groups. Later studies by Bowman et al. (2000a,b), using analysis of 16S rRNA gene (16S rDNA) clones, revealed that

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highly diverged prokaryotes also exist in Antarctic lakes. The prokaryotic communities consisted of both archaea and bacteria, including over 200 distinct phylotypes (i.e. groups of clones with high sequence similarity). These findings enabled better appreciation of prokaryotic diversity and biological material conversions in Antarctic lakes, and suggest that these habitats may represent valuable microbial resources for thermolabile (low-temperature adapted) enzymes and examples of biological adaptation to cold environments.

The chemo-limnological characteristics of Antarctic coastal lakes suggest that some were formed by separation from the ocean during the uplift of the continent after the last glacial period (Pienitz et al., 2008). Lake "Nurume-Ike", located in the center of the Langhovde area, is a typical marine relic and meromictic lake (Sano et al., 1977). Its water column consists of three to four layers (Kudoh et al., 2008) as follows. The surface layer (0–3 m) is slightly saline and is influenced by melt ice and water input from the surrounding catchment. The salinity of the second and third layers (3–11 m) is similar to that of the ocean. The bottom layer (>11 m) has one and a half times the salinity of the ocean, and has a completely reduced environment and high concentration of hydrogen sulfide, suggesting that there has been no substantial exchange of water between the lake and the sea recently (Kudoh et al., 2008).

In this study, we aimed to expand knowledge of the biodiversity of Antarctic prokaryotes by the analysis of 16S rDNA clones derived from the Lake Nurume-Ike sediment. We also compared the prokaryotic communities of Lake Nurume-Ike with other Antarctic meromictic lakes located in Vestfold Hilds, Eastern Antarctica, reported by Bowman et al. (2000a).

2. Materials and methods

2.1. Sampling and DNA extraction

Lake Nurume-Ike is located in the Langhovde area (69° 13′ 25″ S, 39° 40′ 01″ E), about 30 km south of Syowa Station. The maximum depth is about 17 m, and major and minor diameters are about 300 and 150 m, respectively. A sample of anoxic sediment at 13 m depth of the lake was obtained on 10 February 2004, using an Ekman-Birge-type bottom sampler. From this, a community DNA sample was extracted and purified from 5.0 g of the sediment using the Ultraclean Soil DNA Kit Mega Prep (Mo-bio) according to the manufacturer's instructions.

2.2. Amplification and sequencing of 16S rDNA clones

Archaeal and bacterial 16S rRNA genes were amplified by PCR using oligonucleotide primers as follows: archaeal forward primer 5′-TTCCGGTTGATCCYGCCGGA (A21F), bacterial forward primer 5′-AGAGTTTGATCCTGGCTCAG (B27F), and universal reverse primer 5′-GGYTACCTTGTTACGACTT (U1492R). Amplification by PCR comprised 30 cycles of 30 s at 94 °C, 30 s at 58 °C, 2 min at 72 °C, and a final extension of 5 min at 72 °C using Ex *Taq* DNA polymerase (Takara-bio). The PCR products were purified by using Sephacryl S-400 (Amersham Biosciences) in spin columns and cloned into the pT7Blue vector (Novagen). *E. coli* DH5α cells were transformed with the plasmid library, and plated onto LB plates including 100 mg/mL ampicillin, 40 mg/mL X-gal and 0.5 mM IPTG. Individual white colonies were randomly picked and were sub-cultured in 200 μl of LB medium including 100 mg/mL ampicillin. The aliquot of each culture was then used for PCR amplification of each 16S rRNA gene clone (16S rDNA clone). About 800 bp of the 5′-region of each 16S rDNA clone (207 archaeal clones and 324 bacterial clones) was sequenced.

2.3. Identification of 16S rDNA clones and phylogenetic analysis

The 16S rDNA sequences of 531 clones were submitted for BLAST searching of 16S rRNA sequences (BLASTN, http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al., 1990) to identify individual clones. PCR derived chimera sequences were checked by using the CHIMERA_CHECK program provided by Niels Larsen, Ribosomal Database Project (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). We also searched for chimera sequences by manually checking the sequence alignments using GENETYX Ver.9.0 software (Genetyx). In order to analyze phylogenetic relationships among the clones and previously reported 16S rDNA sequences, Neighbor-joining trees including bootstrap probabilities (1000 samplings) were constructed using the CLUSTAL W Ver.1.83 program (Thompson et al., 1994) and the GENETYX Ver.9.0 software. The homologous coverage (biodiversity coverage) C was determined with the following equation: $C = 1-(N/n)$, where N is the number of phylotypes and n is the total number of analyzed clones (Good, 1953; Singleton et al., 2001). In the present study, clones with similarity of 98.0%

and above were group together into the same phylotype. The nucleotide sequences of 56 phylotypes are available in the DDBJ/EMBL/GenBank databases under the accession numbers; AB534620–AB534675.

