Extremophiles (2009) 13:411–423
DOI 10.1007/s00792-009-0227-5

ORIGINAL PAPER

Bacterial diversity in the snow over Tibetan Plateau Glaciers

Yongqin Liu · Tandong Yao · Nianzhi Jiao · Shichang Kang · Baiqin Xu · Yonghui Zeng · Sijun Huang · Xiaobo Liu

Received: 7 September 2008 / Accepted: 31 December 2008 / Published online: 22 January 2009

Abstract Bacterial diversity and cell abundance in the snow of the four glaciers (Guoqu, Zadang, East Rongbuk and Palong No. 4) located in different climatic zones of the Tibetan Plateau were investigated through culture-independent molecular analysis of 16S rRNA gene clone library and flow cytometry approaches. Cell abundance ranged from 0.68×10^3 to 720×10^3 cells mL^{-1}, with higher values in the northern glaciers than in the southern ones. Bacterial diversity was unexpectedly high in the snow habitats of the world's highest plateau, with 15 common genera distributed widely among the glaciers. The bacterial diversity in the snow at different glaciers was related to the surrounding environments. The Guoqu Glacier, to the north near the desert zone and with the lowest temperature, preserved more bacteria closely related to a cold environment and soil than the other glaciers. However, in the Palong No. 4 Glacier located in the south warm region around vegetation, most bacteria were phylogenetically related to plant-associated bacteria.

Keywords Bacteria · Abundance · Diversity · Snow · Glacier · The Tibetan Plateau

Communicated by T. Matsunaga.

Y. Liu (✉) · T. Yao · S. Kang · B. Xu
Institute of Tibetan Plateau Research,
Chinese Academy of Sciences (CAS),
100085 Beijing, China
e-mail: yqliu@itpcas.ac.cn

N. Jiao · Y. Zeng · S. Huang · X. Liu
State Key Laboratory of Marine Environmental Science,
Xiamen University, 361005 Xiamen, China

Introduction

Snow is a significant climatic and ecological system that covers ca 35% of the Earth's surface permanently or for varying times during the year (Miteva 2007). Snow ecological systems are dynamic nutrient and microbial reservoirs (Jones 1999). Microbes play important roles in snow ecology (Hoham and Duval 2001) and have drawn more and more attention in recent years. Snow algae at different glaciers have been studied in detail. The results show that snow algae serve as primary producers that sustain heterotrophic communities on the glaciers (Hoham and Duval 2001; Painter et al. 2001; Stibal et al. 2006; Takeuchi 2001; Yoshimura et al. 1997). However, snow bacteria are less studied. In the polar region, Carpenter et al. (2000) obtained rRNA gene sequences related to Thermus-Deinococcus-like organisms, and report the low rates of bacterial DNA and protein synthesis in South Pole snow. Amato et al. (2007) report the bacterial concentrations are about 2×10^4 cells mL^{-1} in snow cover at Spitsbergen, Svalbard, and recovered strains belonging to the α-, *β*-, *γ*-*Proteobacteria*, *Firmicutes* and *Actinobacteria* (Amato et al. 2007). In the low and mid latitudes, Segawa et al. (2005) studied the flora and biomass in snow from the Tateyama Mountain, Japan, and suggest that seasonal variations in bacterial snow biomass were due to rapid bacterial growth during summer (Segawa et al. 2005). Bacterial abundance in the snow at the Mount Sonnblick, Austria and in the Tyrolean Alps range from 10^3 to 10^5 cells mL^{-1} (Alfreider et al. 1996; Sattler et al. 2001).

However, there is scarce information concerning bacteria in snow on glaciers over the Tibetan Plateau (abbreviation TP), which contains the most glaciers outside the high latitudes with an area of 4.9×10^5 km^2 (Shi 2000). Snow cover in the TP has influence on the global

climate (Barnett et al. 1989). Glaciers on the TP sensitively indicate global climatic change (Duan et al. 2006; Thompson et al. 2000; Tian et al. 2006). In such a significant region, knowing what kind of bacteria dwell there, and what the relationships between the bacteria and the environment are, is the first step to explore in order to gain an understanding of the snow ecology system. In this study, we investigated the bacterial abundance and diversity as well as nutrients and related chemical parameters in the snow of the four geographically distant glaciers on the TP. Our aim was to first explore the bacterial community features in the snow in the world's highest plateau. Second, we aimed at disclosing the relationship between bacteria and environment at different glaciers over the TP.

Methods

Sampling site

The TP is the highest and largest plateau in the low- and mid-latitude region, with an average elevation of 4,200 m and an area of 2.5 $\times$ 10^6 km^2. The climate and environment of northern and southern parts of the plateau are different. A warm and wet oceanic climate is dominant in the southern plateau and an arid continental climate in the northern part. The vegetation cover in the southern plateau is more than that in the northern part. From south to north, the natural landscapes appear in the following succession: forest, meadow, steppe, and desert (Luo et al. 2004; Zheng 1996).

We collected snow samples from four glaciers located in different climatic and environmental zones over the plateau (Fig. 1): the Guoqu Glacier, Zadang Glacier, East Rongbuk Glacier and Palong No. 4 Glacier. The Guoqu Glacier is located on the northern slope of Mt. Geladaindong, the summit of the Tanggula Mountains and the headstream of the Yangtze River. The region is dominated by desert and steppe, and the climate is semiarid (Zheng 1996).The Zadang Glacier is one of numerous glaciers on the Niqingtanggula Mountains, which is near Lake Nam Co, the largest lake in Tibet. The climate is in transition between semihumid and semiarid. The East Rongbuk Glacier is located on the northern slope of Mt. Everest. It is at the boundary of the Indian monsoon-dominated wet and warm climate in the south and the westerly jet stream-dominated continental climate in the north. The Palong No. 4 Glacier is located at the source region of Palongzhangbu River, one of the major branches of the Brahmaputra. It is on the passage of water vapor transport from the Indian Ocean to the TP. The climate is wet and warm. The region is dominated by mountain forest (Zheng 1996).

Sampling

Four snow pits were dug and sampled from 2005 to 2006 (Table 1). One pit was dug at each glacier. All pits were located at flat firn basin which usually had the deepest snow in the glacier. All snow pits reached the ice layer. The surface in contact with the air was first systematically scratched away using a sterile spoon and discarded. Snow samples for enumerating bacterial abundance were collected using sterile plastic tube (50 mL) embedded into the snow pit wall at 5–15-cm intervals to fill it without any need for extra manipulation to avoid contamination. Samples for concentration measurements of Ca^{2+}, dissolved organic carbon (DOC) and total nitrogen (TN) were collected at the same intervals along the pit wall and put into Whirl Pack bags. Samples for bacterial diversity analysis

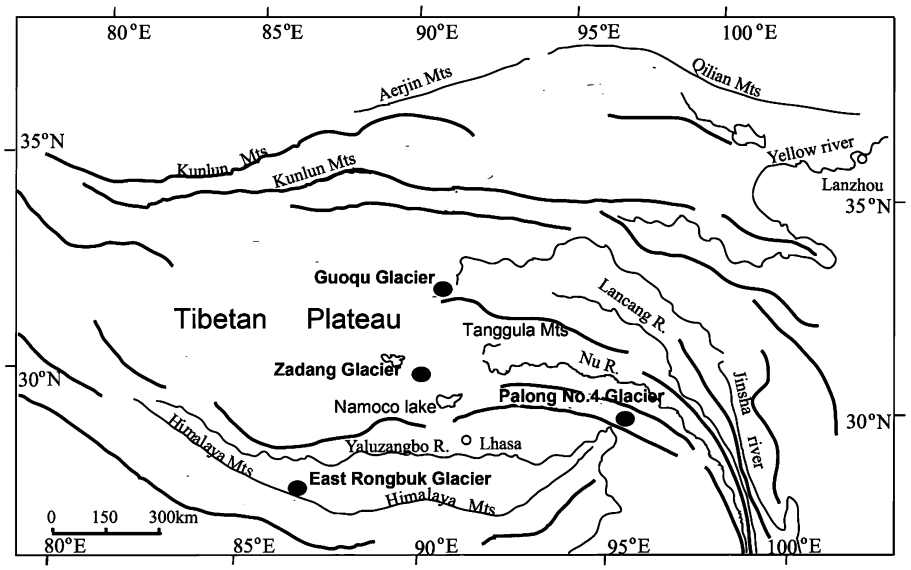

Fig. 1 Location of the Guoqu, Zadong, Rongbuk, and Palong No. 4 Glaciers on the Tibetan Plateau

