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Abundance and community of snow bacteria from three glaciers in the Tibetan Plateau

Shuhong Zhang[1,3], Guangli Yang[1], Yetang Wang[3,4], Shugui Hou[2,3,*]

1. Department of Life Science, Shangqiu Normal University, Shangqiu 476000, China. E-mail: mariazhangsh@yahoo.com.cn
2. School of Geographic and Oceanographic Sciences, Nanjing University, Nanjing 210093, China
3. Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, China
4. Shandong Marine Fisheries Research Institute, Yantai 264006, China

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Abstract

Bacterial abundance and diversity in snow of East Rongbuk, Laohugou and Hailuogou glaciers on the Tibetan Plateau were investigated through epifluorescence microscope and denaturing gradient gel electrophoresis. Cell abundance ranged from 4.0×10^3 to 290.2×10^3 cells/mL. The phylogenetic trees placed the 16S rRNA sequences in four major groups: Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. *Brevundimonas*, *Flavobacterium*, *Hymenobacter*, *Bacillus*, *Polaromonas*, *Rhodoferax* and *Streptomyces* were widely distributed bacteria in glaciers from different cold regions. The remaining five genera of *Hylemonella*, *Delftia*, *Zoogloea*, *Blastococcus* and *Rhodococcus* were endemism, only recovered from our investigated glaciers. It is proposed that the three glaciers on the Tibetan Plateau provide a specific ecological niche for prolonging survival of diverse microbial lineages.

Key words: bacterial diversity; glacial snow; the Tibetan Plateau
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Introduction

Microorganisms in the glaciers have received increasing attention during the past decade. Diverse bacteria have been recovered from the deep ice core of polar and alpine glaciers. The results provide important clues to lifestyles that might be encountered on Mars and Europa (Christner et al., 2000, 2001, 2003; Miteva et al., 2004; Sheridan et al., 2003). A significant number of bacteria beneath glaciers are reported to play important roles in chemical weathering and carbon cycling processes (Cheng and Foght, 2007; Foght et al., 2004; Sharp et al., 1999; Skidmore et al., 2000, 2005). Due to the importance of the Tibetan Plateau in the study of climatic and environmental changes, many research works have focused on microorganisms and their relationships with climatic and environmental changes (Christner et al., 2000; Xiang et al., 2004; Yao et al., 2006; Zhang et al., 2006, 2007a, 2008a). Results from previous studies show that microbial records from ice cores could potentially reflect past climatic and environmental changes.

However, information on glacial snow bacteria is still very limited. The earliest report which document active metabolism of bacteria was about the surface snow of south pole in 2000. Evidence was obtained about low rates of bacterial DNA and protein synthesis which indicates that the organisms were metabolizing at ambient subzero temperatures (–12 to –17°C) (Carpenter et al., 2000). Then, in 2005, bacterial biomass were analyzed in mountain snow from the Tateyama Mountains, Japan. The differences in development observed among bacterial species suggest that their growth was promoted by different nutrients and/or environmental conditions in the snow (Segawa et al., 2005).

In China, there are more than 46,000 glaciers, with a total area of > 59,000 km^2 (Zhu et al., 2003). To our knowledge, snow bacteria were investigated from Cho Oyu (Tong et al., 2008), Guoqu, Zadang, East Rongbuk and Palong No. 4 (Liu et al., 2009) on the Tibetan Plateau. However, for the Laohugou Glacier, a complex valley glacier with extreme continental feature and the largest in the Qilian Mountains (Du et al., 2008), Hailuogou Glacier and the most typical monsoon maritime one in China (Li et al., 2008), bacterial communities have not been previously explored. Our work filled the gap to explore the invaluable bacterial species from the sampling sites and illuminated the spatial differences of bacterial community from glaciers on the Tibetan Plateau.