3. Results

3.1. 16S rDNA clone library construction and screening

A 16S rDNA clone library was constructed from community DNA extracted from sediment sample collected from Lake Nurume-Ike. The anoxic sample possessed a black-green color as results of accumulated sulfides and algal material. DNA yield from 5.0 g of the sediment sample was about 5 mg. A total of 531 clones were screened. Fourteen chimerical clones were detected during the analysis and were not used for further study. A total of 205 archaeal clones consisted of only 3 phylotypes, whereas 312 bacterial clones were classified into 53 phylotypes. The biodiversity coverage of archaeal and bacterial clones were 98% and 83%, respectively.

3.2. Archaeal community structure

The phylogenetic distribution of archaeal clones across 16S rDNA phylogenetic groups is shown in Fig. 1A. The 205 archaeal clones consisted of a single phylotype of Marine benthic group D (MBG-D) and two phylotypes of unclassified Euryarchaea. No phylotype was closely related to any cultured species.

The MBG-D phylotype, ANRA021, encompassed 93% of total archaeal clones. The closely related published environmental clones of ANRA021 have been isolated from similar benthic environments. For example, "25H-270S-24" was isolated from cold seep sediment of the gas-hydrate-bearing Okhotsk sea (Zhang et al., 2008), "BURTON2-A" from Antarctic meromictic lake sediment (Bowman et al., 2000a), "SMI1-GC205-Arc38" from hypersaline gulf of Mexico sediment (Lloyd et al., 2006), "Eel River-TA1f2" from northern California methane seep sediment (Beal et al., 2009), and "Tommeliten-ARCH69FL" from a North sea pockmark (Niemann et al., 2006) (Fig. 2). The 16S rDNA similarities among these environmental clones were 98–99% within about 500 nucleotides.

The phylotype ANRA016 and ANRA109 contributed about 7% of clones obtained, and showed significant similarity of 16S rDNA sequences (91–92%) with only three published sequences as follows; "p706_a_5.13" isolated from hydrothermal sediment of Yonaguni Knoll IV (Nunoura et al., 2007), "104A5" isolated from a microbial mat of the Chefren mud volcano (Omoregie et al., 2008), and "Mn3b-A87" isolated from a northern California methane seep sediment (Beal et al., 2009) (Fig. 2).

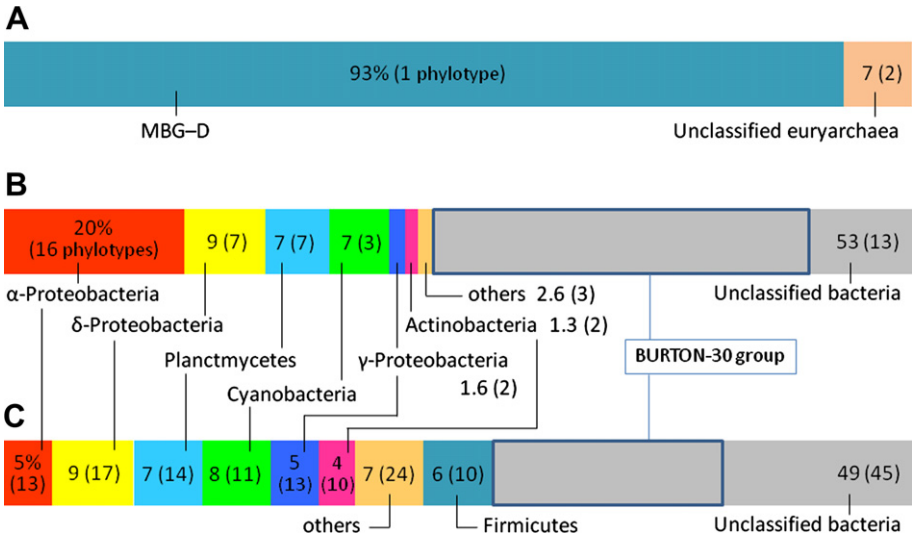

Fig. 1. Phylogenetic distributions of 16S rDNA clones derived from the sediment sample of Lake Nurume-Ike and of five lakes in Vestfold hills. (A) Archaea in Lake Nurume-Ike, (B) Bacteria in Lake Nurume-Ike, (C) Bacteria in Vestfold Hills lakes. Clonal frequencies of individual groups are indicated as the number of phylotypes (in parentheses). The BURTON-30 group, which consisted of ANRB009, ANRB017, BURTON-4, and BURTON-30 phylotypes, is indicated by boxes on the unclassified bars.