Table 1 Location, altitude, and depth of sampled snow pits at four glaciers and the climatic condition in these regions

Glaciers	Location	Altitude/m	Mean temperature/°C	Highest/lowest temperature/°C	Mean precipitation/mm	Snow pit depth/m	Years of the sampled snow
Guoqu	33°34.80′N 91°10.80′E	6,621	2.8	18.8/−28.5	314	0.86	From winter of 2004 to November 2005
Zadang	30°28.57′N 90°38.71′E	5,799	1.6	19.2/−23.5	359	1.52	From winter of 2005 to May 2006
East Rongbuk	28°01.04′N 86°17.02′E	6,520	3.4	23.8/−22.4	174	1.70	From winter of 2003 to April 2005
Palong No. 4	29°13.57′N 96°55.19′E	5,507	9.6	28.6/−11.5	648	2.30	From winter of 2005 to June 2006

Mean and highest temperature, and mean precipitation were the value for 2005 from the nearest meteorological stations to the glaciers, i.e., Tuotuohe (Guoqu Glacier), Bangge (Zadang Glacier), Dingri (East Rongbuk Glacier) and Bomi (Palong No. 4 Glacier) stations

were collected at 15 or 20-cm intervals and placed in sterile Nalgene bottles. Extreme care was taken at all times to ensure minimal contamination. Non-particulating sterile suits, sterile gloves and masks were worn during the entire sampling process. Scoops for collecting snow were sterilized beforehand and used only once for one sample. Snow samples were kept frozen until analysis in the laboratory.

Enumeration of cell abundance

Cell abundance was analyzed using flow cytometry (Beckman Coulter, Epics Altra II). Snow meltwater (1 mL) was fixed with glutaraldehyde (final concentration: 1%) for flow cytometry analysis. SYBR Green I was applied as the nucleic acid stain (Marie et al. 1997). Samples were run on a EPICS ALTRA II flow cytometer (Beckman Coulter), equipped with a 100-mW 488-nm water-cooled argon-ion laser (Cohenrent Inc., USA) and a standard filter set-up. An external sample injector (Harvard Apparatus PHD 2000) was employed for accurate quantification (Jiao et al. 2005). About 1 μm (diameter) fluorescent beads (Polyscience Inc) were added to the sample for internal reference.

Measuring concentrations of Ca^{2+}, DOC and TN

Concentration of Ca^{2+} was measured using a Dionex ion chromatograph model 2010 (Buck et al. 1992). DOC and TN were analyzed using TOC-Vcph (Shimadzu Corp., Japan). Each sample was measured three times with a standard deviation lower than 10%.

Community DNA extraction, PCR amplification, 16S rRNA gene clone library construction

In order to represent the bacterial community features of snow deposited during different seasons at each glacier, we analyzed more than one snow sample at different depths of every snow pit. We chose two samples from the Guoqu Glacier, four samples from the Zadang and Palong No. 4 glaciers, and six samples from the East Rongbuk Glacier according to the depth of snow pit and precipitation in each region. Snow samples were melted overnight at 4°C, and approximately 1 L of meltwater was filtered through a 0.22 μm filter (Millipore). The membrane was immediately treated with 1 mL GTE buffer (25 mmol/L Tris, 10 mmol/L EDTA, 50 mmol/L Glucose, 20 mg/mL, pH 8.0) at 37°C for 2 h. Then proteinase K, NaCl, and sodium dodecyl sulfate were added at final concentrations of 0.2 mg/mL, 0.7 mol/L, and 1%, respectively. The mixture was incubated at 65°C for 1.5 h. After extraction with phenol–chloroform–isoamyl alcohol (25:24:1) and chloroform–isoamyl alcohol (24:1), DNA in the aqueous phase was precipitated with an equal volume of isopropanol overnight at −20°C. After centrifuging and washing with 75% ethanol, the DNA was dissolved in TE buffer. To assess if contamination was introduced during DNA extraction, a negative parallel control was established by filtering 1 L of autoclaved deionized water using the same method as described above. DNA preparations from both the sample and negative control were used as templates to amplify the bacterial 16S rRNA genes with the universal primer pair 27F (forward, 5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1392R (reverse, 5′-ACG GGC GGT GTG TRC-3′) (Brosius et al. 1978; Christner 2002) and La Taq polymerase (TaKaRa Co., Dalian, China). The reaction mixture (30 μL) consisted of 1 U of La Taq (TaKaRa Co., Dalian, China), 0.2 mM each dNTP, 3 μL of 10× buffer, 0.15 mM of each primer and 1 μL (ca. 10 ng) DNA of template. The PCR program was as follows: initial incubation at 94°C for 5 min, followed by 30 cycles (94°C for 1 min, 56°C for 1 min and 72°C for 1.5 min), and then by a final extension at 72°C for 8 min.

The PCR products were purified using an agarose gel DNA purification kit (TaKaRa Co., Dalian, China), ligated into pGEM-T vector (TaKaRa Co., Dalian, China), and then transformed into *E. coli* DH5α. The presence of the inserts was checked using colony PCR. About 120–280

clones from each library were picked randomly for re-amplification and restriction digestion with the enzymes *Hha* I and *Afa* I. The digested fragments were visualized on a 3% agarose gel, and different clones were discriminated according to the RFLP patterns. One clone of each RFLP type was sequenced with 27F as the sequencing primer.

Phylogenetic analysis

All sequences obtained were checked for chimeric artifacts using the CHIMERA_CHECK program (Maidak et al. 2001). Clones identified as potential chimeras were discarded. Sequences of four libraries were calculated with DOTUR (Schloss and Handelsman 2005) and those with similarity greater than 97% were grouped into one operational taxonomic unit (OTU). Sequences were assigned to the genus level grouping with 80% confidence using the "Classifier" program of RDP (Cole et al. 2005). The closest neighbors were retrieved from the NCBI (http://www.ncbi.nih.gov/ BLAST) through blasting. A phylogenetic tree including obtained OTUs and their closest relatives was constructed using MEGA software 3.1 (Kumar et al. 2004). Neighbor-joining phylogenies were constructed from dissimilatory distances and pair-wise comparisons with the Jukes-Cantor distance model. Bootstrap analysis of 1,000 replicates was performed.

Statistical analyses

Coverage of clone libraries were calculated using the equation: Coverage $= 1 - (N/\text{individuals})$, where N is the number of clones that occurred only once (Kemp and Aller 2004). Diversity index (*Shannon*) was calculated using the statistical program PAST (http://folk.uio.no/ohammer/past).

Nucleotide sequence accession numbers

The nucleotide sequences of partial 16S rRNA genes obtained in this study have been deposited in the GenBank database under following accession numbers: from DQ323081 to DQ323115, from EU152996 to EU153042, from EU527075 to EU527186 .