1 Materials and methods

1.1 Sample collection

In May 2006, a snow pit with depth of 1.00 m was dug

* Corresponding author. E-mail: shugui@lzb.ac.cn

at East Rongbuk (ER) Glacier (28°01′05″N, 86°57′52″E, 6524 m a.s.l.). In June 2006, other two pits, 1.05 and 1.40 m in depth, were dug at Laohugou Glacier (39°26′N, 96°33′E, 4700 m a.s.l.) and Hailuogou Glacier (29°32′N, 101°56′E, 4807 m a.s.l.), respectively. Surface samples with 0.10, 0.15, and 0.50 m in depth were discarded respectively from glaciers of ER, Laohugou and Hailuogou. The underlying 3 sediments (each of 30 cm) were placed into sterile high density polyethylene containers. Extreme care was taken at all times during the whole sampling processes to against contamination. Nonparticulating sterile suits, sterile gloves and masks were used during the entire sampling process. The same types of empty containers used as negative control were opened during sample collection and handled in the same way as snow samples. The samples were transported in a frozen state with dry ice to a cold room at about –20°C and were stored there before analysis.

1.2 Total cell counts

Treatment procedures of the samples by SYBR Green-II (Molecular Probes, Inc., USA) was modified from Yamagishi et al. (2003). Total cell counts were also carried out according to Yamagishi et al. (2003), with detailed description by Zhang et al. (2008a).

1.3 DGGE analysis

The empty containers used as negative control during snow sample collection were rinsed with 250 mL sterile high purity water. Each of the melted snow sample (300 mL) and the above rinsed water were filtered through hydrophilic polyethersulfone membranes (Pall; 0.22 μm pore size) with a vacuum pump (Ntengwe, 2005). The microorganisms on the membranes were eluted by agitation for 2 min by hand and sonication for another 2 min (model 14; Branson Ultrasonics Corp., USA) (Uga et al., 2003) and suspended in 2.0 mL phosphate-buffered saline. The suspension for the negative control and snow samples was used for the extraction of genomic DNA according to Zhou et al. (1996). The pellets of crude nucleic acid were finally dried and resuspended in 30 μL of sterile deionized water and quantified by UV spectrophotometer (UV 7501, Techcomp, China).

DNA preparations from both the sample and negative control were used as templates and bacterial specific primers 357F-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the V3 hypervariable region (Crump et al., 2003) of the 16S rRNA gene by PCR by the method of Zhang et al. (2008a). PCR products were purified using a QIA quick PCR mini kit (Qiagen, Germany) according to the manufacturer's instructions and quantified by UV spectrophotometer (UV 7501, Techcomp, China).

Two hundred fifty nanogram of purified amplicons were used in DGGE analysis according to Mccaig et al. (2001), with detailed description by Zhang et al. (2008a).

1.4 Sequences of recovered DGGE bands

Each gel slice that contained an obvious DNA band was excised and subjected to a second PCR under the same conditions as described in Section 1.3 (Díez et al., 2001). The amplified products were isolated in gels and purified using a QIA quick PCR mini kit (Qiagen, Germany). The purified DNA fragments were cloned into pGEM®-T Easy Vector (Promega, Madison, Wisconsin, USA). *E. coli* DH5α (Takara) cells was transformed with the cloning vector, and transformants were selected by blue-white selection on Luria-Bertani agar plates containing ampicillin (100 g/mL), X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside; 1 mg/plate), and IPTG (isopropyl-beta-D-thiogalactopyranoside; 2.38 mg/plate). The positive colonies were sequenced directly using primer M13F with a state-of-the-art ABI 3730XL96 capillary sequencer. The sequences obtained were compared to those in the GenBank database by BLAST algorithm to identify sequences with a high degree of similarity and were deposited in the EMBL nucleotide sequence database under accession numbers FM865647 to FM865660, FM865666 to FM865675 and FM865695 to FM865703.