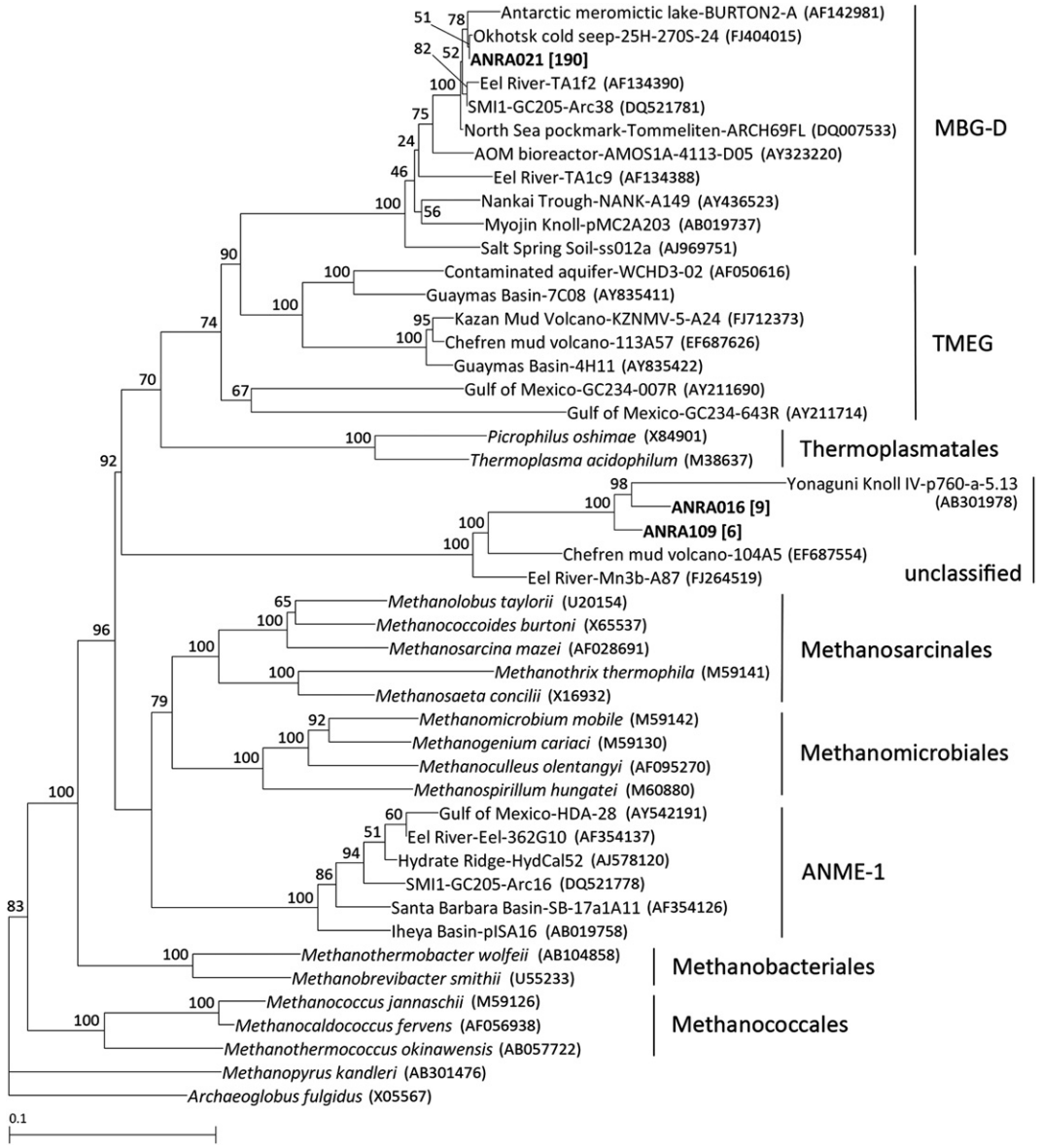

Fig. 2. Phylogenetic tree of archaeal phylotypes in Lake Nurume-Ike and related published sequences. Phylotypes are indicated in bold type with numbers in brackets corresponding to the number of clones. Bootstrap values are indicated at branch node points.

