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Characterization of the Depth-Related Changes in the Microbial Communities in Lake Hovsgol Sediment by 16S rRNA Gene-Based Approaches

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The undisturbed sediment of Lake Hovsgol (Mongolia) is scientifically important because it represents a record of the environmental changes that took place between the Holocene (the present age) and Pleistocene (the last ice age; 12,000 ^{14}C years before present day). Here, we investigated how the current microbial communities change as the depth increases by PCR-denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA genes of the microbial communities. The microbial diversity, as estimated by the Shannon index, decreased as the depth increased. In particular, significant changes in archaeal diversity were observed in the middle depth (at 39~42 cm depth of total 60 cm depth) that marks the border between the Holocene and Pleistocene. Phylotype belonging to *Beta*- and *Gamma*-Proteobacteria were the predominant bacteria and most of these persisted throughout the depth examined. However, as the depth increased, some bacteria (some genera belonging to *Beta*-Proteobacteria, Nitrospira, and OP8-9) were not detectable while others (some genera belonging to *Alpha*-, *Beta*-, *Gamma*-Proteobacteria) newly deteced by DGGE. Crenarchaea were the predominant archaea and only one phylotype belonging to Euryarchaea was found. Both the archaeal and bacterial profiles revealed by the DGGE band patterns could be grouped into four and three subsets, respectively, subsets that were largely divided by the border between the Holocene and Pleistocene. Thus, the diversity of the current microbial communities in Lake Hovsgol sediments decreases with increasing depth. These changes probably relate to the environmental conditions in the sediments, which were shaped by the paleoclimatic events taking place between the Holocene and Pleistocene.

***Keywords*:** Lake Hovsgol, sediment, DGGE, microbial diversity, 16S rRNA gene

Benthic sediment represents one of the most complex microbial habitats on Earth (Priscu *et al.,* 1998). The microorganisms in benthic sediments play important roles in nutrient cycles such as methane production (Chan *et al.,* 2005), sulfate reduction (Li *et al.,* 1999) and ammonia oxidation (Hastings *et al.,* 1998). Consequently, investigating the microbial communities and their function in freshwater sediments will greatly enhance our understanding of the aquatic ecosystem (Tamaki *et al.,* 2005). Since most freshwater sediments are quite heterogeneous ecosystems that give rise to many different environmental niches even on a millimeter scale (Spring *et al.,* 2000), only few studies have analyzed how microbial communities change as sediment depth increases (Urakawa *et al.,* 2000; Koizumi *et al.,* 2003).

Lake Hovsgol (Khubsugul, Mongolia) is one of the largest lake in Asia (Lake Baikal is the largest) (Karabanov *et al.,* 2004). It is famous for being one of the very few ultra-oligotrophic pure lakes in the world (Belykh *et al.,* 2005), and has characteristics of low mineral content (total ion concentration is 210~230 mg/L) and alkalic pH water (pH 8.1~8.5) (Kozlov *et al.,* 1989). Lake Hovsgol is also very interesting from a scientific point of view because it is known to be 2.5~4.0 Ma (million years) old. Since the last glacial maximum (LGM; last ice age) ended 12,000 to 13,000 years ago (Goulden *et al.,* 2003), it is certain that Lake Hovsgol experienced the LGM. Analysis of the lake's sediments (Nara *et al*., 2005) has revealed that during the LGM represented by the >40~50 cm-depth sediments, (Fedotov *et al.,* 2004), there was no accumulation of planktonic/benthic diatoms, chrysophyte cysts, sponge spicules and zooplankton in the lake (Fig. 2). After the LGM, however, nutrient loading from the watershed resumed and the organisms listed above began to accumulate again, as revealed by the 0~40 cm-deepth sediments (Fig. 2). Thus, the stratified sediments of Lake Hovsgol represent a record of past climatic changes that have impacted significantly on the ecology of the lake.

We speculated that these different depth-related environmental conditions may constitute specific niches for microorganisms and that the microbial communities currently living

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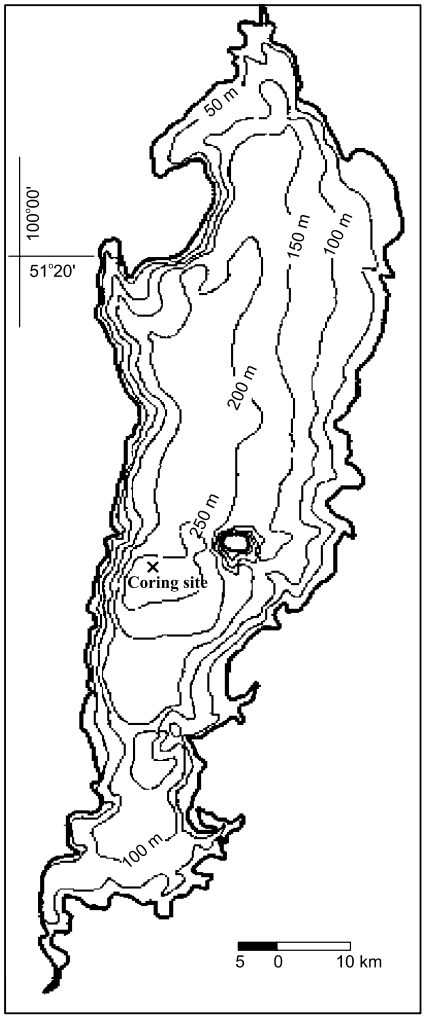

Fig. 1. Map of Lake Hovsgol with sampling site.

in the Lake's sediments may consequently vary with sediment depth. Since the current microbial diversity in the stratified sediments of Lake Hovsgol has not yet been investigated, we subjected the microbial communities in various sediment depths to PCR-denaturing gradient gel electrophoresis (DGGE) analysis.

The 'great plate count anomaly' paradigm refers to the observation that often the most prevalent microorganisms cannot be cultivated (Staley and Konopka, 1985). This anomaly has caused microbial ecologists to move from agar media to PCR-based approaches to estimate microbial diversity and analyze the structure of microbial communities (Rondon *et al.,* 1999; Hugenholtz, 2002). Such molecular biological techniques employ certain molecular markers, such as 16S rRNA gene or its encoding gene, to detect and identify particular microorganisms. Analysis of the structure of microbial communities has been aided in particular by the introduction of DGGE (Muyzer *et al.,* 1993). In this study, we used DGGE analysis of 16S rRNA genes to study the depth-related changes in the microbial communities in the sediment layers of Lake Hovsgol. How the paleoclimatic history of the lake shaped the physico-chemical properties of the sediments, and how these properties have in turn molded the microbial populations we detected is discussed.

Materials and Methods

Sample collection

Sampling of the sediment was carried out in March 2004 at one central location (50°57′18.4″N, 100°21′32.7″E, water depth; about 260 m) of Lake Hovsgol by using a Benthos gravity corer (Fig. 1). The core immediately was transported to the local laboratory, and frozen until next procedure. The core in the plastic pipe was sectioned with 3 cm interval down to a depth of approximately 60 cm. The each sliced core was re-sampled at each center position of core and packed into sterile plastic tubes and stored at -70°C until analysis.

DNA extraction from sediment samples and purification

Total genomic DNA of the frozen sediment samples was isolated by using the bead-beating method as described previously (Yeates *et al.*, 1998). All DNA samples were treated with RNase A (Sigma, USA) and purified by using the ethanol precipitation method. The extracted DNAs were further purified by using the UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories, USA) with the following modifications: the bead-beating step was excluded, and DNA solution was added to Solution MD1 instead of the Micro-Bead Solution.

PCR-Amplification of bacterial and archaeal 16S rRNA genes

PCR amplification of bacterial 16S rRNA genes was performed by using the GC338f-518r primer combination as described Muyzer *et al*. (1993) and performed using amplifying condition of Henckel *et al*. (1999). Nested PCR amplification of archaeal 16S rRNA genes was carried out by using the arch20f-958r primer set (DeLong, 1992; DeLong *et al.,* 1999) for the first round of amplification and the archGC340f-519r set (Ovreas *et al.,* 1997) for the second round of amplification as described previously (Ferris *et al.,* 1996).