Results

Ca^{2+} concentration, DOC and TN in the snow at different glaciers

Ca^{2+} concentration, DOC and TN of the snows varied with depth in the snow pits (Fig. 2). Ca^{2+} concentration and DOC exhibited similar trend with the cell abundance at the Guoqu Glacier. But at the other three glaciers, Ca^{2+}

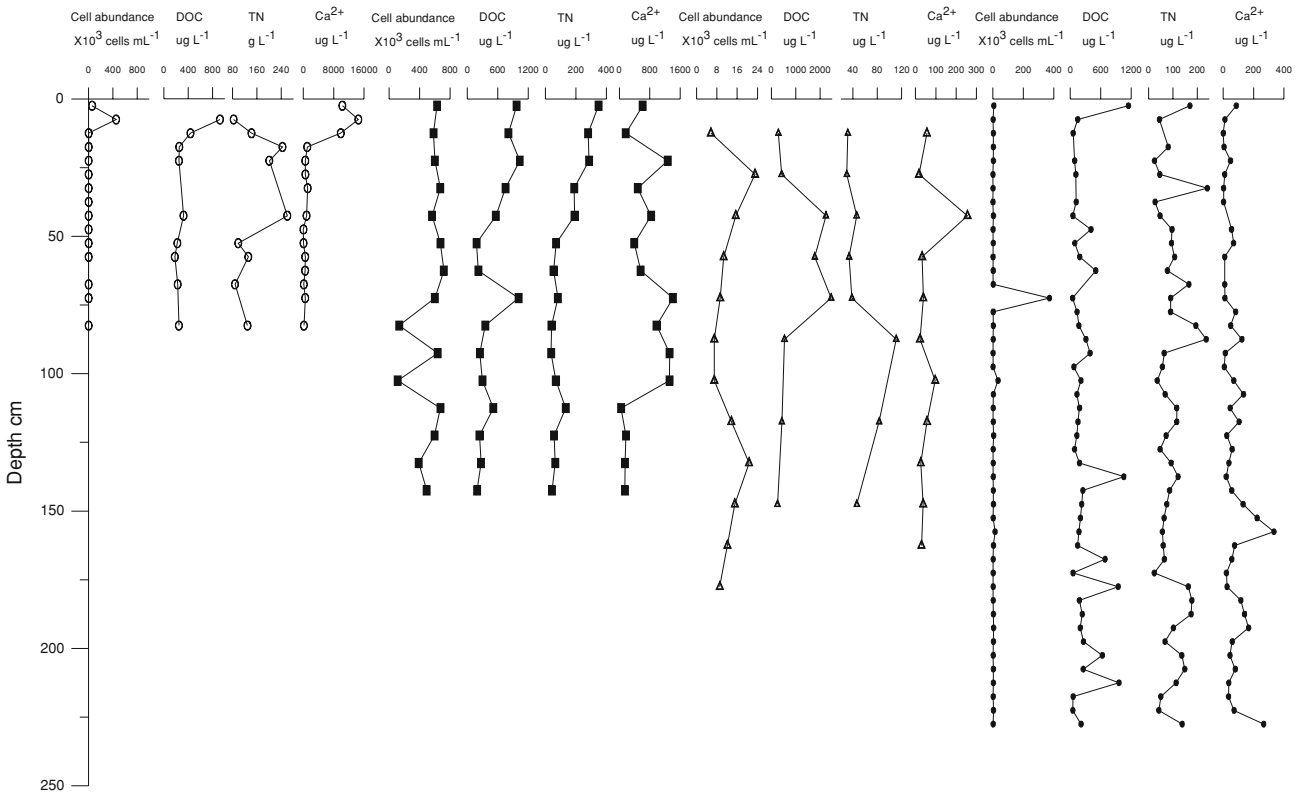

Fig. 2 Cell abundance, concentrations of DOC, TN and Ca^{2+} in different layers of the Guoqu (**a**), Zadong (**b**), Rongbuk (**c**), and Palong No. 4 Glaciers (**d**)

concentration, DOC, and TN showed different change patterns along the depth (Fig. 2).

The Ca^{2+} concentrations of snow at the northern glaciers were higher than that at the southern glaciers. The mean value of Ca^{2+} concentrations at the Guoqu Glacier was the highest among the four glaciers, while the snow at the East Rongbuk Glacier had the highest concentration of DOC, and the TN concentrations were similar at different glaciers (Table 2).

Cell abundance in snow at different glaciers

Cell abundance ranged from 0.68 to 720 $\times$ 10^3 cells mL^{-1} (Fig. 2). Among 88 snow samples, cell abundances of 55 samples were less than 5 $\times$ 10^3 cells mL^{-1}; 4 samples were between 5 and 10 $\times$ 10^3 cells mL^{-1}; 10 samples were in 10 $\times$ 10^3 cells mL^{-1}; and 17 samples were more than 100 $\times$ 10^3 cells mL^{-1}.

The two spike values of cell abundances at the Guoqu Glacier corresponded to the highest Ca^{2+} concentrations. Snow at the Zadang Glacier was of highest abundance; all of the values were more than 10^5 order. Cell abundances at the Palong No. 4 and East Rongbuk glaciers were similar. However, the latter exhibited wide variations along the depth, from 0.68 to 370 $\times$ 10^3 cells mL^{-1} (Fig. 2).

16S rRNA gene clone library analysis

Four bacterial 16S rRNA gene clone libraries of the snows from different glaciers were constructed and a total of 829 clones were subjected to RFLP screening (Table 2). One clone was chosen from each pattern and subjected to sequence analysis. In total, 227 sequences were obtained and identified as normal 16S rRNA gene sequences after being checked using the CHIMERA_CHECK program. All sequences were grouped into 164 OTUs. Coverages of the four libraries were all more than 75% (Table 2), indicating that the number of clones sequenced in this study provided a good inventory of the bacterial 16S rRNA gene sequences in the snow from various glaciers. The negative control did not yield any amplification products, supporting the credibility of the DNA extraction procedures.

The bacterial 16S rRNA gene sequences in snow could be classified into 13 (sub) phylum and unclassified (Fig. 3), with the *γ-Proteobacteria* group dominating (Fig. 4a). All sequences in snow from the TP were near to the sequences in the GenBank database with identity values of 91–99%. One hundred and thirty-two sequences representing 91.1% of the total clones had identity values higher than 97%. The nearest neighbors of snow bacterial sequences were isolated from versatile sources. A majority of the sequences (81% of the total) were similar to sequences recovered from cold environments (glaciers, Antarctic soil, lake/sea ice in the Antarctic or Arctic, permafrost), soil and the aquatic environment (Table 3).

Bacterial genetic diversity in the snow varied at different glaciers (Fig. 4b). At the Guoqu Glacier, members of Bacteroidetes and *β-Proteobacteria* were dominant. Sequences of the Bacteroidetes affiliated to the genus *Hymenobacter* except that one was unclassified. The genus *Hymenobacter* contains oligotrophic soil bacteria, some of them isolated from cold environments in the Antarctic (Balows et al. 1992).In this study, G2-11 and G6-26 related closely with bacteria sourced from soil (EF516581, identity value 97%) and the Puruogangri Glaciers at the TP (DQ418532, identity value 97%). The *β*-Proteobacteria included six sequences, among them four sequences representing 92% of the clones closely related to bacteria in the glacial environment. G6-215 was similar to sequences from the Tianshan No. 1 Glacier, China (EF423322) and the Franz Josef Glacier, New Zealand (AY315177) with an identity value of 98%; G6-212 and G6-58 were similar with two sequences (EU263716 and EU263699, identity value 99%) from the Kuytun Glacier, China; G6-52 was similar with sequences from the Kuytun Glacier (EF263783) and John Evans Glacier, Canada (DQ228409) with an identity value of 96%.

