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ORIGINAL PAPER

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Bacterial community along a historic lake sediment core of Ardley Island, west Antarctica

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Abstract The bacterial community in a historic lake sediment core of Ardley Island, Antarctica, spanning approximately 1,600 years, was investigated by molecular approaches targeting the 16S rRNA gene fragments. The cell number in each 1 cm layer of the sediment core was deduced through semi-quantification of the 16S rRNA gene copies by quantitative competitive PCR (QC-PCR). It was found that the total bacterial numbers remained relatively stable along the entire 59 cm sediment core. Denaturing Gradient Gel Electrophoresis (DGGE) analysis and sequencing of PCR-amplified 16S rRNA gene fragments were performed to analyze the bacterial diversity over the entire column. Principle coordinates analysis suggested that the bacterial communities along the sediment core could be separated into three groups. There were obvious bacterial community shift among groups of 1–20 cm, 21–46 cm and 46–59 cm. Diversity indices indicated that the bacterial community in the 21–46 cm depth showed the highest species diversity and uniformity. The main bacterial groups in the sediments fell into 4 major lineages of the gram-negative bacteria: the α, γ and δ subdivision of *Proteobacteria*, the *Cytophaga-Flavobacteria-Bacteroides*, and some unknown sequences. The gram-positive bacteria *Gemmatimonadetes*, *Firmicutes* and *Actinobacteria* were also detected. The results demonstrated the presence of highly diverse bacterial community population in the Antarctic lake sediment core. And the possible influence of climate and penguin population change on the bacterial community shift along the sediment core was discussed.

Keywords Antarctica · Bacterial community · DGGE · Lake sediment

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Introduction

Bacterial populations are the major contributors in substance cycling on earth, such as the transformation of organic carbon, including sulfate and iron reduction, CO_2 fixation, glucose mineralization, amino acid uptake (Isaksen and Jorgensen 1996; Kostka et al. 1999; Tan and Ruger 1989; Vetter and Deming 1994). Culture-independent molecular methods have greatly enhanced our knowledge on microbial diversity as it has been revealed that majority of the microbes in environments retrieved by molecular methods could not or have not been cultivated in laboratory (Amann et al. 1995). The microbial communities in several different Antarctic environments have been investigated such as in Antarctic continental shelf sediments (Bowman et al. 2003), glacial meltwater lake sediment (Sjoling and Cowman 2003) and Antarctic marine benthos (Reichardt 1987). However, the microbial diversity in Ardley Island, Antarctica, which is situated at about 500 m east of the coast of Fildes Peninsula, King George Island, and defined as one of the sites of special research interests by the Scientific Committee on Antarctic Research (SCAR), has not been investigated yet.

On Ardley Island, Antarctica, large numbers of breeding penguins live. In the breeding period, it is estimated that penguins on the Ardley Island discharged about 139 tons of droppings and some of them were deposited in the Y2 lake as transferred by ice or snow-melt water (Sun et al. 2000; Sun and Xie 2001). By analyzing the geochemical elements in a sediment core

collected from the Y2 lake, it was found that the deposition of penguin droppings had a significant effect on the geochemical composition of the sediment core. Changes in sediment geochemistry reflected the fluctuations in penguin numbers and it was suggested that variations in climate had an impact on penguin populations (Sun et al. 2000, 2004b). Sun et al. (2004a) also studied the seal population changes during the past 1,500 years using the above biogeochemical method. Further molecular biological investigation by measuring the chitinase gene copy numbers in a lake sediment core Y2-4 suggested that the fluctuation of chitinolytic bacterial number correlated significantly with the geochemical elements (Xiao et al. 2005). However, the microbial diversity in the sediment core is still unknown, the impacts of the climate and the penguin droppings on the microbial community of the lake sediment need to be studied further.

In this study, the bacterial diversity in the lake sediment core Y2-4 was investigated by 16S rRNA gene sequence analysis. The bacterial communities in each 1 cm section of the sediment core were determined by Denaturing Gradient Gel Electrophoresis and sequencing. This is a beginning to understand the microbial communities in the lake sediment of Ardley Island, Antarctica.