DGGE analysis of bacterial and archaeal diversities

DGGE was performed by using the D-Code System (Biorad, USA). Amplified DNAs were loaded onto 8% (w/v) polyacrylamide gels with 40 to 60% (Archaea) and 30 to 60% (Bacteria) denaturing gradient (where 100% of the denaturant consists of 7 M urea and 40% formamide) in 1× TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA, pH 8.3) and subjected to 14 h of electrophoresis at 60°C and 80 V. Thereafter, the gels were soaked in SYBR Green I nucleic acid stain (Bioneer, Korea) and photographed with UV transillumination.

Sequencing and phylogenetic analysis of excised DGGE bands

Dominant bands were excised with razor blades from the DGGE gels and soaked overnight in 20 μl of 0.1× TE buffer solutions. One microliter of each solution was PCR-reamplified and directly sequenced. The sequences were taxonomically assigned by using BLAST on the GenBank nucleotide database [National Center for Biotechnology Information

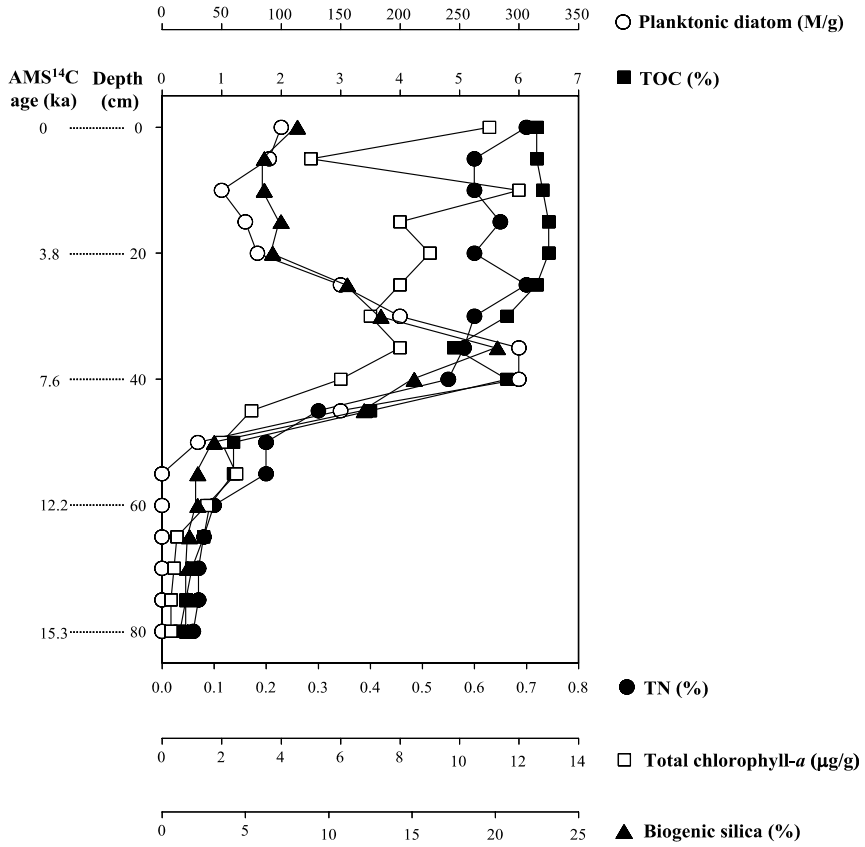

Fig. 2. Depth-related changes in total organic carbon (TOC, black squares), total nitrogen (TN, black circles), planktonic diatoms (open circles), total chlorophyll-*a* (open squares) and biogenic silica (black triangles) in Lake Hovsgol sediment (redrawn from references 29 and 42).

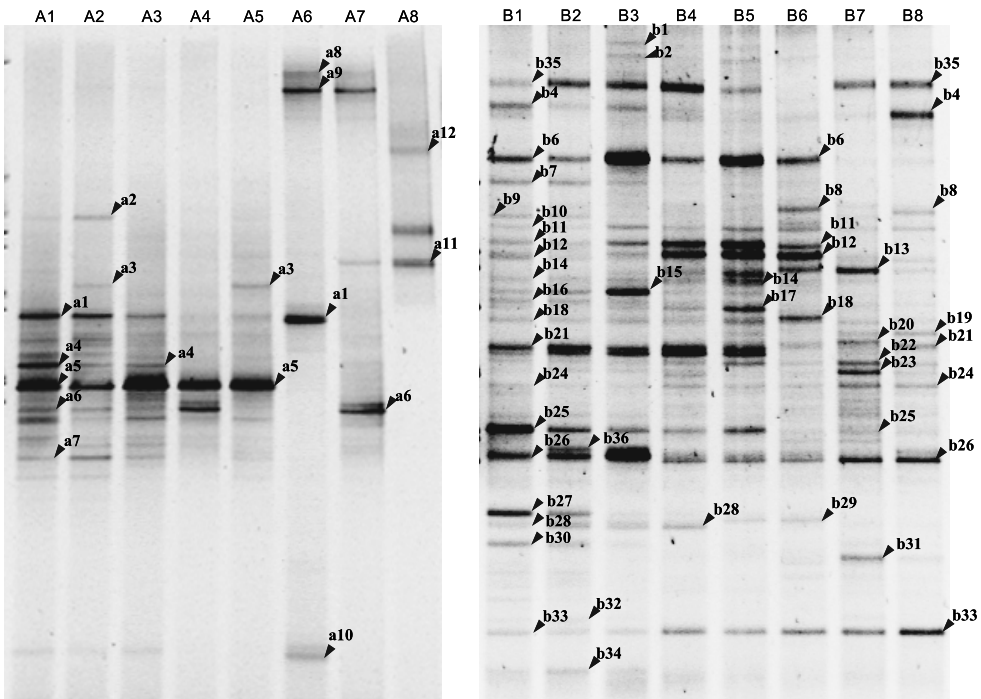

Fig. 3. DGGE analysis of PCR-amplified 16S rDNA fragments from the archaeal (A) and bacterial (B) communities in the sediments of Lake Hovsgol. Lanes 1, 0~3 cm; 2, 6~9 cm; 3, 12~15 cm; 4, 21~24 cm; 5, 30~33 cm; 6, 39~42 cm; 7, 48~51 cm; 8, 57~60 cm.

(NCBI)] (Altschul *et al.,* 1990). Sequences of 16S rRNA genes were aligned and a phylogenetic tree was constructed by using CLUSTAL X software (Thompson *et al.,* 1997).

Nucleotide sequence accession numbers

The nucleotide sequences obtained detected in this study are available from the GenBank nucleotide database at the NCBI website (http://www.ncbi.nlm.nih.gov) under accession numbers DQ452578-DQ452612.