2 Results

2.1 Bacterial concentrations in the three glaciers

Concentration of total bacteria deposited from ER Glacier was in the range of 4.0×10^3–13.0×10^3 cells/mL (Fig. 1). That from Laohugou Glacier varied between 238.1×10^3 and 290.2×10^3 cells/mL and that from Hailuogou Glacier varied between 80.0×10^3 and 139.8×10^3 cells/mL (Fig. 1).

2.2 Phylogenetic diversity of 16S rRNA sequences from DGGE bands of glacial snow in ER

The bacterial communities associated with the three glacial snow were examined by DGGE analysis. Several major bands (Fig. 2) were excised and sequenced. The sequences of the samples from ER glacial snow were affiliated with three phylogenetic groups: β-Proteobacteria, Actinobacteria and Firmicutes (Fig. 3). β-Proteobacteria was predominant, with ER-UC-5 not strongly affiliated with any gene sequences available in public databases.

The organisms of Firmicutes fell into four major lineages. Each of ER-UC-9 and ER-UC-5 formed one cluster. ER-UC-9 had 93% sequence similarity to uncultured *Hymenobacter* sp. (DQ076431). ER-UC-5 had 95% sequence similarity to *Polaromonas aquatica* (AM039831). The two isolates were not clustered together with their nearest neighors, respectively, indicating that they were possibly new taxons within the β-Proteobacteria phylum. ER-UC-3 formed the third cluster, with 100% high similarity to glacier bacterium FJI50 (AY315174). However, no specific genus could be found to match with this isolate. ER-UC-2, ER-UC-4 and ER-UC-8 formed the fourth cluster. ER-UC-4 had *Rhodoferax ferrireducens* (AM265401) as the nearest neighbor, with 96% sequence similarity. Whereas phylogenetic analysis showed that the two sequences did not cluster together. ER-UC-2 had 98% sequence identity to that of *Polaromonas naphthalenivorans* CJ2

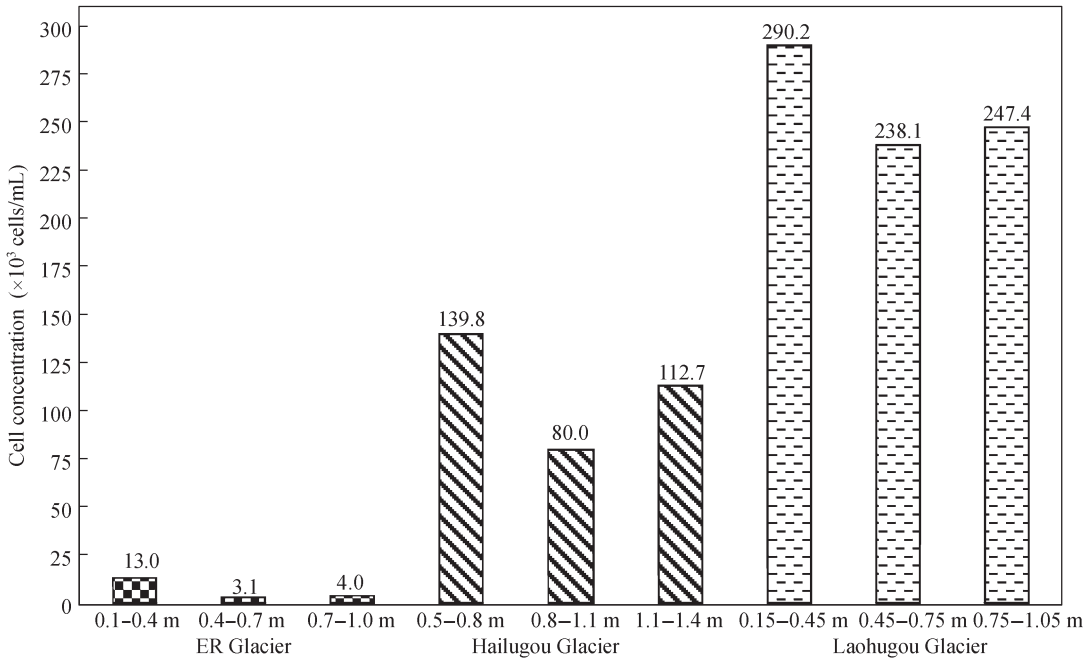

Fig. 1 Bacterial concentration in different layers of three glacial snow pits on the Tibetan Plateau. ER: East Rongbuk.