Materials and methods

Sample collection and description

The lake sediment core Y2-4 in Ardley Island (62°13°S, 58°54°W), spanning around 1,600 years (Xiao et al. 2005), was used in this study. Core Y2-4 [near Y2 core (Sun et al. 2000)], 59.5 cm in length, was taken by driven a clean PVC pipe with 12 cm diameter into the soft substrate of the lake floor on Ardley Island during the 18th Chinese Antarctic Research Expedition. The sediments were stored at −20°C during the shipping process and in lab until been sectioned at 1.0 cm interval in clean bench. The inner part of each section was transferred to a separate sterile falcon tube for microbial study to avoid contaminations.

DNA extraction

Sediment DNA extraction was carried out by the method of soil DNA extraction with some modifications (Wang et al. 2004). The sediment core samples were suspended in artificial seawater (ASW; containing 3.0 g NaCl, 0.7 g KCl, 5.3 g $MgSO_4 \cdot 7H_2O$, 10.8 g $MgCl_2 \cdot 6H_2O$, 1.0 g $CaSO_4 \cdot 2H_2O$ per liter), vortexed and centrifuged for 5 min at 150*g* under 4°C. The suspension and centrifugation step was repeated three times and the supernatant was collected together and maintained on ice until centrifuged again at 15,000*g*, 4 C for 30 min to concentrate the microbes. The microbial pellets were collected to extract DNA using Soil DNA Isolation Kit (Mobio Inc., Solana Beach, CA, USA) followed by manufacture's instruction.

PCR and Quantification of bacteria in the sediment by QC-PCR

Bacteria primers: Eubf993 (5′-GCACAAGCGGTGGA GCATGTGG) and Eubr1387 (5′-GCCCGGGAACGT ATTCACCG) and archaeal-specific primers: Arch21F (TTCCGGTTGATCCYG CCGGA) and Arch958R (YCCGGCGTTGAMTCCAATT) (DeLong, 1992; Kawai et al. 2002) synthesized by Sangon Inc., Shanghai, China were used to amplify about 450 and 900 bp of bacterial and archaeal 16S rRNA gene fragments. PCR reaction system included 5 μl 10 × PCR buffer, 4 μl dNTPs (2 mM), 0.5 μl of each primer (10 μM), ~ 20 ng template and 2 U *Taq* polymerase in 50 μl reaction system. The thermal cycle was taken in a T3 thermal cycler (Biometra, Goettingen, Germany) with PCR condition: 95°C 5 min—95°C 60 s, 55°C 40 s, 72°C 60 s, 35cycles—72°C 10 min.

The bacterial numbers in the sediment core were estimated by quantification of the 16S rRNA gene copies using quantitative competitive PCR (QC-PCR) method as described by Wang et al. (2005). In brief, the 450 bp partial 16S rRNA gene fragment located at 933–1387 region of 1.5 kb 16S rRNA gene was amplified with the primer Eubf933 and Eubr1387 from a bacteria strain WP8, cloned into the pGEM-T vector to form plasmid pE106 (Wang et al. 2005), then pE106 was digested with *Bam*HI and used as the positive control. The competitor DNA was got from the linear plasmid pE106, digested with *Kpn*2I to get 300 bp deletion in the region between the PCR primer pair f933 and r1387, self-ligated, and then digested with *Bam*HI. The quantities of pE106 and pE106 *Δ* 300 bp were determined by using spectrophotometer (Ultrospec 2100, Amersham pharmacia).

PCR-DGGE analysis

The PCR-DGGE procedure including PCR amplification of 16S rRNA gene fragment using GC-clamp PCR primers (Kawai et al. 2002), DGGE gel running, DGGE band recovery, cloning, and sequencing (Sangon Inc., Shanghai, China) was carried out as described by Wang et al. (2005). The electrophoresis conditions for DGGE were optimized for this study. The gel was run at 60°C for 30 min at 30 V, and subsequently for 12 h at 100 V. The cloned bands were named as AC01 to AC35.