The bacterial diversity at the Zadang Glacier was the highest among the four sites studied (Table 2). Clones affiliated to the α-, *β*-, *γ-Proteobacteria*, *Actinobacteria*, and Bacteroidetes were similar (accounting for 14–18% of

Table 2 Mean value of cell abundance, dissolved organic carbon (DOC), total nitrogen (TN), Ca^{2+} concentration, and bacterial 16S rRNA gene libraries in snow at the four glaciers

Glaciers	Cell abundance/ cell mL^{-1}	DOC/μg L^{-1}	TN/μg L^{-1}	Ca^{2+}/μg L^{-1}	16S rRNA gene libraries				
					Name	Clones	OTUs	Shannon H	Coverage (%)
Guoqu	3.5 $\times$ 10^4	337	153	2,723	G	110	29	2.5	86.4
Zadang	5.4 $\times$ 10^5	510	130	658	ZD	223	93	4.0	76.7
East Rongbuk	1.2 $\times$ 10^4	1,052	52	64	RBL	218	25	2.2	95.4
Palong No. 4	1.1 $\times$ 10^4	275	97	69	PL	278	58	3.1	90.6

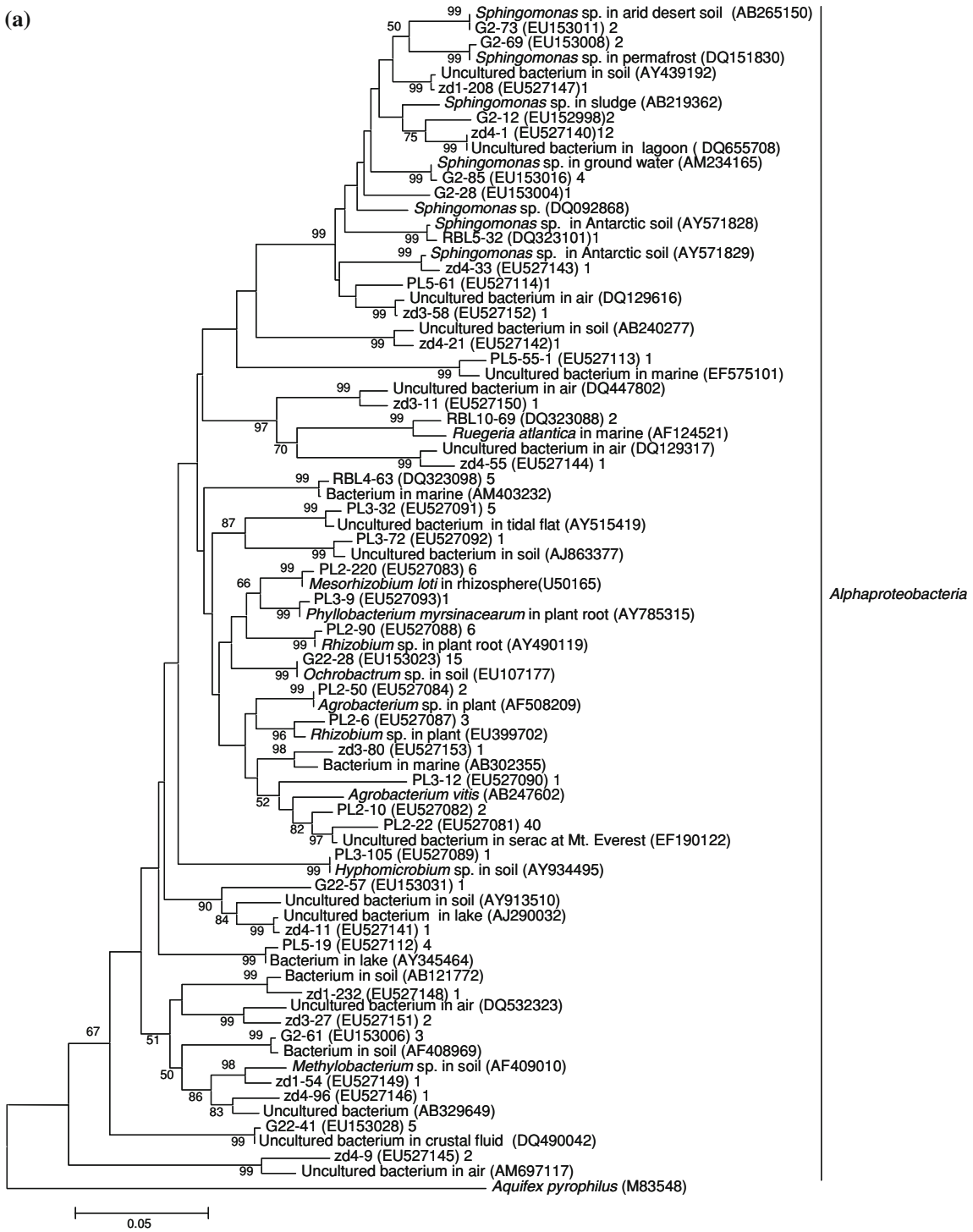

Fig. 3 Neighbor-joining tree showing the phylogenetic relationships of bacterial 16S rRNA gene sequences from snow to closely related sequences from the GenBank database. One representative clone type within each OTU is shown and the number of clones within each OUT is shown at the end (after the GenBank accession number). Their closest sequences and source information are listed. Clone sequences from this study are coded as library name and clone number. **a** is the first subtree showing all *Alphaproteobacteria*, **b** is the second subtree showing all sequences of *Beta* and *Gammaproteobacteria*, **c** is the third subtree showing all sequences of *Actinobacteria*, **d** is the fourth subtree showing all sequences of *Acidobacteria*, Bacteroidetes, *Chloroflexi*, *Cyanobacteria*, *Deinococcus-Thermus*, *Firmicutes*, *Planctomycetes*, TM7, *Verrucomicrobia* and unclassified sequences