3.3. Bacterial community structure

The distribution of 312 bacterial clones is shown in Fig. 1B. The phylogenetic tree of Proteobacteria and related environmental clones is shown in Fig. 3, and the tree of other bacterial classes is shown in Fig. 4. One hundred and forty-eight bacterial clones consisted of 40 phylotypes and were grouped within 9 classes. Another 164 clones consisting of 13 phylotypes did not cluster within any known bacterial group.

α-Proteobacteria was the most dominant class in Lake Nurume-Ike sediment, contributing 20% of total bacterial clones, and consisted of 16 phylotypes and 63 clones. Within this α-Proteobacteria group, ANRB092 and ANRB140 showed significant sequence similarity (95–98%) with Antarctic isolates *Staleya guttiformis* and *Sulfitobacter brevis* (Labrenz et al., 2000). The phylotypes ANRB179, ANRB106, ANRB175, and ANRB132, were identified as the members of the genus *Oceanicola*, *Rhodobaca*, *Mesorhizobium*, and *Sphingopyxis*, respectively. Other phylotypes were not affiliated with any known genus.

The second-most abundant bacterial class was d-Proteobacteria, accounting for 9% of the clones. This

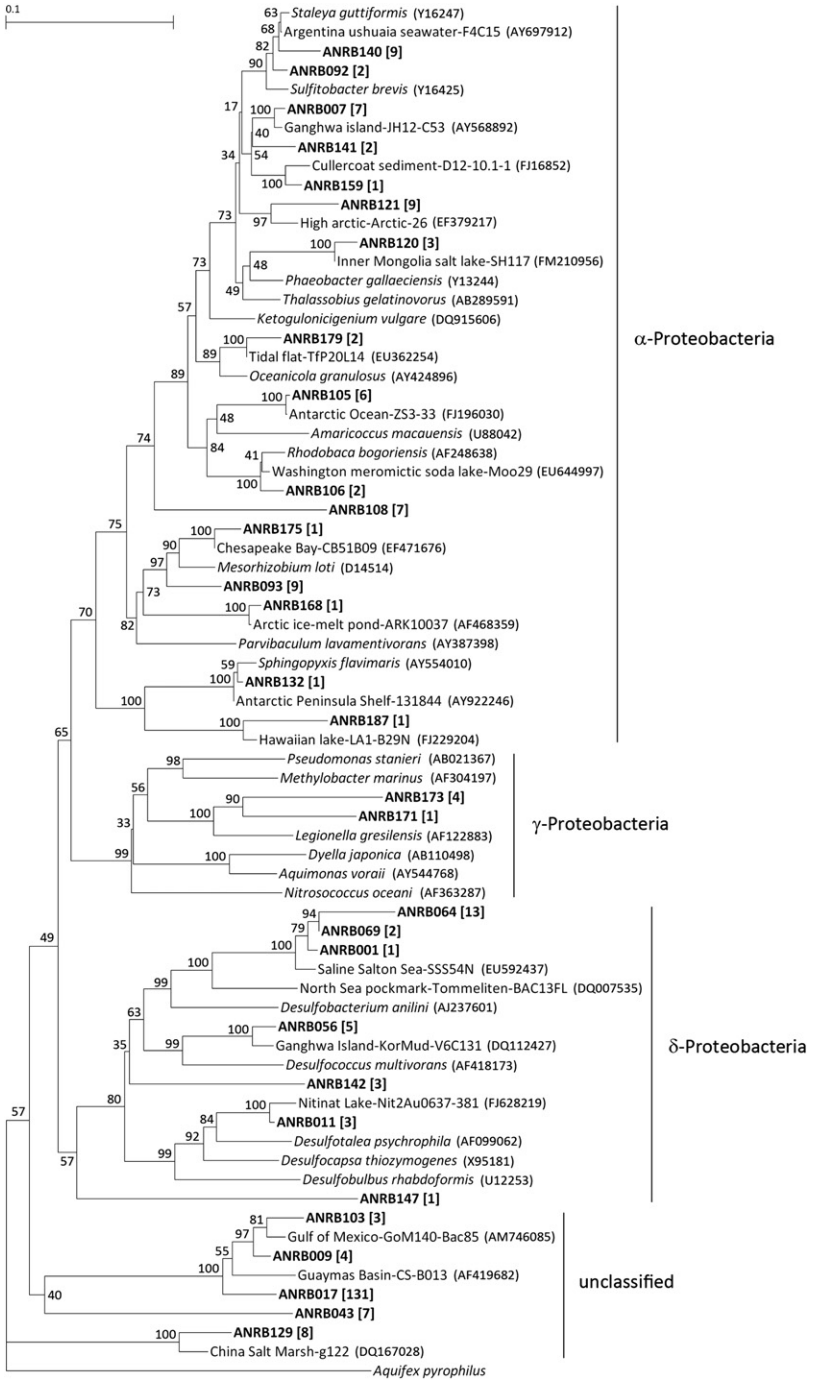

Fig. 3. Phylogenetic tree of phylotypes of Proteobacteria and unclassified lineages in Lake Nurume-Ike with related published sequences. Phylotypes are indicated in bold type with numbers in brackets corresponding to the number of clones. Bootstrap values are indicated at branch node points.

group consisted of seven phylotypes in which no clone was identified as a cultured species. Some of the clones were closely related with published environmental clones isolated from hypersaline Salton Sea sediment (Dillon et al., 2009), salt marsh sediment in Ganghwa island (DQ112427/DNA data base), or the anoxic basin of Nitinat Lake (Schmidtova et al., 2009) (e.g. ANRB001, 069, 056, 011 in Fig. 3).