Clustering and diversity analysis of DGGE profiles

Similarities between the DGGE profiles of samples and the band intensities of each lane were analyzed by BioNumerics software (BioSystematica, UK). For DGGE profile analysis, each lane was chosen separately, common bands were selected as positions for normalization to compensate for differences in migration distance due to gel heterogeneity. The normalized banding patterns were then used to generate dendrograms by calculating the Pearson product moment correlation coefficient and by employing the unweighted pair group method with arithmetic averages (UPGMA) clustering. For diversity analysis, densitometric curves from each lane were acquired by using the Bionumerics software. A matrix was then constructed with this information and used to calculate a set of numerical values that described the diversity of the prokaryotic communities. As a measure of the structural diversity of prokaryotic communities, the Shannon index (Koizumi *et al.,* 2003) of general diversity

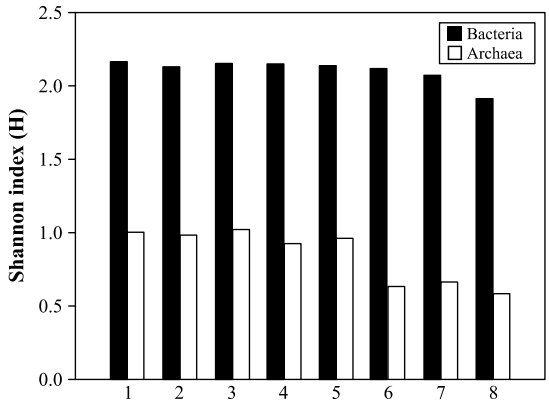

Fig. 4. Change of Shannon index values throughout the depth of lake Hovsgol sediment. Shannon index values (H) were calculated on the basis of the number and intensity of the bands on the gel tracks. Lanes 1, 0~3 cm; 2, 6~9 cm; 3, 12~15 cm; 4, 21~24 cm; 5, 30~33 cm; 6, 39~42 cm; 7, 48~51 cm; 8, 57~60 cm.

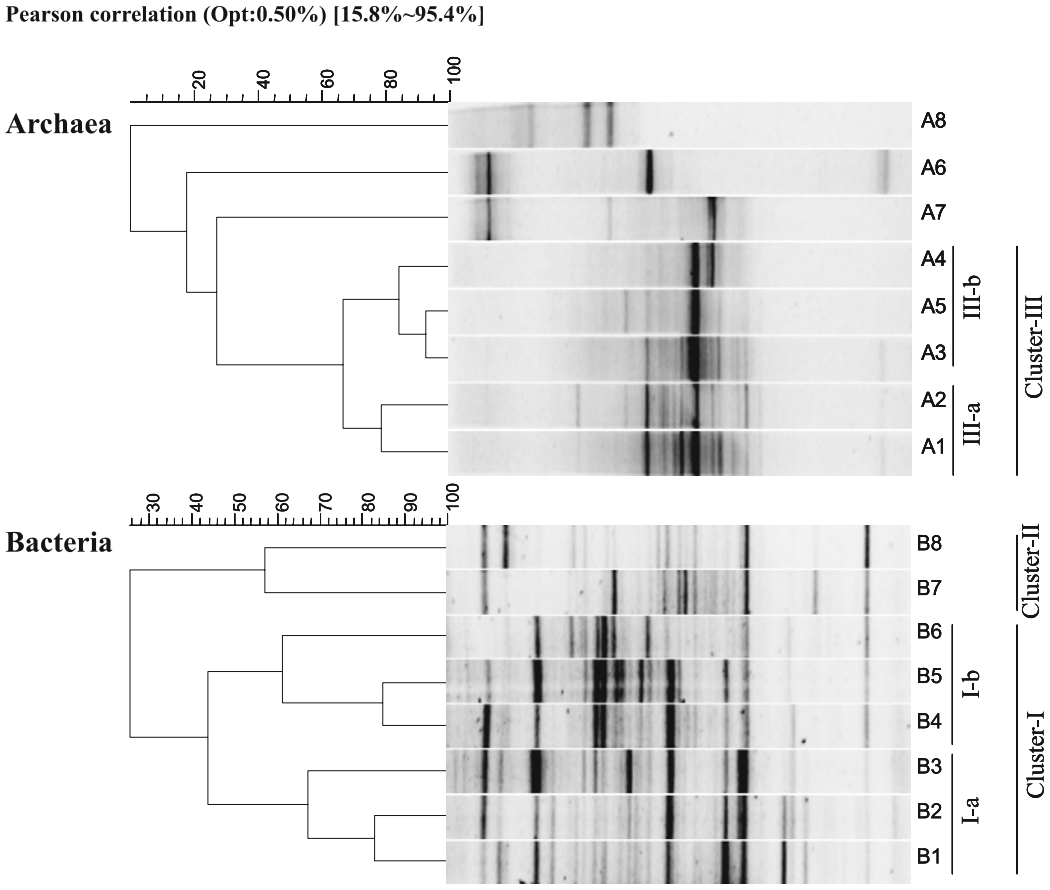

Fig. 5. Dendrogams of the bacterial and archaeal communities in the different sediment layers. These dendrograms were constructed by using Pearson's curve-based correlations and the UPGMA clustering method (BioNumerics software; BioSystematica, UK). Lanes 1, 0~3 cm; 2, 6~9 cm; 3, 12~15 cm; 4, 21~24 cm; 5, 30~33 cm; 6, 39~42 cm; 7, 48~51 cm; 8, 57~60 cm.

(H) was calculated by using the formula $H=-\Sigma Pi \cdot \ln(Pi)$, in which Pi is the importance probability of the bands in a track. H was calculated on the basis of the bands on the gel tracks, namely, on the intensities of the bands (as judged by peak heights in the densitometric curves). The importance probability (Pi) was calculated as $Pi=ni/N$, where ni is the height of the ith peak and N is the sum of all peak heights in the densitometric curve.

Results

DGGE analysis of the bacterial and archaeal communities in the sediments of Lake Hovsgol

To investigate how the microbial population in Lake Hovsgol changes with sediment depth, we subjected eight 3 cm-thick layers of the sediments (up to about 60 cm deep) to DGGE analysis using 16S rRNA gene. Although bacterial 16S rRNA genes were successfully amplified by a direct PCR step, archaeal 16S rRNA gene amplification only occurred upon nested PCR using the arch20f-958r primer set for the first round amplification step and the archGC340f-519r set for the second round amplification step. This indicates that the archaeal community in the sediments is quantitatively much smaller than the bacterial community.

The DGGE patterns revealed by the bacterial PCR products were also more diverse than those of the archaea (Fig. 3), as on average 20.8 and 9.1 DGGE bands were obtained from each sediment layer, respectively. Since the number and thickness of the bands in a DGGE profile can represent species diversity (Muyzer, 1999), the DGGE band patterns obtained from each 3 cm thick-sediment were used to calculate the Shannon index of diversity (H). This index allowed us to determine and compare the microbial diversity in each environmental niche. Figure 4 shows that H dropped with increasing sediment depth, suggesting that diversities of both bacterial and archaeal communities decreased as the sediment depth increased. In particular, the archaeal diversity index fell markedly at the 39~42 cm depth; this low diversity was also observed in the deeper sediments. It is also clear that the archaeal DGGE profiles of the deeper sediments (samples 6~8; 39~60 cm) differ from the profiles of the upper layers (Fig. 3A), as many of bands of the upper layers disappeared and small number of new bands detected. In contrast, most of the bacterial DGGE bands were observed in all layers, although a few bands did disappear with others newly appearing as depth increased (Fig. 3B). Thus, the composition of the archaeal community is more clearly affected by the depth-related environmental changes of the sediments than the bacterial community.

To measure more clearly how the microbial communities change with sediment depth, we subjected the DGGE profiles to UPGMA cluster analysis (Fig. 5). This revealed that the members of the bacterial communities in the two deepest layers (B7; 48~51 cm and B8; 57~60 cm) fall into a different cluster (Cluster II) relative to the communities in the upper layers, which make up Cluster I (B1-6; 0~42 cm). The entire densitometric curves for each lane were then compared numerically by calculating Pearson product moment correlation coefficients (r). When *r* equals 100, this indicates that the dominant species in the samples being compared are identically distributed. For the Lake Hovsgol sediments, the bacterial r values ranged from 43.77% to 84.64% (Fig. 5). This analysis revealed that the Cluster I bacterial profiles could be further subdivided into two sub-clusters, namely, Cluster I-a (B1-3; 0~15 cm) and Cluster I-b (B4-6; 21~42 cm). When the lanes within Cluster I-a or Cluster I-b were compared (intra-cluster comparisons), the r values exceeded 61.15%, which indicates very similar DGGE profiles. Moreover, these two clusters were related more closely to each other (r=43.77%) than to Cluster II (r=25.49%).