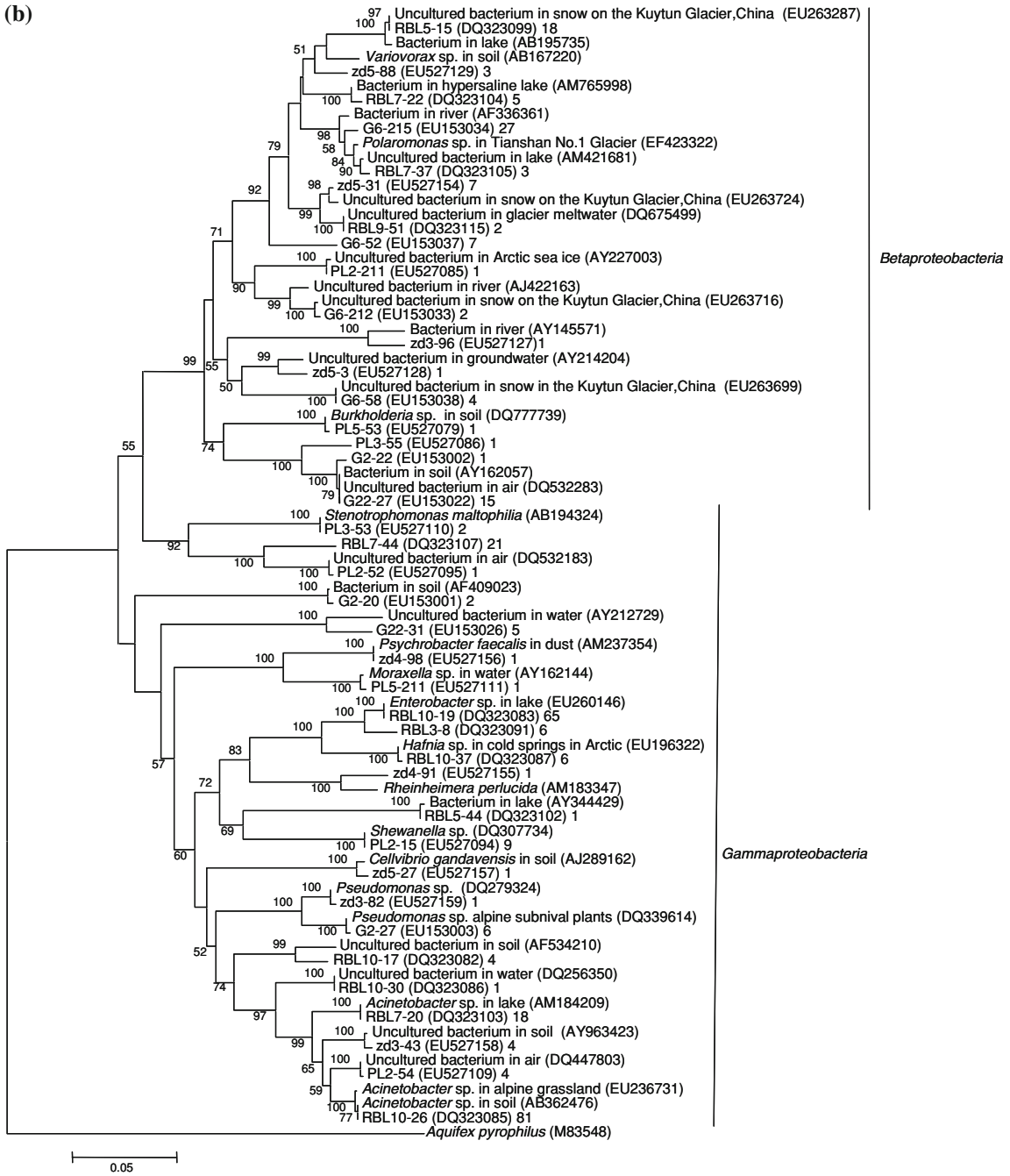

Fig. 3 continued

the total). The genus *Sphingomonas* was dominant in the α-*Proteobacteria*, accounting for nearly half of the total clones. Clones belonging to the genus *Polaromonas* were the most abundant in the *β-Proteobacteria*. The genus *Acinetobacter* in the *γ-Proteobacteria* dominated absolutely, accounting for 83% of the total clones. The *Actinobacteria* embraced 11 genera and seven unclassified sequences. Each genus played a similar role. Sixty percent of clones were correlated with psychrophilic (psychrotrophic) bacteria isolated from glacier, sea ice, high mountain lakes, or the Antarctic. In the Bacteroidetes, the major genus was *Hymenobacter*. Fifty-seven percent of clones were correlated with sequences from cold environments.

In the snow at the East Rongbuk Glacier, the *γ-Proteobacteria* group was the most abundant and diverse. The

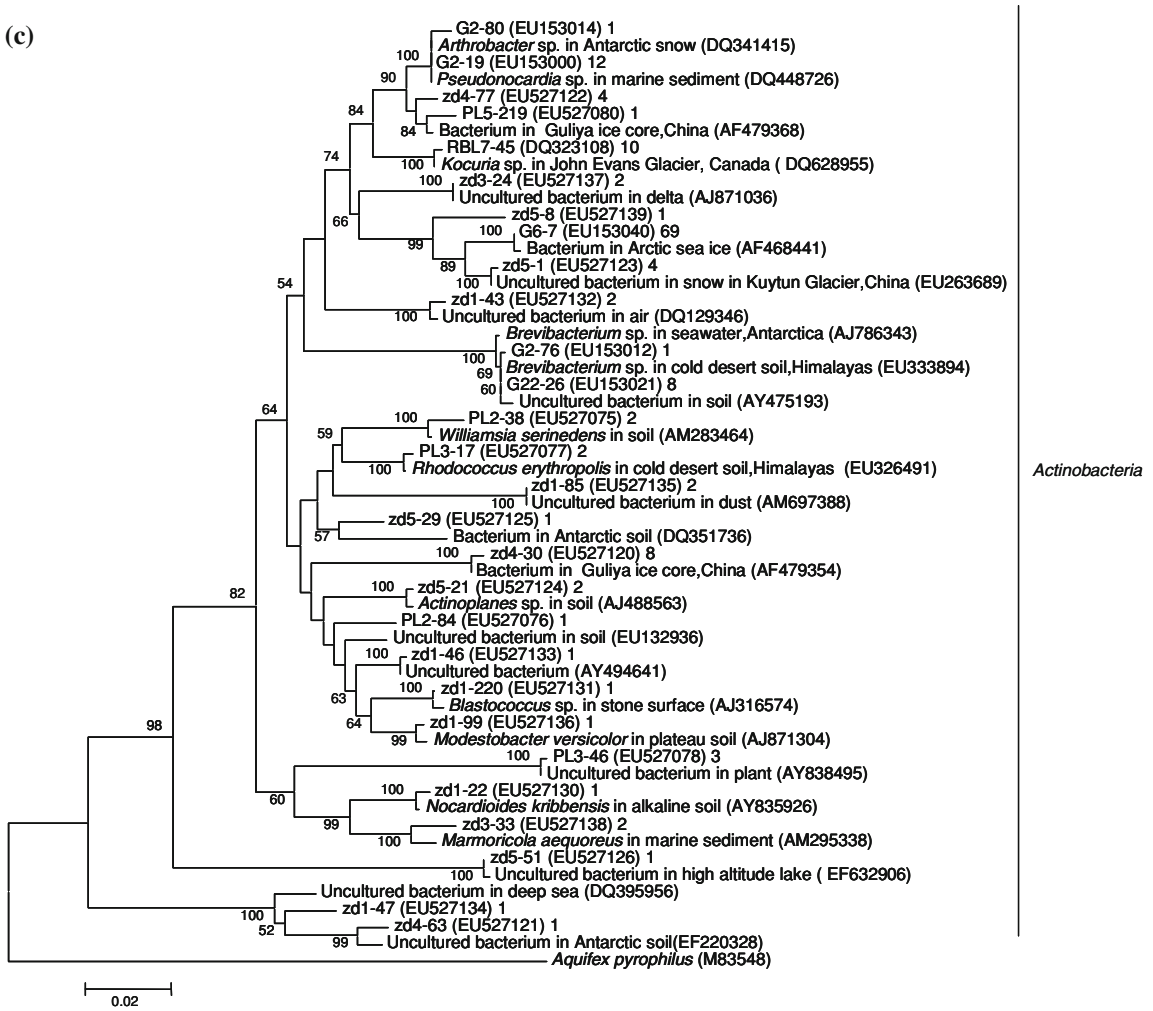

Fig. 3 continued

genera *Acinetobacter* and *Enterobacter* were dominant. Clones belonging to these two genera accounted for 43 and 45% of the total. In the genus *Acinetobacter*, RBL7-20 was closely related to a glacial ice core bacterium in Greenland (DQ147599) and an Arctic sea ice bacterium (AF468386) with an identity value of 99%. RBL10-26 was similar to soil bacterium (EU603514, identity value 99%). Only one sequence was in the genus *Enterobacter* whose nearest neighbor was isolated from an oligotrophic lake (EU260134, identity value 99%).

At the Palong No. 4 Glacier, the *α-Proteobacteria* and *Actinobacteria* were dominant. The genus *Rhizobium* was dominant in the *α-Proteobacteria*. Three of four sequences in the genus related to bacteria which inhabited plants. The other one (PL 2-22) was similar with bacteria in glacial serac ice (EF190122) with an identity value of 98%. Sequence PL2-220 in the genus *Mesorhizobium*, PL5-19 in the genus *Pannonibacter*, and PL3-9 in the genus *Phyllobacterium* were all also near to plant bacteria with identity values more than 98%. In the *Actinobacteria*, five sequences representing 93% of total clones connected with bacteria isolated from cold environments. The others (PL3-46, PL2-38) were related with plant and soil bacteria.

Common genera of the four libraries

Among a total of 83 genera, 23 genera occurred in more than one library. Genera The genera *Sphingomonas* in the *α-Proteobacteria* and *Polaromonas* in the *β-Proteobacteria* were the genera common to the four libraries. Eight genera existed in three libraries, i.e., *Bradyrhizobium* and *Ochrobactrum* in the *α-Proteobacteria*, *Acidovorax*, *Curvibacter*, and *Ralstonia* in the *β-Proteobacteria*, *Acinetobacter* in the *γ-Proteobacteria*, *Brevibacterium* in the *Actinobacteria*, and *Bacillus* in the Firmicutes. The other 13 genera occurred in two libraries.