Data analysis and phylogenetic tree construction

The 16S rRNA gene sequences recovered from this study were first checked for chimeras by using the Ribosomal Database Project's CHECK-CHIMERA program. The

validated sequences were blasted in the DDBJ data bank to search for related sequences. Principle coordinates analysis (PCA) was used to investigate the variation (presence/absence of bands) in the DGGE banding patterns. The diversity and evenness indices were conducted as described (Stach et al. 2003; Yan et al 2003).

Phylogenetic tree was constructed from a matrix of pairwise genetic distances by the maximum-parsimony algorithm and the neighbor joining method using the program DNAMAN (version 5.1; Lynnon, Biosoft), and 1,000 trials of bootstrap analysis was used to provide confident estimates for phylogenetic tree topologies.

Nucleotide sequence accession numbers

Thirty five nucleotide sequences have been deposited in the GenBank database under accession numbers from AJ582232 to AJ582266 (AC01 to AC35).

Results

Bacterial quantification in the sediment core

No PCR band was obtained from all the 59 sediment layer samples using the archael-specific primers, whereas specific PCR amplification was successful using the bacterial-specific primers (photos not shown). This indicated that the microbial community in the Y2-4 lake sediment core was exclusively composed of bacteria. The bacterial numbers in each 1 cm section of the sediment core were deduced from the number of 16S rRNA gene copies determined by QC-PCR as described in the Materials and methods. The 16S rRNA gene copy number per gram soil kept relatively stable in the whole sediment core within the range from 10^7 to 10^8 copies. The TOC contents in the sediment core fluctuated significantly (Xiao et al. 2005), the little fluctuation of the 16S rRNA gene copies did not correlate with the TOC contents of the sediment core ($r = 0.25$, $n = 59$), suggesting that the input of organic carbon had little effects on the total cell number in the Y2-4 sediment core.

DGGE profiles

The optimized DGGE profile was applied to obtain the distinct DGGE bands from complex samples (examples see Fig. 1). DGGE bands in each 1 cm layer were recorded and cloned for sequencing (Table 1). The bands in each 5 cm of the sediment core were pooled and the band patterns were analyzed by PCA (Figs. 2, 3). PCA analysis produced the plots in which the entries are spread according to their relatedness. On the PCA plots, the DGGE band patterns were clustered separately into three groups. There were two community shifts between layers 20 and 21 cm (Fig. 2, points 4–5) and between 45 and 46 cm (Fig. 2, points 9–10). The band patterns in the depth of 1–20 cm always tightly clustered (Fig. 2, points 1–4); and within the depth of 21–45 cm (Fig. 2, points 5–9) and 46–59 cm (Fig. 2, points 10–12), the DGGE patterns were relatively similar and the samples were clustered together on the PCA plots.

Among the three groups, the 21–45 cm community displayed highest diversity and evenness (Table 1). According to Zhou's suggestion (Zhou et al. 2002), that value for 1/D below about 50 indicate typical dominance profiles, the 1–20 cm and 46–59 cm communities showed dominance, whereas the 21–45 cm community had more evenly distributed species than the other two communities. The 1–20 cm and 46–59 cm community had similar diversity, evenness and richness indices (Table 1).

Bacterial communities as revealed by DGGE band sequences

Thirty-five DGGE bands were excised from the gels, cloned and sequenced as described above. Analysis of the band sequences revealed that the main bacteria in the sediment core were composed of α, γ and δ subdivision of *Proteobacteria*, *Cytophaga-Flavobacteria-Bacteroides*, *Gemmatimonadetes*, *Firmicutes*, *Actinobacteria* of gram-positive bacteria, and some unknown sequences (Table 2). Most of the sequences had highest similarities to those of uncultured organisms obtained from lakes, sea, mining waster, soil and glacier (Table 2). *Proteobacteria*, particularly γ-subdivision of *Proteobacteria* were abundant in the top layers of sediment. The γ-*Proteobacteria* detected were *Pseudomonas* sp., *Acinetobacter* sp. and *Escherichia coli*. Clones which were closely related with *uncultured Desulfocapsa* and their relatives of δ-*Proteobacteria* were only detected in the middle layers (Table 2). *Cytophaga-Flavobacteria-Bacteroides* were widely distributed in the sediment core except in the last 14 layers. *Gemmatimonadetes*, *Firmicutes*, *Actinobacteria* of gram-positive bacteria were present in the entire sediment column. Ten of the 35 clones which had low similarities to any known sequences in database scattered in the layers. The cloned sequences together with their nearest neighbors in the GenBank were presented in Table 2, and a phylogenetic tree showing the relationship of the clones with their related reference sequences was shown in Fig. 2.