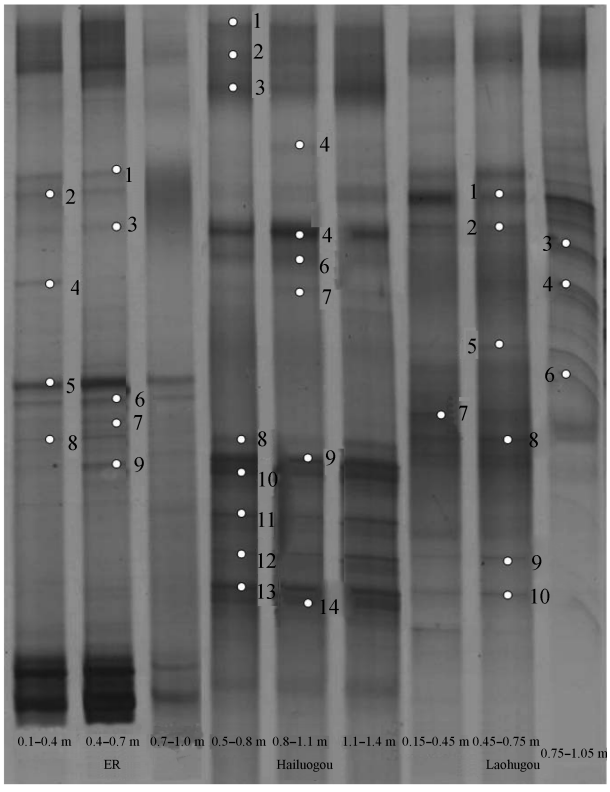

Fig. 2 DGGE profile of PCR product amplified from 16S rRNA (V3) gene of three glacial snow pits from ER, Hailuogou and Laohugou.

(AY166684). ER-UC-8 was related to *Hylemonella* sp. 32AD14 (AB242706), with 96% sequence similarity, indicating a possibe new taxon within β-Proteobacteria phylum. One bacterial sequence of ER-UC-7 was affiliated with Actinobacteria. It exhibited a similarity of 99% with *Rhodococcus* sp. ZY-2006b (DQ986367). Two bacterial sequences were associated with Firmicutes, into which *Bacillus* sp. was dominated. ER-UC-1 had 100% high sequence identity to that of *Bacillus* sp. (Y13066). ER-UC-6 was related to *Bacillus* sp. GB02-25 (DQ079010), with 97% sequence similarity.

2.3 Phylogenetic diversity of 16S rRNA sequences from DGGE bands of glacial snow from Laohugou

The isolates retrieved from Laohugou glacial snow were composed of Actinobacteria, α-Proteobacteria, β-Proteobacteria, and Bacteroidetes (Fig. 4). *Brevundimonas* sp. (α-Proteobacteria) dominated among sequences from the glacial snow.

Regarding Actinobacteria, a total of four sequences were close to *Rhodococcus* sp. LHG-UC-2 had 98% sequence similarity to *Rhodococcus* sp. 28/19 (DQ310477). LHG-UC-3 had *Rhodococcus* sp. 11/16a (DQ310479) as the nearest neighbor, with 99% sequence similarity. Both LHG-UC-4 and LHG-UC-6 showed 100% sequence similarity to *Rhodococcus* sp. ZY-2006b (DQ986367).