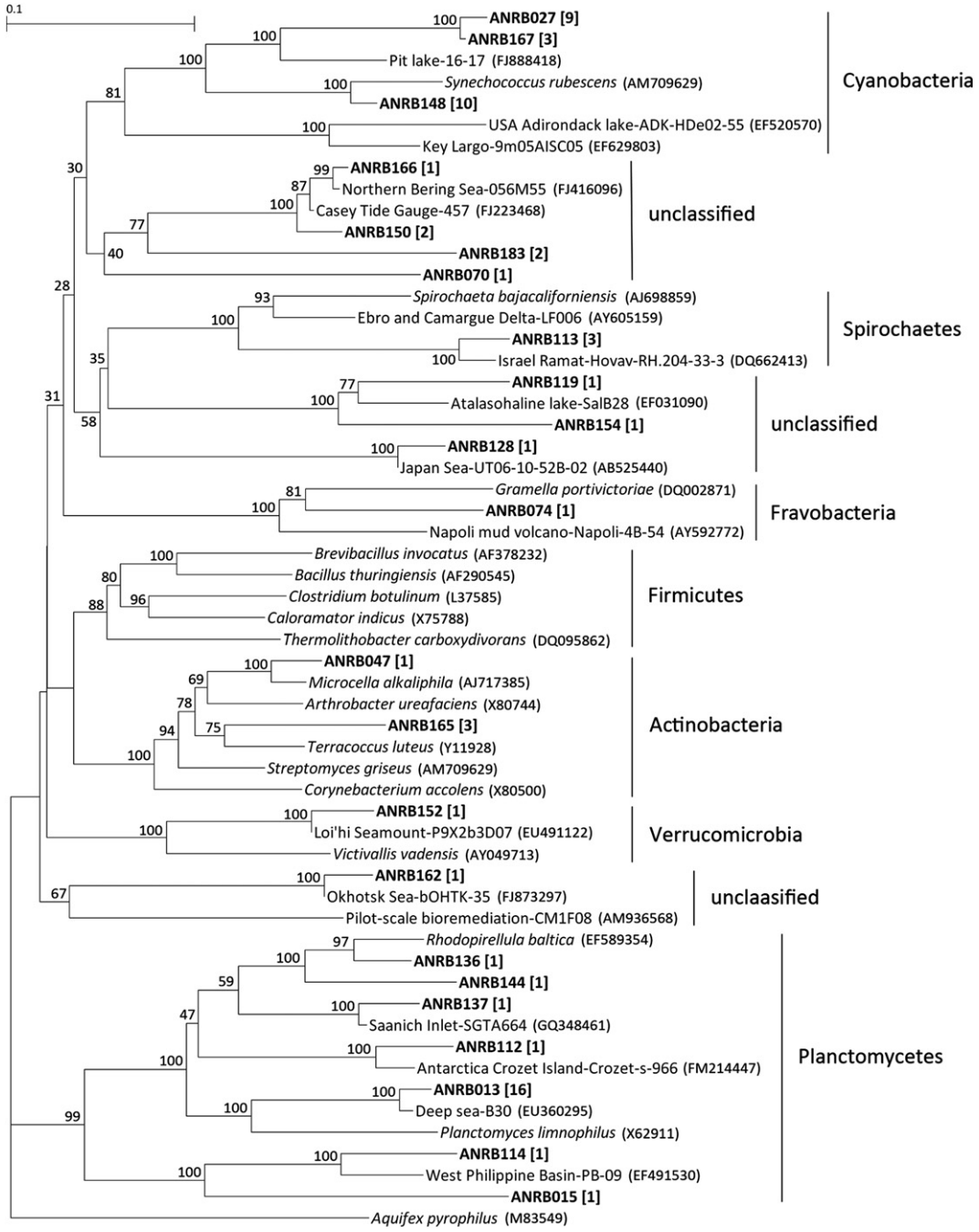

Fig. 4. Phylogenetic tree of bacterial phylotypes and unclassified lineages (except Proteobacteria) in Lake Nurume-Ike with related published sequences. Phylotypes are indicated in bold type with numbers in brackets corresponding to the number of clones. Bootstrap values are indicated at branch node points.

Five clones of two phylotypes were affiliated with g-Proteobacteria based on the phylogenetic analysis. However, their sequences did not showed significant similarity with any published 16S rDNA sequences of g-Proteobacteria.

A number of Lake Nurume-Ike bacterial clones also grouped with Planctomycetes and Cyanobacteria. The phylotype ANRB136 belonging to Planctomycetes was related with *Rhodopirellula baltica*. Other Planctomycetes clones did not show significant similarity with

any known species, however, some of them (ANRB137, 122, 013) were related with published environmental clones isolated from the oxygen-minimum zone of Saanich Inlet (Walsh et al., 2009), Antarctic Crozet island marine sediment (FM214447/DNA data base), and deep-sea sediment of the West Pacific (Wang et al., 2008) (Fig. 4). Cyanobacterial clones were classified into three phylotyopes, of which one phylotype (ANRB148) showed 93% sequence similarity with the sequence of *Synechococcus rubescens*. Other two phylotypes did not showed high sequence similarity with any published cyanobacterial 16S rDNA sequences.