Discussion

The Ardley Island, Antarctica, is one of the sites of special scientific interest defined by SCAR. The site has the largest population of Gentoo penguins (*Pygoscelis papua*) within the South Shetland Island (Sun and Xie 2001). The penguins discharged large amount of droppings which served as the main nutrient source on the island. The lake sediment core Y2-4 used in this study has been intensively affected by penguin droppings (Xiao et al. 2005). Here, the bacterial communities in

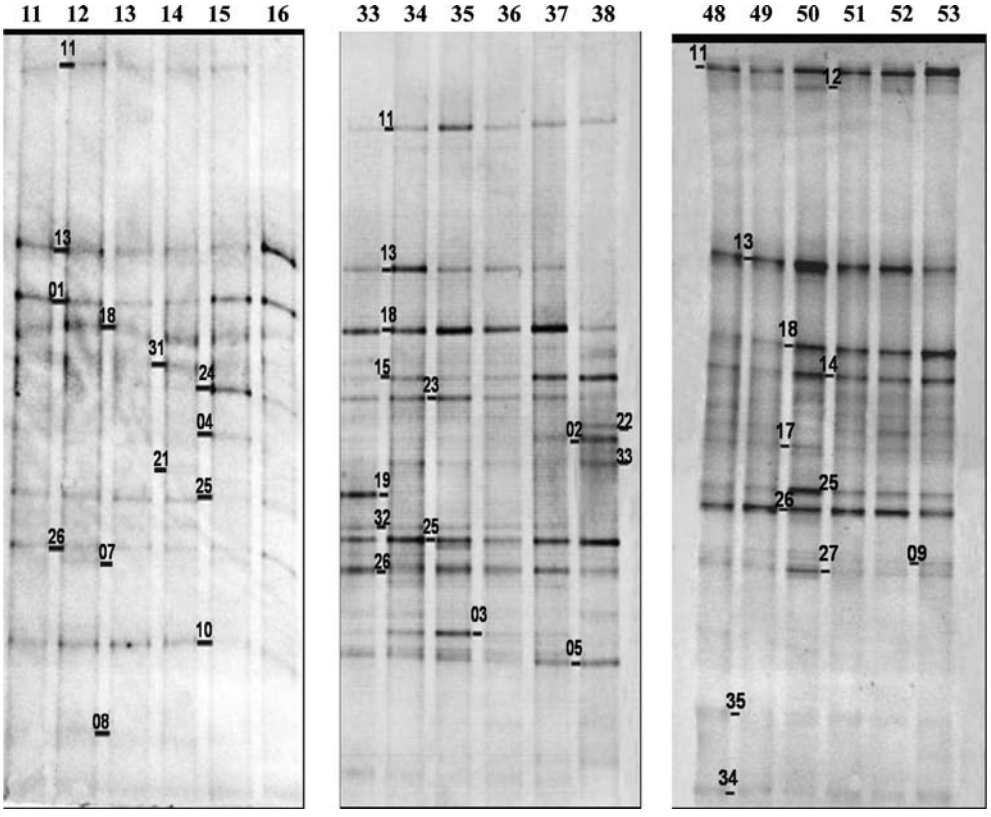

Fig. 1 The DGGE profile of the selected samples from lake sediments in Ardley Peninsula, Antarctic. DGGE profiles from sediment layers 11–16 cm, 33–38 cm and 48–53 cm depth were shown as examples. *Lane number* represents the sediment layer. The DNA bands which were excised from the gel, cloned and sequenced were indicated. The number of the bands corresponds to that of the clones showed in Table 2 (band number corresponds to the AC number of clones). Not all bands cloned in this study are shown here as they were not in the gel of the selected layers

this specific environment were reported, the impacts of the input penguin droppings on the bacterial community of the lake sediments were analyzed.