In the case of the archaea, UPGMA cluster analysis revealed that the DGGE profiles of the top five layers were very similar to each other and differed from those of the three bottom layers; the bottom three layers also did not resemble each other. As a result, the top five layers could be grouped into Cluster III while the lowest three layers formed individual groups. Calculation of the Pearson coefficients to compare the archaeal DGGE patterns revealed r values that ranged from 17.60% to 92.29% (Fig. 5). These r values allowed Cluster III to be divided into two sub-clusters, namely, Cluster III-a (A1-2; 0~9 cm) and Cluster III-b (A3-6; 12~42 cm). These observations support our observations above that suggest the depth-related environmental changes in Lake Hovsgol sediments affect the archaeal communities more severely than the bacterial communities.

Phylogenetic profiling of DGGE band clones

All DGGE bands that were obvious to the naked eye were excised from the gel, PCR-reamplified and subjected to nucleotide sequencing. Repeatedly excised bands located at the same position in the gel that showed identical nucleotide sequences were named, counted, and indicated as one representative. Thirty six sequences were obtained from bacterial DGGE bands, and twelve sequences from archaea (Fig. 3). Since some full-length bacterial sequences cannot be determined due to chimerism or co-migration of DGGE fragments (Qiu *et al.,* 2001), incomplete sequences were not used for analysis (Table 1). In conclusion, total 23 and 12 sequences were obtained from the bacterial and archaeal DGGE bands, respectively.

Comparison of the 16S rRNA gene sequences derived from DGGE bands with those in the GenBank database revealed the sequenced bacterial fragments were clustered into eight phylogenetic groups (Table 1 and Fig. 6A). These consisted of *Alpha*-Proteobacteria (YDB31), *Beta*-Proteobacteria (YDB11, 13, 14, 17, 20, 23, 24, 25, and 26), *Gamma*-Proteobacteria (YDB1, 2, 6, 8, 21, and 35), Firmicutes (YDB4 and 29), Nitrospira (YDB27 and 30), Actinobacteria (YDB33), and OP8 & 9 (YDB28 and YDB15). The Proteobacteria-related sequences were most similar to those of uncultured proteobacterial clones that had been isolated from marine sediment (YDB31) (Asami *et al.,* 2005), an uranium-contaminated aquifer (YDB14 and YDB17), deep subsurface sediment (YDB24), subseafloor (YDB26), cold-tolerant alpine soil (YDB1 and YDB6) (Meyer *et al.,* 2004), the Northern Baltic sea (YDB2) (Kisand *et al.,* 2002) and the Sub-Antarctica (YDB21). The sequences of YDB4 and YDB29 in the Firmicutes group matched those of the facultative psychrophilic low G+C Gram-positive bacterium HTA563 (100%) from deep sea mud (Takami *et al.,* 1997)

Table 1. Phylogenetic analysis of the 16S rRNA sequences of the DGGE bands obtained from the sediment layers of Lake Hovsgol (The position of each band in the DGGE gel is shown in Fig. 3.)

Clone	Accession number	Phylogenetic group	Nearest neighbor	Identity (%)	Source of the closest relative	Nearest isolate	Identity (%)
YDB1	DQ452578	Gamma	*Pseudomonas* sp. BG2dil (AY263468)	97%	alpine soil (Meyer *et al.*, 2004)	*Pseudomonas frederiksbergensis* isolate OUCZ24 (AY785733)	97%
YDB2	DQ452579	Gamma	*Pseudomonas* sp. GOBB3-207 (AF321048)	97%	estuary (Kisand *et al.*, 2002)	*Pseudomonas* sp. B65 (AF332541)	97%
YDB4	DQ452580	Firmicutes	Low G+C Gram-positive bacterium isolate HTA563 (AB002641)	100%	sea mud (Takami *et al.*, 1997)	*Staphylococcus epidermidis* (AY308046)	100%
YDB6	DQ452581	Gamma	*Pseudomonas* sp. BE3dil (AY263472)	100%	alpine soil (Meyer *et al.*, 2004)	*Pseudomonas* sp. An23 (PSP55116)	100%
YDB8	DQ452582	Gamma	*Pseudomonas* sp. NMX (AF482685)	98%	soil sediment (Coates *et al.*, 2002)	*Pseudomonas* sp. HS300 (AY940129)	98%
YDB11	DQ452583	Beta	Uncultured beta proteobacterium clone Gitt-KF-125 (AJ532686)	100%	uranium mill tailings (Selenska-Pobell, 2002)	*Janthinobacterium agaricidamnosum* (Y08845)	99%
YDB13	DQ452584	Beta	Uncultured beta proteobacterium clone AKYG404 (AY922005)	98%	farm soil (Tringe *et al.*, 2005)	*Zoogloea ramigera* (D14257)	96%
YDB14	DQ452585	Beta	Uncultured beta proteobacterium clone B-BH93 (AY622261)	98%	subsurface soil (Reardon *et al.*, 2004)	*Janthinobacterium* sp. Rud09 (AY788980)	98%
YDB15	DQ452586	OP9	Uncultured bacterium clone Amsterdam-2B-61 (AY592418)	98%	deep-sea mud volcanoes	NA	
YDB17	DQ452587	Beta	Uncultured beta proteobacterium clone C-CY80 (AY622237)	95%	subsurface soil (Reardon *et al.*, 2004)	*Janthinobacterium agaricidamnosum* (Y08845)	95%
YDB20	DQ452588	Beta	Uncultured bacterium clone 4-21 (AF351229)	98%	aquifer waters (Bakermans and Madsen, 2002)	*Dechloromonas* sp. FL9 (AF288773)	95%
YDB21	DQ452589	Gamma	*Vibrio* sp. U32 (AY864627)	96%	Sub-Antarctica	*Vibrio* sp. U32 (AY864627)	96%
YDB23	DQ452590	Beta	Uncultured bacterium clone 2-7 (AY548943)	99%	ANAMMOX sludge	*Ralstonia solanacearum* (AY216796)	97%
YDB24	DQ452591	Beta	Uncultured proteobacterium clone NANK-B57 (AY436534)	100%	marine sediment core (Newberry *et al.*, 2004)	*Ralstonia* sp. Q3-8/14 (AY216798)	100%
YDB25	DQ452592	Beta	Uncultured *Thiobacillus* sp. clone 44a-B2-21 (AY082471)	98%	mine drainage	*Thiobacillus thioparus* (M79426)	96%
YDB26	DQ452593	Beta	Uncultured bacterium clone 33-FL54B99 (AF469368)	100%	subseafloor (Huber *et al.*, 2003)	*Ralstonia* sp. 50 (AY177368)	100%
YDB27	DQ452594	Nitrospira	Uncultured bacterium clone ITKB-228 (AB198852)	98%	subsurface sediment	*Leptospirillum ferrooxidans* (X86776)	84%
YDB28	DQ452595	OP8	uncultured bacterium clone SH050-1C2 (AY781382)	100%	lake sediment	NA	
YDB29	DQ452596	Firmicutes	Unidentified bacterium clone Neu2P3-93 (AJ518357)	99%	sediment (Wobus *et al.*, 2003)	*Clostridium scatologenes* (AJ427628)	99%
YDB30	DQ452597	Nitrospira	Uncultured bacterium clone ITKB-228 (AB198852)	99%	subsurface sediment	*Leptospirillum ferrooxidans* (X86776)	84%
YDB31	DQ452598	Alpha	Uncultured alpha proteobacterium clone KY40 (AB116424)	98%	marine sediment (Asami *et al.*, 2005)	*Rhodobium bactotapetarum* (AJ510235)	94%
YDB33	DQ452599	Actinobacteria	Uncultured bacterium isolate SSDG gel band 9 (AY362838)	100%	waste water (Lee *et al.*, 2004)	*Propionibacterium acnes* (AY642054)	99%
YDB35	DQ452600	Gamma	*Pseudomonas* sp. An15 (AJ551153)	100%	deep sea sediment	*Pseudomonas* sp. An15 (AJ551153)	100%