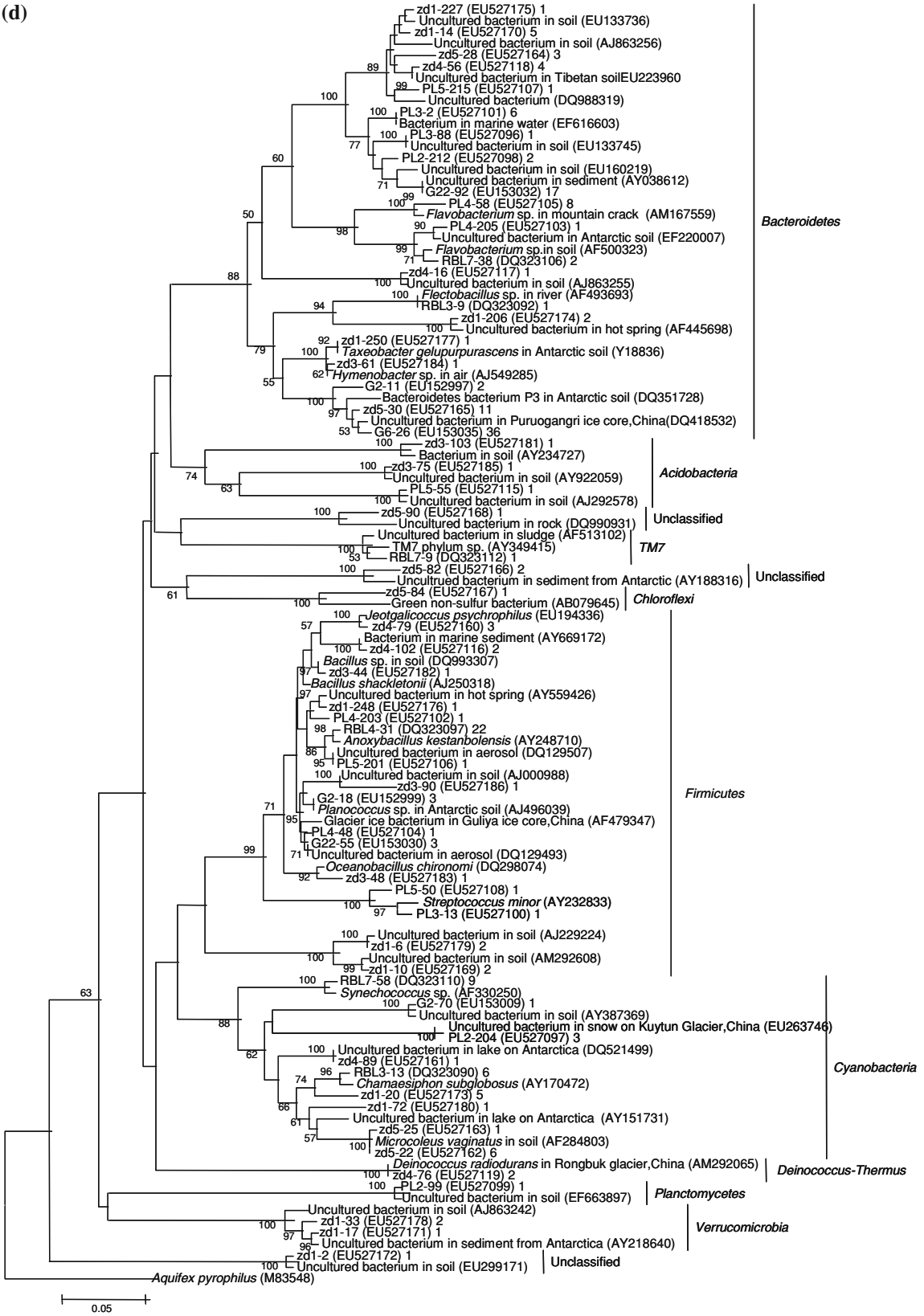

Fig. 3 continued

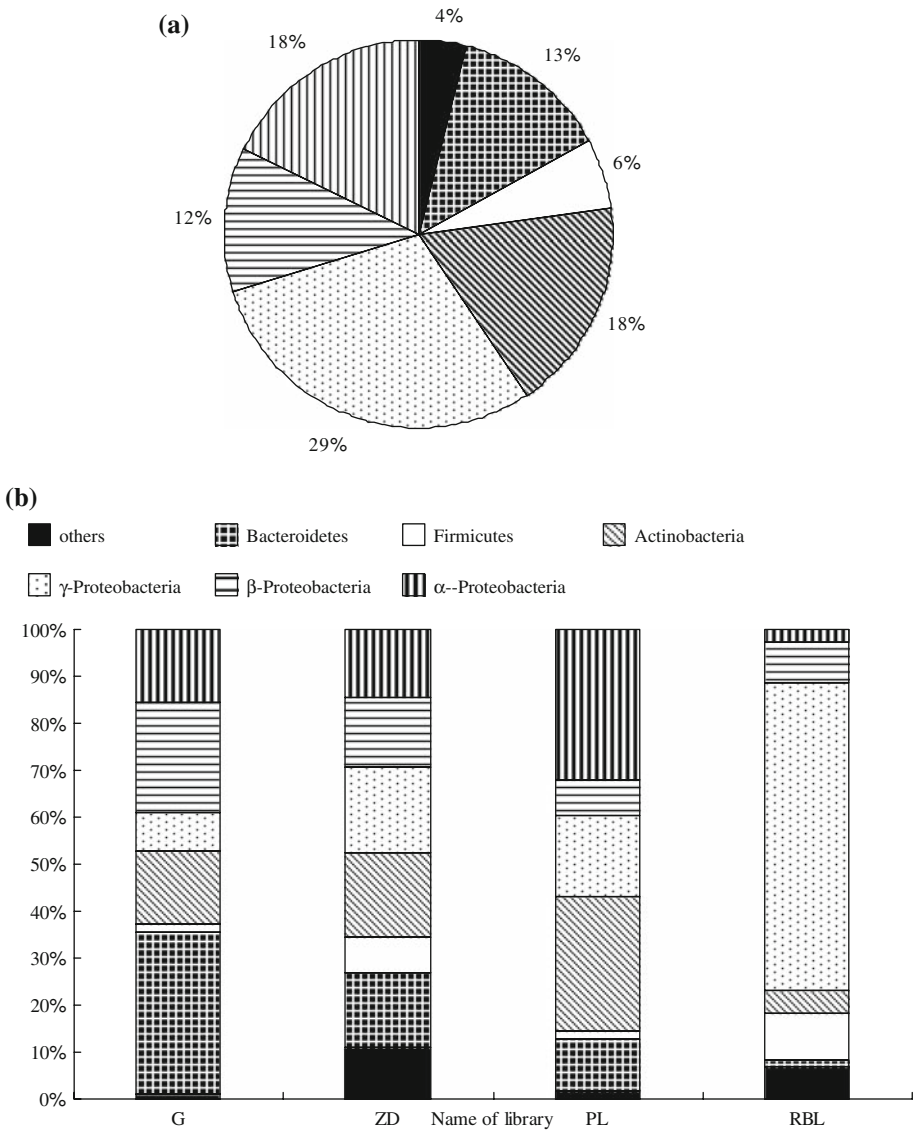

Fig. 4 Distribution of major phyla (subphyla) of bacterial 16S rRNA gene sequences in all four clone libraries (**a**) and individual clone library from the snow over four Tibetan Plateau Glaciers (**b**)

Table 3 Classification of the environment of the nearest neighbor of snow bacteria at the Guoqu (G), Zadang (ZD), East Rongbuk (RBL) and Palong No. 4 (PL) Glaciers