The microbial community in the sediments was investigated by molecular approaches targeting the 16S rRNA gene sequences. Bowman et al. (2000) detected highly unusual archaeal clones in sediment from Vestfold Hills lake in Antarctica using 16S rRNA-based clone library analysis. We used archaeal-specific primer Arch21F and Arch958R (DeLong 1992) to do PCR using our samples, but no bands were obtained in all 59 sediment layers examined (photo not shown). It is surprising that no archaea was detected in the Y2 lake sediments as archaea was found widely distributed in nature. The bacterial numbers in the sediment core were estimated by semi-quantification of the 16S rRNA gene copies in the environment. The sediments were found containing around 10^7 to 10^8 copies of 16S rRNA gene fragments per gram soil. The cell number over the entire sediment core which was dated around 1,600 years, remained relatively stable. The data indicated that the input of penguin droppings into the sediment had little effect on the whole bacterial counts. This could be easily explained as the sediment core is a nutrient-rich environment containing high TOC contents (Xiao et al.

Table 1 Characteristics and diversity indices of three sediment core communities

Community	No. of distinct sequences[a]	H′[b]	1/D[c]	Evenness[d]	Richness[e]
1–20 cm	21	1.21	25.33	0.48	25 ± 6
21–45 cm	28	2.26	56.22	0.74	30 ± 10
46–59 cm	19	1.16	8.99	0.32	19 ± 8

[a]Number of bands in the section of the core
[b]Shannon–Wiener index; higher number represents more diversity
[c]Reciprocal of Simpson index; higher number represents more diversity
[d]As evenness approach 1, the population is more evenly distributed
[e]Statistical prediction of the number of different species of 16S rDNA gene

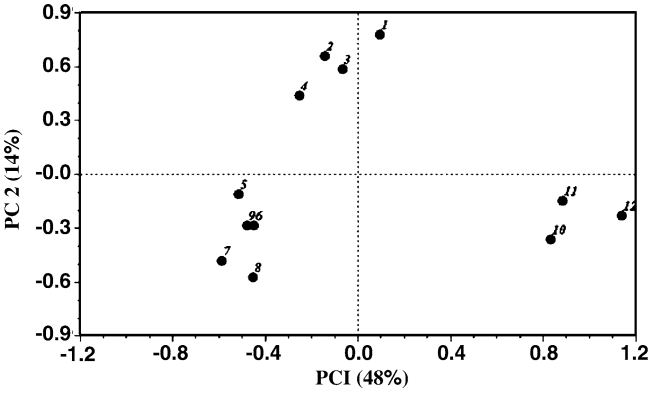

Fig. 2 Principal-coordinate plots of DGGE profiles showing the similarity of prominent bands in Y2 lake sediments core at various depths. The samples were analyzed by PCA with every five layers. Symbols: *1* 1–5 cm; *2* 6–10 cm; *3* 11–15 cm; *4* 16–20 cm; *5* 21–25 cm; *6* 26–30 cm; *7* 31–35 cm; *8* 36–40 cm; *9* 41–45 cm; *10* 46–50 cm; *11* 51–55 cm; *12* 56–59 cm