Table 1. Continued

Clone	Accession number	Phylogenetic group	Nearest neighbor	Identity (%)	Source of the closest relative	Nearest isolate	Identity (%)
YDA1	DQ452601	Marine Benthic group C	Uncultured crenarchaeote VAL81 (AJ131315)	99%	forest lake (Jurgens *et al.*, 2000)	NA	
YDA2	DQ452602	Marine Benthic group C	Uncultured archaeon clone St_D_05 (AY531752)	99%	lake sediment	NA	
YDA3	DQ452603	Marine Benthic group C	Uncultured crenarchaeote VAL81 (AJ131315)	97%	forest lake (Jurgens *et al.*, 2000)	NA	
YDA4	DQ452604	Marine Benthic group C	Uncultured archaeon clone MKCSM-bx10 (DQ363782)	97%	mangrove soil	NA	
YDA5	DQ452605	Marine Benthic group C	Uncultured archaeon clone ARC-U3SP-10 (AY456738)	99%	soil (Castro *et al.*, 2004)	NA	
YDA6	DQ452606	Marine Benthic group C	Uncultured crenarchaeote clone X4Ar42 (AY607267)	99%	rice field soil (Lueders *et al.*, 2004)	NA	
YDA7	DQ452607	Marine Benthic group C	Uncultured archaeon clone ARC-U3SP-10 (AY456738)	95%	soil (Castro *et al.*, 2004)	NA	
YDA8	DQ452608	Group 1.1a	Uncultured archaeon clone ODP1230A22 (AB177110)	99%	marine sediment (Inagaki *et al.*, 2006)	NA	
YDA9	DQ452609	Group 1.1a	Uncultured archaeon clone 1.8mBB.27 (AY661852)	99%	marine sediment (Sorensen *et al.*, 2004)	NA	
YDA10	DQ452610	Euryarchaea	Uncultured archaeon cloneASN24 (AB161351)	100%	soil (Kasai *et al.*, 2005)	NA	
YDA11	DQ452611	Group I.1b	Uncultured Cenarchaeote clone HPA-85 (AY430142)	99%	stromatolite (Burns *et al.*, 2004)	NA	
YDA12	DQ452612	Group I.1b	Uncultured crenarchaeote clone MWS38 (AY522902)	100%	mesophilic soil (Sliwinski and Goodman, 2004)	NA	

* Organisms for which a related isolate in their own phylum could not be found on the basis of the 16S rRNA sequence are marked as NA

Table 2. The number of microorganisms belonging to the major bacterial and archaeal groups found in the sediment layers of Lake Hovsgol (Lanes 1, 0~3 cm; 2, 6~9 cm; 3, 12~15 cm; 4, 21~24 cm; 5, 30~33 cm; 6, 39~42 cm; 7, 48~51 cm; 8, 57~60 cm)

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
α-Proteobacteria	0	0	0	0	0	1	1	1
β-Proteobacteria	7	7	7	8	8	6	6	5
γ-Proteobacteria	3	3	5	5	5	4	3	3
Actinobacteria	1	1	1	1	1	1	1	1
Firmicutes	1	1	1	1	2	2	1	1
Nitrospira	2	2	2	0	0	0	0	0
OP8,9	2	2	2	2	1	0	0	0
Marine Benthic Group C	6	7	5	3	5	1	1	0
Group 1.1a	0	0	0	0	0	2	2	0
Group 1.1b	0	0	0	0	0	0	1	2
Euryarchaea	1	1	1	0	0	1	0	0

and the uncultured bacterium Neu2P3-93 (99%) from the sediment of a reservoir (this latter bacterium was closely related to *Clostridium scatologenes*). The DGGE bands of YDB27 and YDB30, which could be grouped into the phylum *Nitrospira* (≥98% similarity), were most similar in sequence to isolate of *Leptospirillum ferrooxidans* (84%); *L. ferrooxidans* is known as an iron-oxidizing bacterium that is often found in the deep underground of mines (Schrenk *et al.,* 1998). YDB15 and YDB28 resembled OP9 and OP8, respectively, which were detected in the anoxic zone of the Cariaco Basin (Madrid *et al.,* 2001) and in anaerobic sludge (Chouari *et al.,* 2005). Table 2 reveals that the diverse bacterial phylotypes are found in most of the sediment layers. However, the bacterial community did change somewhat as the depth of the sediments increased (Table 2). While most Proteobacteria were found throughout the sediments, the

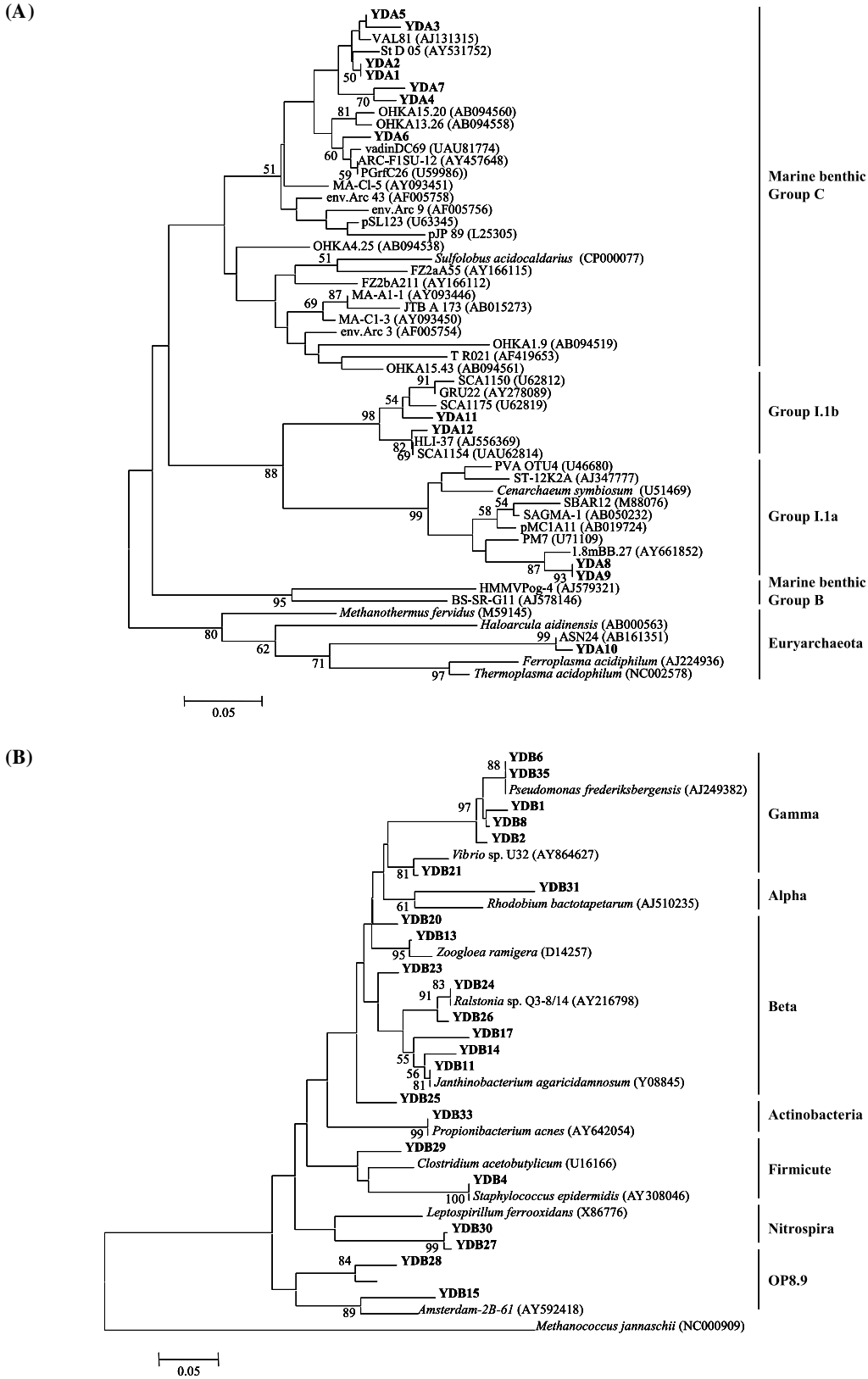

Fig. 6. Neighbor joining phylogenetic trees of the Lake Hovsgol archaea (A) and bacteria (B) that were identified on the basis of partial 16S rDNA sequences. The phylogenetic tree was constructed by using the CLUSTAL X package program as described in 'Materials and Methods'. The scale bar represents 0.05 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 100 replications) greater than 50% are shown at the branch points.