As shown in the phylogenetic tree of Fig. 3, some unclassified lineages, which consisted of many Nurume-Ike clones and some published environmental clones, appeared outside the cluster of Proteobacteria. Within these lineages, the phylotype ANRB017 showed highest clonal frequency as a single phylotype existed. Interestingly, this clone was closely related with BURTON-30 group which is also dominant in the Vestfold lakes (Fig. 1B, C) and was assigned as a low G + C Gram-positive division by Bowman et al. (2000a). Other related published environmental clones of this phylotype were "CS-B013" isolated from hydrothermal sediments in the Guaymas Basin (Teske et al., 2002) and "GoM140-Bac85" isolated from cold seep sediment of Gulf of Mexico (AM706485/DNA data base). Three unclassified lineages also branched near the clusters of Cyanobacteria, Spirochaetes, and Verrucomicrobia on the other bacterial phylogenetic tree (Fig. 4).

4. Discussion

Lake Nurume-Ike is a typical marine relic meromictic Antarctic lake. This lake seems to be large enough to maintain relatively stable environmental conditions compared with other smaller and shallower lakes, which may contribute to forming a stable environment for microorganisms. The bottom water and sediment of Lake Nurume-Ike is anoxic, and has one and a half times the salinity of the ocean. We used this anoxic sediment to obtain mixed DNA to analyze its prokaryotic community structure. However, it must be recognized that these sediment-based clone libraries may represent not only anaerobic benthic prokaryotes but also a proportion of aerobic taxa that have fallen from the mixed layers above.