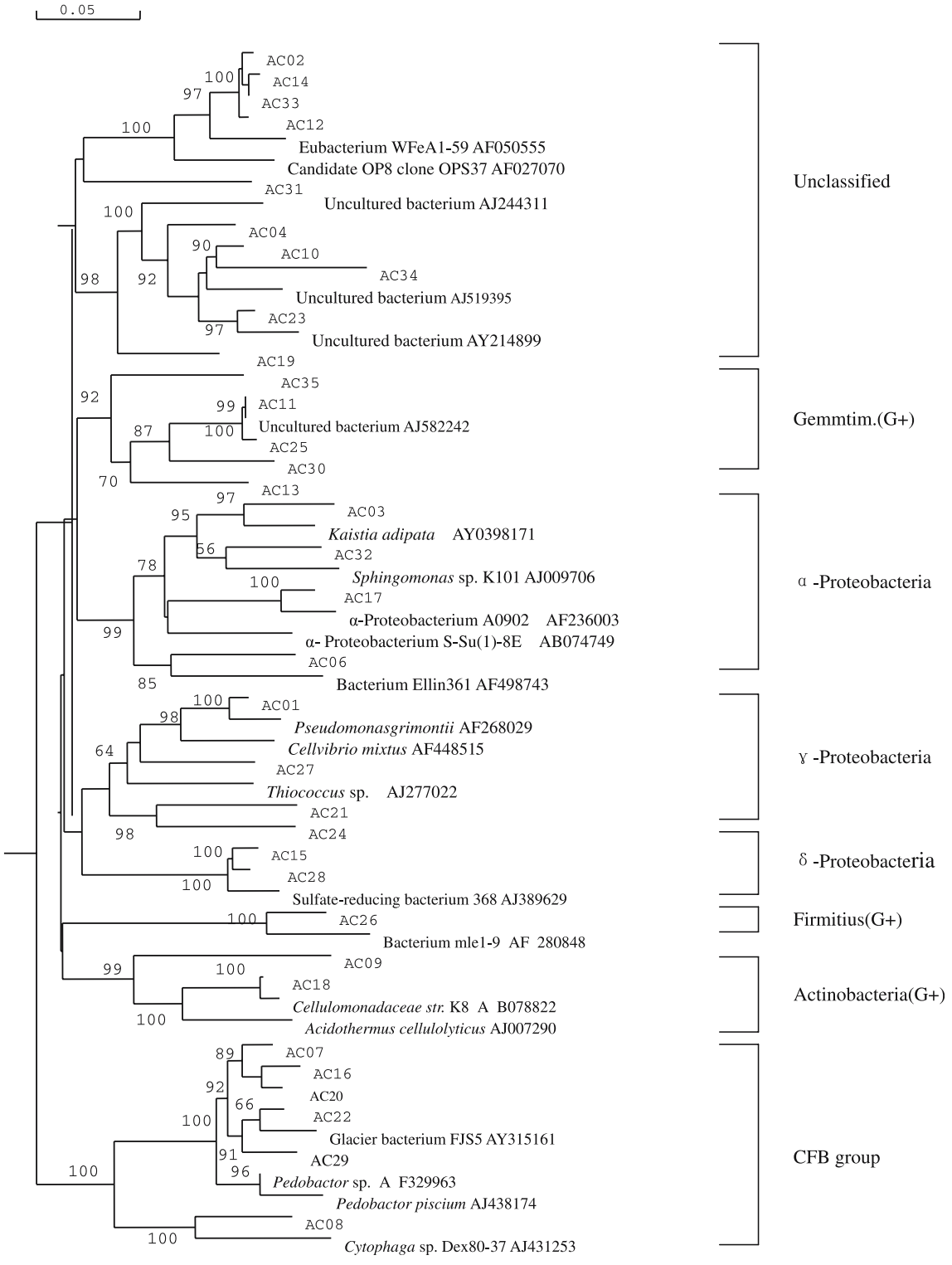

Fig. 3 Phylogenetic tree showing the relationship of retrieved clones in this study with references The phylogenetic tree was constructed as described in the Materials and methods. Only bootstrap values more than 50 were illustrated

2005), therefore, the growth of the bacterial community would not be significantly affected by the fluctuation of nutrient addition. On the other hand, it has been found that the numbers of chitinolytic bacteria in the sediment core were greatly influenced by the input of penguin droppings (Xiao et al. 2005), implied that the growth of the chitinolytic bacteria relied largely on the supply of nutrient sources.