Janthinobacterium-like β-proteobacterial clones were only found in the shallower layers, the deepest being the 39-42 cm layer. In contrast, the *Zoogloea*-like *Beta*-Proteobacteria and the *Rhodobium*-like *Alpha*-Proteobacteria were not found near the surface, being first detected at 21∼24 cm and 39∼42 cm, respectively. Similarly, while the Actinobacteria were found throughout the sediments, the Nitrospira and OP clones were no longer found in sediments deeper than the 12∼15 cm and 30∼33 cm layers, respectively.

Archaeal DGGE bands were also sequenced and analyzed. The Crenarchaea formed the predominant archaeal group observed in Lake Hovsgol sediments and only one phylotype belonging to Euryarchaea (YDA10) was found. This euryarchaeal clone was grouped with the uncultured freshwater sediment archaeon ASN24 (100%), which was isolated from petroleum-contaminated soil (Kasai *et al.,* 2005). With regard to the Crenarchaea, the YDA1, 2, 3, 4, 5, 6, and 7 clones were grouped into marine benthic Crenarchaea group C, while YDA8 and 9 were grouped into marine Crenarchaea group 1.1a and YDA11 and 12 were grouped in the soil Crenarchaea group 1.1b (Table 1 and Fig. 6A). Unlike the bacterial community, however, each band was found in only a limited number of sediment samples rather than in all or many of them (Table 2). In addition, each phylogenetic group was found at a particular depth (Table 2). For example, while the marine benthic group C Crenarchaea predominated in the sediments, this group gradually became less frequently detected as the depth decreased. In addition, while the Group 1.1a and 1.1b Crenarchaea found at the depth of 39∼42 cm, the Euryarchaea disappeared.

Discussion

Lake Hovsgol is 2.5∼4.0 Ma (million years) old (Goulden *et al.,* 2003) and has never been subjected to anthropogenic stress (Belykh *et al.,* 2005). Analyses of its sediment layers have revealed that consecutive paleoclimatic transitions have generated different environmental niches. Lake Hovsgol was glaciated during the LGM of the Pleistocene, at which point the level of the lake was 200 m lower than it is at present (Fedotov *et al.,* 2002). During this period, the water of the lake did not run out and water circulation did not occur. The low water level and volume during the LGM drastically changed the lake water chemistry as it elevated the carbonate concentrations (Karabanov *et al.,* 2004). Such high salinity, along with the sudden change of climate, the cold temperatures, and limited water circulation, markedly reduced the productivity of the organisms living in the lake. The water level and circulation in the lake recovered around 12 ^{14}C ka (about 12,000 years ago, as determined by radiocarbon C^{14} dating), at which point the ecosystem of the lake is believed to have regenerated (Prokopenko *et al.,* 2003).

Nutrient availability is a primary factor that can change the microbial communities in lake sediments, as Parkes *et al*. (2005) have revealed that high levels of organic matter in the sediment greatly stimulates prokaryotic activity and results in highly diverse prokaryotic populations. In Lake Hovsgol during the LGM, the environmental conditions resulted in a marked decline in nutrient availability. This is revealed by measurements of the chlorophyll-*a* concentrations in the sediments; Nara *et al.* (2005) found that these concentrations were 10-fold higher in the upper sediments of Lake Hovsgol than in its >50 cm sediments. This change was paralleled by decreases in total organic carbon, total nitrogen, biogenic silica concentrations, planktonic diatom numbers, and benthic diatom numbers (Karabanov *et al.,* 2004), which indicates that macro-organisms like diatoms largely disappeared during the LGM and only returned at a depth of about 50 cm. Significantly, the 40∼50 cm deep Lake Hovsgol sediments have been estimated to be 12,000 years old (Fedotov *et al.,* 2004); this is exactly the border between the Holocene (present age) and Pleistocene (the last glacial age).

In our study, we found the microbial communities in the Lake Hovsgol sediments are much less diverse in terms of both bacteria and archaea in the 39∼60 cm sediment layers (as estimated by the Shannon index) compared to the communities in the upper layers (Fig. 4). In particular, the archaeal communities in the 0∼33 cm layers were almost twice diverse than the communities found in the 39∼60 cm layers. UPGMA analysis of the DGGE profiles of the sediment communities also showed that both prokaryotic communities could be separated at the border between Holocene and Pleistocene that is represented by the 39∼42 cm layer. This suggests that the phylogenetic analysis of DGGE clones from Lake Hovsgol sediments reveals the existence of a fairly diverse and stratified bacterial and archaeal community, and the different nutrient concentrations in the sediment strata, which were shaped by paleoclimatic events, may modulate the diversity of the microbial communities currently living in the sediment strata of Lake Hovsgol.

The 16S rRNA gene sequences retrieved from the DGGE bands allowed us to partially identify the microorganisms in the various depth sediments and to determine their phylogenetic affiliations. With regard to the bacterial community in the Lake Hovsgol sediments, it generally resembled the communities found in other lakes with similar habitats. However, although *Delta*-Proteobacteria and CFB bacteria are frequently detected in the sediments of other lakes (Glockner *et al.,* 2000; Humayoun *et al.,* 2003; Demergasso *et al.,* 2004), they were not detected in the sediments of Lake Hovsgol. Phylogenetic analysis of the archaeal 16S rRNA sequences obtained from Lake Hovsgol sediments revealed two groups, namely, euryarchaea and uncultured crenarchaea (Fig. 6). These crenarchaeal and euryarchaeal groups have also been reported to be present in the sediments of other lakes. Although we tried to detect methanogen using PCR with specific primers (Marchesi *et al.,* 2001), we could not detect the presence of methanogen (data not shown), which suggests that anaerobic digestion coupled with sulfate reduction and methane production may not occur in Lake Hovsgol sediments. According to the study of Fedotov *et al.* (2004), the sediments of Lake Hovsgol are anoxic from just below the surface and contain many metals; in particular, P, Mn, Fe, and Ba levels increase from the bottom to the surface. It is possible that these elements are reduced in the organic matter-rich sediments into their ionic forms and become precipitated in the oxygenated zones near the surface. That metal elements such as Fe increase

in concentration as the sediments get closer to the surface may also be related to the presence of bacteria belonging to the genus *Leptospirillum*. These iron-oxidizing bacteria occupy the upper sediments (0∼15 cm) of Lake Hovsgol (Table 1 and Fig. 3B). In addition, *Rhodobium*, which is capable of photosynthetic hydrogen production, was absent from the sediments overlying the 39∼42 cm layer while *Janthinobacterium*, a nitrate reducer, is only present in this layer and the sediments above it. The appearance of *Leptospirillum* and *Janthinobacterium* in the upper sediments is probably related to changed nutrient levels in the sediments. Thu, the climatic changes that took place between the Holocene and Pleistocene promoted the bloom of primary producers such as diatoms and consequently altered the microbial communities in the sediments and increased their growth.