From the archaeal clone library, we identified only three phylotypes, consisting of a single MBG-D phylotype and two unclassified euryarchaeal phylotypes, in spite of obtaining 205 archaeal clones. We obtained no clones affiliated with methanogens, terrestrial miscellaneous euryarchaeal group (TMEG), archaeal anaerobic methanotrophs (ANME), or crenarchaeal lineages. One possible reason for absence of methanogens is oxidation–reduction potential (ORP) of the Lake Nurume-Ike sediment, which is about −0.18 V (Kudoh et al., 2008), while methanogens are known to require an ORP below −0.33 V for their growth (Harvey et al., 1986). Interestingly, few archaeal phylotypes were also reported in other Antarctic meromictic lake sediments (Bowman et al., 2000a,b) with, for instance, one to three archaeal phylotypes being detected from five lakes in the Vestfold Hills in East Antarctica. This suggests that only a few archaeal species dominate in the Lake Nurume-Ike sediments. We were concerned about the efficacy of PCR primers A21F and U1492R for amplification of the archaeal 16S rDNAs. However, this primer set has successfully detected a variety of archaea, including uncultured lineages mentioned above in many studies, although Lloyd et al. (2006) also showed that the primer 21F gave some biased amplification of archaeal 16S rDNAs and lower archaeal diversity than another primer, A8F (5′-TCCGGTTGATCCTGCC). While a few more archaeal lineages are likely to exist in the Lake Nurume-Ike sediment than were detected in our analysis, it seems that the archaeal diversity in individual Antarctic lakes is generally lower than that of ocean sediments.

The MBG-D phylotype was most abundant not only in the Lake Nurume-Ike sediment but also in Antarctic Lake Burton and Taynaya Bay. This uncultured euryarchaeal lineage is widely distributed in ocean sediments, such as continental shelf anoxic sediment (e.g. Vetriani et al., 1998), salt marsh subsurface sediment (e.g. Munson et al., 1997), and subsurface marine sediment (e.g. Vetriani et al., 1999) including a methane hydrate site (Inagaki et al., 2006). The MBG-Ds of Lakes Nurume-Ike and Burton were probably derived from the ocean, and also provide biological evidence that these lakes are marine relics.

In contrast with the limited archaeal diversity, 312 bacterial clones were classified into 53 phylotypes. This was not surprising, since high bacterial diversity of anoxic sediments of Antarctic lakes has already reported by Bowman et al. (2000a,b). However, our results offer a broader coverage of biodiversity, which reached 83% as a result of a larger number of clones being analyzed from a single sediment sample. This coverage value is at least two times greater than obtained in previous studies. This result significantly expands current views of

bacterial diversity in Antarctic lakes. For example, α-Proteobacteria was found to be more diverse than demonstrated previously. Additionally, two phylotypes were closely related with *S. guttiformis* and *S. brevis*, both of which were isolated from the mixed layer of Antarctic meromictic Lake "Ekho" (Labrenz et al., 2000). These species are strictly aerobic, clearly indicating that the sediment of Lake Nurume-Ike also includes deposition of cells living naturally in the aerobic layers above in the water column.

The bacterial diversity in anoxic sediments of Lake Nurume-Ike was compared with those of other Antarctic meromictic lakes (Lakes Ace, Burton, Clear, Pendant, Scale) reported by Bowman et al. (2000a). These five lakes are also saline and are located in the Vestfold Hills (68° 27–38′ S, 77° 59′–78° 14′ 01″ E), about 1500 km from Lake Nurume-Ike. The populations of the bacterial classes in the Lake Nurume and the Vestfold Hills lakes are shown in Fig. 1. α-Proteobacteria clones in Lake Nurume-Ike were more frequent than in the Vestfold lakes, and there was no difference in the frequencies of d-Proteobacteria, Planctomycetes, and Cyanobacteria among the lakes. These results indicate that the bacterial community of Lake Nurume-Ike sediment consists of numerous phylogenetic groups and has a diversity comparable to the diversity of other Antarctic lakes communities reported by Bowman et al. (2000a). However, most of the clones obtained from Lake Nurume-Ike showed less than 97% sequence similarity with those from the Vestfold Hills. Furthermore, Firmicutes clones, which were one of major groups in the Vestfold Hills lakes, were not detected from Lake Nurume-Ike. The major difference between the methods used by Bowman et al. (2000a) and the current study was in the sequences of forward primers used in the amplification of 16S rDNA by PCR. Bowman et al. (2000a) used 530F instead of the 27F used here. However, we have found no publication describing differences in performance between these two PCR primers and, at this time, it is difficult to speculate on the possible reasons underlying the different distributions of Firmicutes clones between the lakes in the two studies.

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