The bacterial diversity in the sediment core was analyzed by PCR-DGGE method. There are many

Table 2 The cloned sequences and their distribution

Clone (accession no.)	Closest relative (accession no.)	Percentage similarity	Source	Band distribution[a]
α-Proteabacteria				
AC03(AJ582234)	*Kaistia adipata* AY039817	94%	Soil	21–45 cm
AC06(AJ582237)	Bacterium Ellin361 AF498743	96%	Soil	17–30 cm
AC17(AJ582248)	*A*-Proteobacterium A0902 AF236003	98%	Drinking-water biofilm	46–59 cm
AC32(AJ582263)	*Sphingomonas* sp. K101 AJ009706	92%	Groundwater	30–37 cm
γ-Proteabacteria				
AC01(AJ582232)	*Pseudomonas grimontii* AF268029	98%	Natural mineral water	1–20 cm
AC21(AJ582252)	*Acinetobacter* sp. TB40 AY278938	99%	Sea	1–20 cm
AC24(AJ582255)	*Escherichia coli* AY043392	99%	Respiratory secretions	1–20 cm
AC27(AJ582258)	*Pseudomonas reactans* AF255337	99%	Mushroom sporophores	46–59 cm
δ-Proteabacteria				
AC15(AJ582246)	*Desulfotalea psychrophila* AF099062	96%	Hot spring	21–45 cm
AC28(AJ582259)	*Desulfocapsa* sp. Cad626 AJ511275	98%	Chemocline of lake	21–25 cm, 39–42 cm
CFB group				
AC07(AJ582238)	*Pedobacter* sp. LMG 10343 AF329963	96%	Soil	1–20 cm
AC08(AJ582239)	*Cytophaga* sp. Dex80–37 AJ431253	89%	East Pacific rise	1–25 cm
AC16(AJ582247)	Glacier bacterium FJS5 AY315161	94%	Glaciaer	1–10 cm
AC20(AJ582251)	*Sphingobacterium* sp. AY167837	96%	Epilithon	1–10 cm
AC22(AJ582253)	Glacier bacterium FJS5 AY315161	98%	Glaciaer	1–10 cm, 40–46 cm
AC29(AJ582260)	*Pedobacter piscium* AJ438174	94%	Glaciaer	21–30 cm
Gemmatimonadetes				
AC11(AJ582242)	*Gemmatimonas aurantiaca* AB072735	96%	Lake	1–59 cm
AC13(AJ582244)	Uncultured *Gemmantimon*. AJ582242	96%	Lake	1–59 cm
AC25(AJ582256)	*Gemmatimonas* sp. AJ577104	90%	Lake	1–59 cm
AC30(AJ582261)	Uncultured *Gemmantimon*. clone AY851840	90%	Lake	40–47 cm
AC35(AJ582266)	Uncultured bacterium AY218696	98%	Lake	46–59 cm
Firmicutes				
AC26(AJ582257)	Syntrophic bacterium AB106354	90%	Anaerobic sludge	1–59 cm
Actinobacteria				
AC05(AJ582236)	Uncultured *Holophaga* sp. AM072424	93%	Uranium mining waste	21–45 cm
AC09(AJ582240)	*Acidothermus cellulolyticus* AJ007290	90%	Acidic mining waste	46–59 cm
AC18(AJ582249)	*Cellulomonadaceae str*. K8 AB078822	99%	Soil	1–59 cm
Unclassified				
AC02(AJ582233)	Uncultured clone HS9–75 AY221615	93%	Soil	36–45 cm
AC04(AJ582235)	Uncultured soil bacterium AY326539	93%	Soil	1–20 cm
AC10(AJ582241)	Uncultured soil bacterium AF507416	97%	Soil	1–20 cm
AC12(AJ582243)	Candidate OP8 clone OPS37 AF027070	91%	Hot spring	46–59 cm
AC14(AJ582245)	OP8 clone OPS12 AF027065	94%	Hot spring	46–59 cm
AC19(AJ582250)	Unidentified bacterium AB015272	96%	Lake	21–33 cm
AC23(AJ582254)	Uncultured bacterium AY214899	97%	Soil	21–45 cm
AC31(AJ582262)	Uncultured soil bacterium AF507766	88%	Soil	1–20 cm
AC33(AJ582264)	Eubacterium WFeA1-59 AF050555	94%	Polluted aquifer	21–45 cm
AC34(AJ582265)	Uncultured bacterium Riz6I AJ244311	99%	Oilseed rape	46–59 cm