Environment of the nearest neighbor	Total		G		ZD		RBL		PL	
	Sequences (%)	Clones (%)	Sequences (%)	Clones (%)	Sequences (%)	Clones (%)	Sequences (%)	Clones (%)	Sequences (%)	Clones (%)
Cold environment	33	43	41	71	37	39	36	16	28	55
Soil	30	25	41	17	29	33	16	26	29	20
Aquatic environment	18	24	14	6	17	14	44	58	19	12
Plant	5	4	3	5					14	9
Air	6	2			10	6			3	1
Others	7	3			8	8	4	4	7	2

Discussion

Comparison of snow cell abundance on the Tibetan Plateau to other regions

The microbial abundance of mountainous and polar region snow is reported in the range 0.2×10^3–100×10^3 cells mL^{-1}. Snow bacterial populations were from about 0.2×10^3–5×10^3 cells mL^{-1}in Antarctic (Carpenter et al. 2000; Garrison et al. 1986), at about 60×10^3 cells mL^{-1} in the Arctic (Amato et al. 2007), and from 6×10^3 to 230×10^3 cells mL^{-1}in high mountain regions (Sattler et al. 2001; Segawa et al. 2005). Cell abundances in the snow on the TP were in the above range. Most of snow samples (55 of 88) have similar abundance to those in the Antarctica, and less than those in the Arctic and other high mountains. Nearly 30% of the samples contained similar or higher bacteria than that in the Arctic and other high mountains. Mean values of cell abundance at the Guoqu, East Rongbuk, and Palong No. 4 glaciers were slightly lower than those at Arctic and on mid latitude mountains. However, the mean value of abundance at the Zadang Glacier was ten times that at the Arctic and mountains.

Relationship between cell abundance and environment on the Tibetan Plateau

Cell abundance in the snow at the northern glaciers was higher than that at the southern ones (Table 2). The snow bacterial abundance is decided by a multiplicity of factors including source supply of bacteria from the atmosphere, bacterial growth, and condensation by snow melting. Researches report that bacteria attached to aeolian dust can be transported to glaciers (Abyzov et al. 1998; Yao et al. 2006). Bacterial concentration was positively correlated with the density of mineral particles in snow (Segawa et al. 2005). In order to study the relationship between bacterial abundance and dust in snow, we analyzed the Ca^{2+} concentration, an index of dust concentration transported into glaciers (Yao et al. 2004). Comparing cell abundances in the snow at four glaciers, the Guoqu and Zadang glaciers, which had relatively high Ca^{2+} concentrations, contained more abundant bacteria than the Rongbuk and Palong No. 4 glaciers (Table 2). Two spikes of bacterial abundance at the Guoqu Glacier were likely connected with the dust storm event indicating the highest Ca^{2+} concentrations (Yao et al. 2004). If we do not consider Ca^{2+} concentrations in the snow deposited during dust storm event at the Guoqu Glacier, the Ca^{2+} concentrations in the snow at the Zadang Glacier were the highest among four glaciers, and about tenfold of that at the Rongbuk and Palong No. 4 glaciers. The bacteria at the Zadang Glacier were correspondingly the most abundant and ten times higher than the Rongbuk and Palong No. 4 glaciers at nearly all depths. This result indicated that bacterial abundances in the snow over the TP were strongly influenced by dust input into glaciers. Within glaciers, it was found that a significant correlation existed between bacterial abundance and dust at the Guoqu Glacier($r = 0.75$, $n = 14$, $P < 0.01$). However, no such correlations were observed at the other three glaciers. This result suggested that dust played an important role in transporting bacteria to the Guoqu Glacier, which located in the north and under the influence of westerly jet stream carried dust from arid and mid arid regions. Meanwhile, bacteria were possibly transported by not only mineral particles but also organic particles from the aquatic environment and plant at other glaciers close to lakes or vegetation.

Segawa et al. (2005) report psychrophilic bacteria rapidly growing in the snow. The bacterial growth condition in the snow at the TP is still not well documented yet. In our study, snow in the southern glaciers did not contain more bacteria than in the northern glaciers, although southern glaciers with better conditions for bacterial survival and growth, i.e., being warm and wet. Bacterial abundance was not correlated with concentrations of DOC and TN at any of the glaciers as well. However, the bacterial growth condition is in need of more study in the future.

Common genera widespread distribution in snow over the Tibetan Plateau

Among the diverse snow bacteria, some genera showed widespread distribution in glaciers of the TP. The *Acinetobacter* bacteria occur in seven glaciers over the plateau, including the Manlan and Puruogangri Glaciers in the north (Xiang et al. 2004; Zhang et al. 2006), the Zadang Glacier at the center, the East Rongbuk Glacier in the south, the Guliya Glacier(Christner et al. 2003) in the west and the Palong No. 4 Glacier in the east. Bacteria in the genera *Sphingomonas* and *Polaromonas* existed not only in the four glaciers in our study but also in the Malan (Xiang et al. 2004) and Puruogangri Glaciers (Zhang et al. 2006). The genus *Bacillus* was found in five glaciers, i.e., the Guoqu, Zadang, Palong No. 4, Malan (Xiang et al. 2004), and Guliya glaciers (Christner et al. 2003).Genera *Ochrobactrum* and *Ralstonia* were recovered from five glaciers: three of our studied glaciers, the Malan (Xiang et al. 2004), or Guliya (Christner et al. 2003) glaciers. The genera *Bradyrhizobium*, *Acidovorax*, *Curvibacter*, and *Brevibacterium* were common in three of our glaciers, and the genera *Rhodoferax* and *Kocuria* existed in both the Zadang and East Rongbu glaciers, and the Puruogangri (Zhang et al. 2006) and Malan glaciers (Xiang et al. 2004). Besides being in the Guoqu and Zadang Glaciers, the genera

Pseudomonas, Arthrobacter, and *Hymenobacter* existed in the Guliya, Malan, or Puruogangri Glaciers. Bacteria belonging to the above genera were distributed widely in glaciers located at different regions of the plateau. This implies that the same selective mechanism occurs across the plateau, and that these bacteria have certain abilities to endure the harsh living conditions such as low temperature, low nutrients, high light, and UV irradiation. For example, the *Sphingomonas* bacteria have the ability to utilize a wide range of organic compounds and to grow and survive under low-nutrient or starvation conditions. Bacteria in most of these genera (*Bradyrhizobium*, *Sphingomonas*, *Polaromonas*, *Acinetobacter*, *Pseudomonas, Arthrobacter, Hymenobacter*, *Rhodoferax*, *Kocuria*) have also been isolated from Antarctic or Arctic glaciers (Christner et al. 2000; Miteva et al. 2004).

Relationship between bacterial diversity and environment of the Tibetan Plateau

Snow bacteria at different glaciers had specific characteristics of their own. The Guoqu Glacier at the north of the Tanggula mountains near the desert zone with the lowest temperature preserved more bacteria closely related to cold environment and soil than other glaciers (Table 3). Most bacteria were affiliated to the genera *Hymenobacter*, *Arthrobacter,* and *Polaromonas*, which are soil bacteria with strains isolated from cold environments. On the contrary, in the Palong No. 4 Glacier located at the south warm region around vegetation, fewer bacteria were similar to psychrophilic or psychrotrophic bacteria, but more bacteria were related to plant-associated bacteria (Table 3). The plant-associated genera *Mesorhizobium, Pannonibacter, Phyllobacterium,* and *Rhizobium* only occurred in this glacier. In the East Rongbuk Glacier at the highest latitude among the four glaciers, there is less soil dust input (Liu et al. 2006), and the abundance of soil bacteria was the least. Most bacteria here were related to aquatic bacteria, indicating the influence of the Indian monsoon and the number of lakes at the end of the glacier. Snow bacteria showed connection with their located geographic site and the influence of various atmosphere currents.