DGGE method can clearly indicate the existence of common but potentially uncultivated groups of microorganisms in natural environment, it does not permit the more precise identification of these microbes to the species or sub-species level (Table 1 and Fig. 6). Moreover, we cannot determine from the partial sequence information yielded by DGGE how the microorganisms in the lake sediments function or interact. A more comprehensive understanding of the physiology of these organisms and their complex biogeochemical processes will require their cultivation, isolation and characterization (Torsvik *et al.,* 2002; Zengler *et al.,* 2002; Bae *et al.,* 2005).

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References

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.

Asami, H., M. Aida, and K. Watanabe. 2005. Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. *Appl. Environ. Microbiol.* 71, 2925-2933.

Bae, J.W., S.K. Rhee, J.R. Park, B.C. Kim, and Y.H. Park. 2005. Isolation of uncultivated anaerobic thermophiles from compost by supplementing cell extract of *Geobacillus toebii* in enrichment culture medium. *Extremophiles* 9, 477-485.

Bakermans, C. and E.L. Madsen. 2002. Diversity of 16S rDNA and naphthalene dioxygenase genes from coal-tar-waste-contaminated aquifer waters. *Microb. Ecol.* 44, 95-106.

Belykh, O.I., E.G. Sorokovikova, I.V. Tikhonova, and A.F. Fedotov. 2005. Abundance, morphological diversity, and spatial distribution of autotrophic picoplankton in Lake Hovsgol (Mongolia). *Aquat. Ecosyst. Health Manage.* 8, 461-473.

Burns, B.P., F. Goh, M. Allen, and B.A. Neilan. 2004. Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. *Environ. Microbiol.* 6, 1096-1101.

Castro, H., A. Ogram, and K.R. Reddy. 2004. Phylogenetic characterization of methanogenic assemblages in eutrophic and oligotrophic areas of the Florida Everglades. *Appl. Environ. Microbiol.* 70, 6559-6568.

Chan, O.C., P. Claus, P. Casper, A. Ulrich, T. Lueders, and R. Conrad. 2005. Vertical distribution of structure and function of the methanogenic archaeal community in Lake Dagow sediment. *Environ. Microbiol.* 7, 1139-1149.

Chouari, R., D. Le Paslier, P. Daegelen, P. Ginestet, J. Weissenbach, and A. Sghir. 2005. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ. Microbiol.* 7, 1104-1115.

Coates, J.D., K.A. Cole, R. Chakraborty, S.M. O'Connor, and L.A. Achenbach. 2002. Diversity and ubiquity of bacteria capable of utilizing humic substances as electron donors for anaerobic respiration. *Appl. Environ. Microbiol.* 68, 2445-2452.

DeLong, E.F. 1992. Archaea in coastal marine environments. *Proc. Natl. Acad. Sci. USA* 89, 5685-5689.

DeLong, E.F., L.T. Taylor, T.L. Marsh, and C.M. Preston. 1999. Visualization and enumeration of marine planktonic archaea and bacteria by using polyribonucleotide probes and fluorescent in situ hybridization. *Appl. Environ. Microbiol.* 65, 5554-5563.

Demergasso, C., E.O. Casamayor, G. Chong, P. Galleguillos, L. Escudero, and C. Pedros-Alio. 2004. Distribution of prokaryotic genetic diversity in athalassohaline lakes of the Atacama Desert, Northern Chile. *FEMS Microbiol. Ecol.* 48, 57-69.

Fedotov, A.P., E.P. Chebykin, S.M. Yu, S.S. Vorobyova, O.E. Yu, L.P. Golobokova, T.V. Pogodaeva, T.O. Zheleznyakova, M.A. Grachev, D. Tomurhuu, T. Oyunchimeg, T. Narantsetseg, O. Tomurtogoo, P.T. Dolgikh, M.I. Arsenyuk, and M. De Batist. 2004. Changes in the volume and salinity of Lake Khubsugul (Mongolia) in response to global climate changes in the upper Pleistocene and the Holocene. *Palaeogeogr. Palaeoclim. Palaeoecol.* 209, 245-257.

Fedotov, A.P., M. De Batist, E. Shapron, K. De Reiker, T. Paule, and M.A. Grachev. 2002. Seismic survey of sediments of Lake Hovsgol. *Doklady of the Russian Academy of Sciences* 382, 261-263 (in Russian).

Ferris, M.J., G. Muyzer, and D.M. Ward. 1996. Denaturing gradient gel electrophoresis profiles of 16S rRNA-defined populations inhabiting a hot spring microbial mat community. *Appl. Environ. Microbiol.* 62, 340-346.

Glockner, F.O., E. Zaichikov, N. Belkova, L. Denissova, J. Pernthaler, A. Pernthaler, and R. Amann. 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl. Environ. Microbiol.* 66, 5053-5065.

Goulden, C.E., O. Tumurtogoo, and E. Karabanov. 2003. The geological history and geography of Lake Hovsgol, Mongolia. The Geology, Biodiversity and Ecology of Lake Hovsgol (Mongolia).

Hastings, R.C., J.R. Saunders, G.H. Hall, R.W. Pickup, and A.J. McCarthy. 1998. Application of molecular biological techniques to a seasonal study of ammonia oxidation in a eutrophic freshwater lake. *Appl. Environ. Microbiol.* 64, 3674-3682.

Henckel, T., M. Friedrich, and R. Conrad. 1999. Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. *Appl. Environ. Microbiol.* 65, 1980-1990.

Huber, J.A., D.A. Butterfield, and J.A. Baross. 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiol. Ecol.* 43, 393-409.

Hugenholtz, P. 2002. Exploring prokaryotic diversity in the genomic era. *Genome Biol.* 3:REVIEWS0003.

Humayoun, S.B., N. Bano, and J.T. Hollibaugh. 2003. Depth distribution of microbial diversity in mono lake, a meromictic soda lake in California. *Appl. Environ. Microbiol.* 69, 1030-1042.

Inagaki, F., T. Nunoura, S. Nakagawa, A. Teske, M. Lever, A. Lauer, M. Suzuki, K. Takai, M. Delwiche, F.S. Colwell, K.H. Nealson, K. Horikoshi, S. D'Hondt, and B.B. Jorgensen. 2006. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. USA* 103, 2815-2820.

Jurgens, G., F. Glockner, R. Amann, A. Saano, L. Montonen, M. Likolammi, and U. Munster. 2000. Identification of novel Archaea in bacterioplankton of a boreal forest lake by phylogenetic analysis and fluorescent *in situ* hybridization (1). *FEMS Microbiol. Ecol.* 34, 45-56.

Karabanov, E., D. Williams, G. Khursevich, A. Prokopenko, M. Kuzmin, L. Tkachenko, E. Kerber, A. Gvozdkov, V. Sideleva, S. Fedenya, E. Solotchina, O. Khlustov, E. Bezrukova, P. Letunova, and S. Krapivina. 2004. Ecological collapse of Lake Baikal and Lake Hovsgol ecosystems during the Last Glacial and consequences for aquatic species diversity. *Palaeogeogr. Palaeoclim. Palaeoecol.* 209, 227-243.

Kasai, Y., Y. Takahata, T. Hoaki, and K. Watanabe. 2005. Physiological and molecular characterization of a microbial community established in unsaturated, petroleum-contaminated soil. *Environ. Microbiol.* 7, 806-818.

Kisand, V., R. Cuadros, and J. Wikner. 2002. Phylogeny of culturable estuarine bacteria catabolizing riverine organic matter in the northern Baltic Sea. *Appl. Environ. Microbiol.* 68, 379-388.

Koizumi, Y., H. Kojima, and M. Fukui. 2003. Characterization of depth-related microbial community structure in lake sediment by denaturing gradient gel electrophoresis of amplified 16S rDNA and reversely transcribed 16S rRNA fragments. *FEMS Microbiol. Ecol.* 46, 147-157.