[a]The distribution of cloned bands in the sediment layers were shown

molecular methods available for surveying microbial populations. Compared with other methods, DGGE is powerful in its high efficiency of analyzing large number of samples simultaneously. Therefore, DGGE method was chosen for our investigation of bacterial community in the historic sediment core. However, it should be still aware that this method has several limitations such as only short PCR fragment can be used and bands peaks can represent several different species because of co-migration in DGGE gels. The bacterial communities were found to have two main shifts over the entire sediment core by PCA analysis. It was found that the first 20 cm layers were clustered together, 21–45 layers grouped, and the last 14 cm layers clustered. This shift of bacterial community structure along the 59.5 cm-long column, probably reflected a vertical change of nutrient sources, temperature of the sediments core (the TOC and P_2O_5 content varied along the sediment core, Xiao et al. 2005). The bacterial communities in the middle (21–45 cm) of the sediment core showed highest species diversity. The top 20 layers appeared to be homogenous in the composition of bacterial community. This is probably partly due to the results of active bio-turbation (Bowman et al. 2003), which introduced fresh nutrients from the lakewater–sediment interface, and resulted in reoxidation of components involved in anaerobic respiration, and physically redistributes the bacterial community structure (Turley 2000). The bacterial

community in the last 14 sediment layers clustered together, which corresponded to the Medieval warm time period (Soon and Baliunas 2003). The relatively high TOC, P_2O_5 contents and chitinase gene copies in these layers (Xiao et al. 2005) suggested that this shift of bacterial structure was mediated by the change of input of penguin droppings and/or temperature.

At the surface layers, γ-Proteobacteria and *Cytophaga* of CFB group were found abundant (Table 2), which fit with the notion that *Cytophagales* have abilities to associate and glide on surfaces and to degrade a wide variety of polymeric substances. Similar results were got from the study of permanently cold marine sediments (Ravenschlag et al. 1999). The gram-positive bacteria *Gemmatimonadetes*, *Firmicutes* and *Actinobacteria* seem to be ubiquitous in every layer we examined. Putative sulfur- or sulfide-oxidizing δ-*Proteobacteria* AC28, which had 98% identity with *Desulfocapsa* sp. cad626, was detected in the middle layers. Large amount of the clones were found to be unclassified or uncultured bacteria, indicating that our knowledge on the bacterial world is still rather limited and our trip to the bacterial world of Antarcitc environments just begun.

Microbial diversity studies have been performed in some environments in Antarctica. The bacterial community in a glacial meltwater lake sediment in Antarctica detected by clone analysis fell into six major lineage of the domain bacteria: α, γ and δ and subvision of *Proteobacteria*, *CFB* group, the *Spirochaetacea*, and the *Actinobacteria* (Sjoling and Cowan 2003). In the study of Antarctic continental shelf sediments, the community structure appeared homogenous in the sediment core depths of 1–4 cm. Sequencing of DGGE bands and rRNA probe hybridization analysis revealed that the major community member belong to δ- and γ-*Proteobacteria*, *Flavobacteria*, *Planctoomycetales* and *Archaea* (Bowman et al. 2003). Compared with those studies of sediment core in Antarctica, the bacterial community in Y2 lake sediments core possessed no archaea, but had more contents in *Actinobacteria*, *Gemmatimonadetes* and *Firmicutes*. The cell number and biomass declined with sediment core depth in Antarctic continental shelf sediments, which is different from our results that the 16S rRNA gene copy number kept relatively stable along the Y2 lake sediments core.

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