Conclusion

Abundant bacteria existed in snow at the world's highest plateau, ranging from 0.68 to 720 $\times$ 10^3 cells mL^{-1}. Most of them were similar to those in the Antarctica, and less than those in the Arctic and other high mountains. The abundance of snow bacteria was higher at the northern glaciers than at the southern ones. Cell abundance was related with the input dust concentration but did not show obvious correlation with the nutrient condition.

Unexpected high diverse bacteria dwelled in snow over the TP. Bacterial 16S rRNA gene sequences affiliated to 13 phyla and 82 genera. Among them, 15 common genera distributed widely in glaciers located at different regions of the plateau, implying that the same selective mechanism occurs at plateau. Bacteria in different glaciers showed their own features and connected with their locations, indicating the relationship between bacteria and environment.

Acknowledgments This work was supported by the Ministry of Science and Technology of the People's Republic of China (Grant No. 2005CB422004) and the National Natural Science Foundation of China (Grant No. 40671044, 40471022, 40771187). Professor John Hodgkiss is thanked for his help with English.

References

Abyzov SS, Mitskevich IN, Poglazova MN (1998) Microflora of the deep glacier horizons of central Antarctica. Microbiology 67:66–73

Alfreider A, Pernthaler J, Amann R, Sattler B, Glockner F, Wille A, Psenner R (1996) Community analysis of the bacterial assemblages in the winter cover and pelagic layers of a high mountain lake by in situ hybridization. Appl Environ Microbiol 62:2138–2144

Amato P, Hennebelle R, Magand O, Sancelme M, Delort AM, Barbante C, Boutron C, Ferrari C (2007) Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol Ecol 59:255–264

Balows A, Truper HG, Dworkin M, Harder W, Schleifer KH (1992) The prokaryotes, vol 2, 2nd edn edn. Springer, New York

Barnett TP, Dumenil L, Schlese U, Roeckner E, Latif M (1989) The effect of Eurasian snow cover on regional and global climate variations. J Atmos Sci 46:661–685

Brosius J, Palmer ML, Kennedy JP, Noller FH (1978) Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. Proc Natl Acad Sci USA 75:4801–4805

Buck CF, Mayewski PA, Spencer MJ, Whitlow S, Twickler MS, Barrett D (1992) Determination of major ions in snow and ice cores by ion chromatography. J Chromatogr 594:225–228

Carpenter EJ, Lin S, Capone DG (2000) Bacterial activity in South Pole snow. Appl Environ Microbiol 66:4514–4517

Christner BC (2002) Detection, recovery, isolation and characterization of bacteria in glacial ice and lake Vostok accretion ice. PhD thesis, Ohio State University, Columbus

Christner BC, Mosley-Thompson E, Thompson LG, Zagorodnov V, Sandman K, Reeve JN (2000) Recovery and identification of viable bacteria immured in glacial ice. Icarus 144:479–485

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2003) Bacterial recovery from ancient glacial ice. Environ Microbiol 5:433–436

Cole JR, Chai B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM, Tiedje JM (2005) The ribosomal database project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res 33:294–296

Duan KQ, Yao TD, Thompson LG (2006) Response of monsoon precipitation in the Himalayas to global warming. J Geophys Res 11:D19110. doi:10.1029/2006JD007084

Garrison DL, Sulivan CW, Ackley SF (1986) Sea ice microbial communities in Antarctica. Bioscience 36:243–250

Hoham RW, Duval B (2001) Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: Jones HG, Pomeroy JW, Walker DA, RW H (eds) Snow ecology: an interdisciplinary examination of snow-covered ecosystems. Cambridge University Press, Cambridge, pp 168–228

Jiao NZ, Yang YH, Hong N, Ma Y, Harada S, Koshikawa H, Watanabe M (2005) Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea. Cont Shelf Res 25:1265–1279

Jones HG (1999) The ecology of snow-covered systems: a brief overview of nutrient cycling and life in the cold. Hydrol Process 13:2135–2147

Kemp PF, Aller JY (2004) Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. FEMS Microbiol Ecol 47:161–177

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Liu YQ, Yao TD, Tian LD, Xu BQ, Wu GJ (2006) Glaciochemical records from Naimonia'Nyi ice core in the Himalayas. J Geograph Sci 16:465–471

Luo TX, Pan YD, Ouyang H, Shi PL, Luo J, Yu ZL, Lu Q (2004) Leaf area index and net primary productivity along subtropical to alpine gradients in the Tibetan Plateau. Glob Ecol Biogeogr 13:345–358

Maidak BL, Cole JR, Lilburn TG, Parker CT, Saxman PR, Farris RJ, Garrity GM, Olsen GJ, Schmidt TM, Tiedje JM (2001) The RDP-II (ribosomal database project). Nucleic Acids Res 29:173–174

Marie D, Partensky F, Jacquet S, Vaulot D (1997) Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. Appl Environ Microbiol 63:186–193

Miteva V (2007) Bacteria in snow and glacier ice. In: Margesin R, Schinner F, MJ C, Gerday C (eds) Psychrophiles: from biodiversity to biotechnology. Springer, Berlin, pp 31–50

Miteva VI, Sheridan PP, Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 70:202–213

Painter TH, Duval B, Thomas WH, Mendez M, Heintzelman S, Dozier J (2001) Detection and quantification of snow algae with an airborne imaging spectrometer. Appl Environ Microbiol 67:5267–5272

Sattler B, Puxbaum H, Psenner R (2001) Bacterial growth in supercooled cloud droplets. Geophys Res Lett 28:239–242

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506

Segawa T, Miyamoto K, Ushida K, Agata K, Okada N, Kohshima S (2005) Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains, Japan, analyzed by 16S rRNA gene sequencing and real-time PCR. Appl Environ Microbiol 71:123–130

Shi Y (2000) Glacier and their environment in China. Science Press, Beijing

Stibal M, Sabacka M, Kastovska K (2006) Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb Ecol 52:644–654

Takeuchi N (2001) The altitudinal distribution of snow algae on an Alaska glacier (Gulkana Glacier in the Alaska Range). Hydrol Process 15:3447–3459

Thompson LG, Yao T, Mosley-Thompson E, Davis ME, Henderson KA, Lin PN (2000) A high-resolution millennial record of the South Asian Monsoon from Himalayan ice cores. Science 289:1916–1919

Tian LD, Yao TD, Li Z, MacClune K, Wu GJ, Xu BQ, Li YF, Lu AX, Shen YP (2006) Recent rapid warming trend revealed from the isotopic record in Muztagata ice core, eastern Pamirs. J Geophy Res 111:D13103. doi:10.1029/2005JD006249

Xiang SR, Yao TD, An LZ, Li Z, Wu GJ, Wang YQ, Xu BQ, Wang JX (2004) Change of bacterial community in the Malan Ice Core and its relation to climate and environment. Chin Sci Bull 49:1869–1875

Yao TD, Wu GJ, Pu JC, Jiao KQ, Huang CL (2004) Relationship between calcium and atmospheric dust recorded in Guliya ice core. Chin Sci Bull 49:706–710

Yao TD, Xiang SR, Zhang XJ, Wang NL, Wang YQ (2006) Microorganisms in the Malan ice core and their relation to climatic and environmental changes. Global Biogeochem Cycles 20:GB1004. doi:1010.1029/2004GB002424

Yoshimura Y, Kohshima S, Ohtani S (1997) A community of snow algae on Himalayan glacier: change of algal biomass and community structure with altitude. Arct Alp Res 29:126–137

Zhang X, Yao T, An LZ, Tian LD (2006) A study on the vertical profile of bacterial DNA structure in the Puruogangri (Tibetan Plateau) ice core using denaturing gradient gel electrophoresis. Ann Glaciol 43:160–166

Zheng D (1996) The system of physico-geographical regions of the Qinghai-Xizang (Tibet) plateau. Sci China Ser D 39:410–417