Kozlov, U.P., V.V. Vorobiev, D. Batsuur, O.M. Kozhova, O. Shagdarsyren, A. Dashdorzh, N. Sodnom, B.A. Bogoyavlenskii, V.P. Martinov, and B. Batchargal. 1989. *Atlas of Lake Hubsugul*.

Lee, S.Y., H.G. Kim, J.B. Park, and Y.K. Park. 2004. Denaturing gradient gel electrophoresis analysis of bacterial populations in 5-stage biological nutrient removal process with step feed system for wastewater treatment. *J. Microbiol.* 42, 1-8.

Li, J.H., S. Takii, K.J. Purdy, and H. Hayashi. 1999. Seasonal changes in ribosomal RNA of sulfate-reducing bacteria and sulfate reducing activity in a freshwater lake sediment. *FEMS Microbiol. Ecol.* 28, 31-39.

Lueders, T., B. Pommerenke, and M.W. Friedrich. 2004. Stable-isotope probing of microorganisms thriving at thermodynamic limits: syntrophic propionate oxidation in flooded soil. *Appl. Environ. Microbiol.* 70, 5778-5786.

Madrid, V.M., G.T. Taylor, M.I. Scranton, and A.Y. Chistoserdov. 2001. Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the Cariaco Basin. *Appl. Environ. Microbiol.* 67, 1663-1674.

Marchesi, J.R., A.J. Weightman, B.A. Cragg, R.J. Parkes, and J.C. Fry. 2001. Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. *FEMS Microbiol. Ecol.* 34, 221-228.

Meyer, A.F., D.A. Lipson, A.P. Martin, C.W. Schadt, and S.K. Schmidt. 2004. Molecular and metabolic characterization of cold-tolerant alpine soil *Pseudomonas* sensu stricto. *Appl. Environ. Microbiol.* 70, 483-489.

Muyzer, G. 1999. DGGE/TGGE a method for identifying genes from natural ecosystems. *Curr. Opin. Microbiol.* 2, 317-322.

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

Nara, F., H. Naraoka, T. Watanabe, Y. Tani, Y. Soma, M. Soma, K. Horiuchi, T. Kawai, T. Oda, and T. Nakamura. 2005. Response of phytoplankton productivity to climate change recorded by sedimentary photosynthetic pigments in Lake Hovsgol (Mongolia) for the last 23,000 years. *Quat. Int.* 136, 71-81.

Newberry, C.J., G. Webster, B.A. Cragg, R.J. Parkes, A.J. Weightman, and J.C. Fry. 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, Ocean Drilling Program Leg 190. *Environ. Microbiol.* 6, 274-287.

Ovreas, L., L. Forney, F.L. Daae, and V. Torsvik. 1997. Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl. Environ. Microbiol.* 63, 3367-3373.

Parkes, R.J., G. Webster, B.A. Cragg, A.J. Weightman, C.J. Newberry, T.G. Ferdelman, J. Kallmeyer, B.B. Jorgensen, I.W. Aiello, and J.C. Fry. 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geological time. *Nature* 436, 390-394.

Priscu, J.C., C.H. Fritsen, E.E. Adams, S.J. Giovannoni, H.W. Paerl, C.P. McKay, P.T. Doran, D.A. Gordon, B.D. Lanoil, and J.L. Pinckney. 1998. Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science* 280, 2095-2098.

Prokopenko, A.A., M.I. Kuzmin, G.V. Kalmuchkov, V.F. Geletiy, A.N. Gvozdkov, and P.A. Solotchin. 2003. Changes in the composition of bottom sediments of Lake Hovsgol as an indication of climatics in the Baikal region 5-14 Ka BP. *Doklady Akademii Nauk, Proceedings of Academy of Sciences of Russia* 390, 109 (In Russian).

Qiu, X., L. Wu, H. Huang, P.E. McDonel, A.V. Palumbo, J.M. Tiedje, and J. Zhou. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.* 67, 880-887.

Reardon, C.L., D.E. Cummings, L.M. Petzke, B.L. Kinsall, D.B. Watson, B.M. Peyton, and G.G. Geesey. 2004. Composition and diversity of microbial communities recovered from surrogate minerals incubated in an acidic uranium-contaminated aquifer. *Appl. Environ. Microbiol.* 70, 6037-6046.

Rondon, M.R., R.M. Goodman, and J. Handelsman. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. *Trends Biotechnol.* 17, 403-409.

Schrenk, M.O., K.J. Edwards, R.M. Goodman, R.J. Hamers, and J.F. Banfield. 1998. Distribution of thiobacillus ferrooxidans and leptospirillum ferrooxidans: implications for generation of acid mine drainage. *Science* 279, 1519-1522.

Selenska-Pobell, S., K. Flemming, T. Tzvetkova, J. Raff, M. Schnorpfeil, and A. Geissler. 2002. Bacterial communities in uranium mining waste piles and their interactions with heavy metals, p. 455-464. *In* B.J. Merkel, B. Planer-Friedrich, and C. Wolkersdorfer (eds.), Uranium in the aquatic environment. Springer-Verlag, Berlin, Germany.

Sliwinski, M.K. and R.M. Goodman. 2004. Spatial heterogeneity of crenarchaeal assemblages within mesophilic soil ecosystems as revealed by PCR-single-stranded conformation polymorphism profiling. *Appl. Environ. Microbiol.* 70, 1811-1820.

Sorensen, K.B., A. Lauer, and A. Teske. 2004. Archaeal phylotypes in a metal-rich and low-activity deep subsurface sediment of the Peru Basin, ODP Leg 201, Site 1231. *Geobiology* 2, 151-161.

Spring, S., R. Schulze, J. Overmann, and K. Schleifer. 2000. Identification and characterization of ecologically significant prokaryotes in the sediment of freshwater lakes: molecular and cultivation studies. *FEMS Microbiol. Rev.* 24, 573-590.

Staley, J.T. and A. Konopka. 1985. Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu. Rev. Microbiol.* 39, 321-346.

Takami, H., A. Inoue, F. Fuji, and K. Horikoshi. 1997. Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS*

Microbiol. Lett. 152, 279-285.

Tamaki, H., Y. Sekiguchi, S. Hanada, K. Nakamura, N. Nomura, M. Matsumura, and Y. Kamagata. 2005. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. *Appl. Environ. Microbiol.* 71, 2162-2169.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, 4876-4882.

Torsvik, V., L. Ovreas, and T.F. Thingstad. 2002. Prokaryotic diversity-Magnitude, dynamics, and controlling factors. *Science* 296, 1064-1066.

Tringe, S.G., C. Von Mering, A. Kobayashi, A.A. Salamov, K. Chen, H.W. Chang, M. Podar, J.M. Short, E.J. Mathur, J.C. Detter, P. Bork, P. Hugenholtz, and E.M. Rubin. 2005. Comparative metagenomics of microbial communities. *Science* 308, 554-557.

Urakawa, H., T. Yoshida, M. Nishimura, and K. Ohwada. 2000. Characterization of depth-related population variation in microbial communities of a coastal marine sediment using 16S rDNA-based approaches and quinone profiling. *Environ. Microbiol.* 2, 542-554.

Wobus, A., C. Bleul, S. Maassen, C. Scheerer, M. Schuppler, E. Jacobs, and I. Roske. 2003. Microbial diversity and functional characterization of sediments from reservoirs of different trophic state. *FEMS Microbiol. Ecol.* 46, 331-347.

Yeates, C., M.R. Gillings, A.D. Davison, N. Altavilla, and D.A. Veal. 1998. Methods for microbial DNA extraction from soil for PCR amplification. *Biol. Proced. Online* 1, 40-47.

Zengler, K., G. Toledo, M. Rappe, J. Elkins, E.J. Mathur, J.M. Short, and M. Keller. 2002. Cultivating the uncultured. *Proc. Natl. Acad. Sci. USA* 99, 15681-15686.