Considering α-Proteobacteria, one sequence was affiliated with it. LHG-UC-1 exhibited a high similarity of 100% with *Brevundimonas nasdae* (AM412000). Regarding β-Proteobacteria, a total of 4 sequences forming two clusters were affiliated with it. LHG-UC-7 and LHG-UC-8 were within one cluster of Comamonadaceae family. They had 100% high sequence identity to that of *Hylemonella gracilis* (DQ861289) and uncultured *Delftia* sp. (DQ856785). LHG-UC-9 and LHG-UC-10 formed the other cluster, and were associated with uncultured bacterium (DQ675493), with 98% and 97% sequence similarity, respectively.

One bacterial sequence was affiliated with Bacteroidetes. LHG-UC-5 exhibited a similarity of 99% with

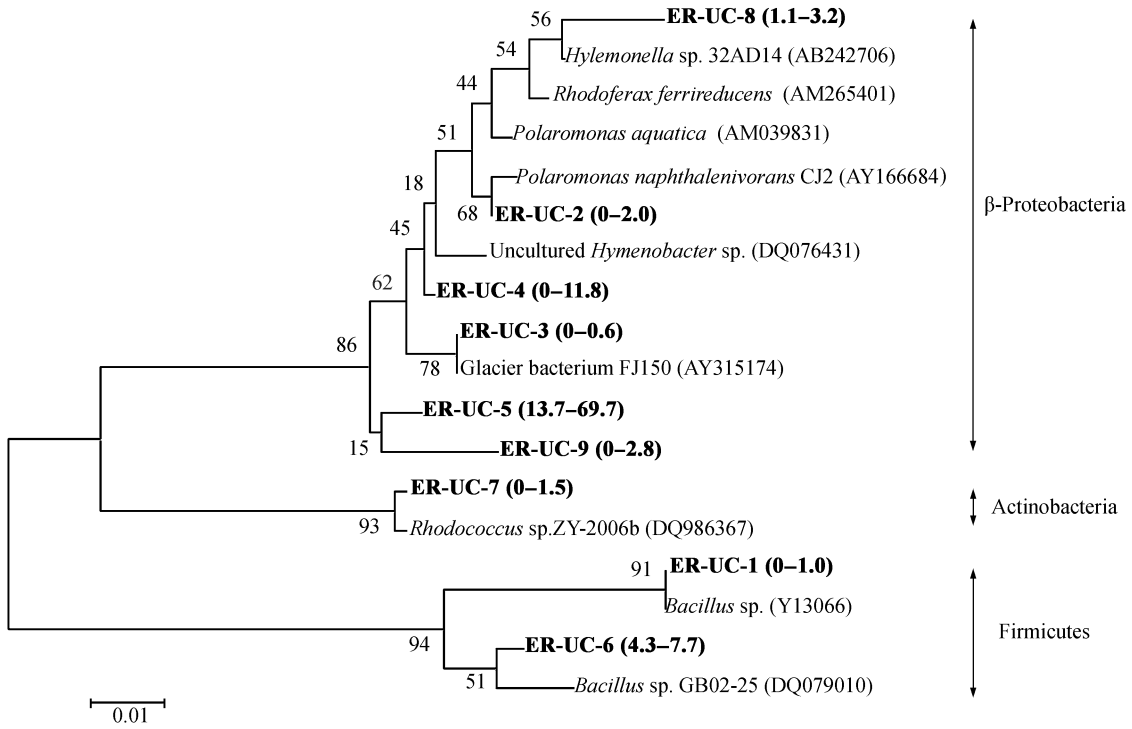

Fig. 3 Neighbor-joining phylogenetic tree based on 16S rDNA sequences from DGGE bands of glacial snow from ER. The numbers of the sequences refer to those in Fig. 2. The numbers in the brackets indicated signal intensity (%) of the corresponding DGGE bands. Only > 50% bootstrap values (1000 replications) were indicated at nodes. Scale bar represents observed number of changes per nucleotide position.

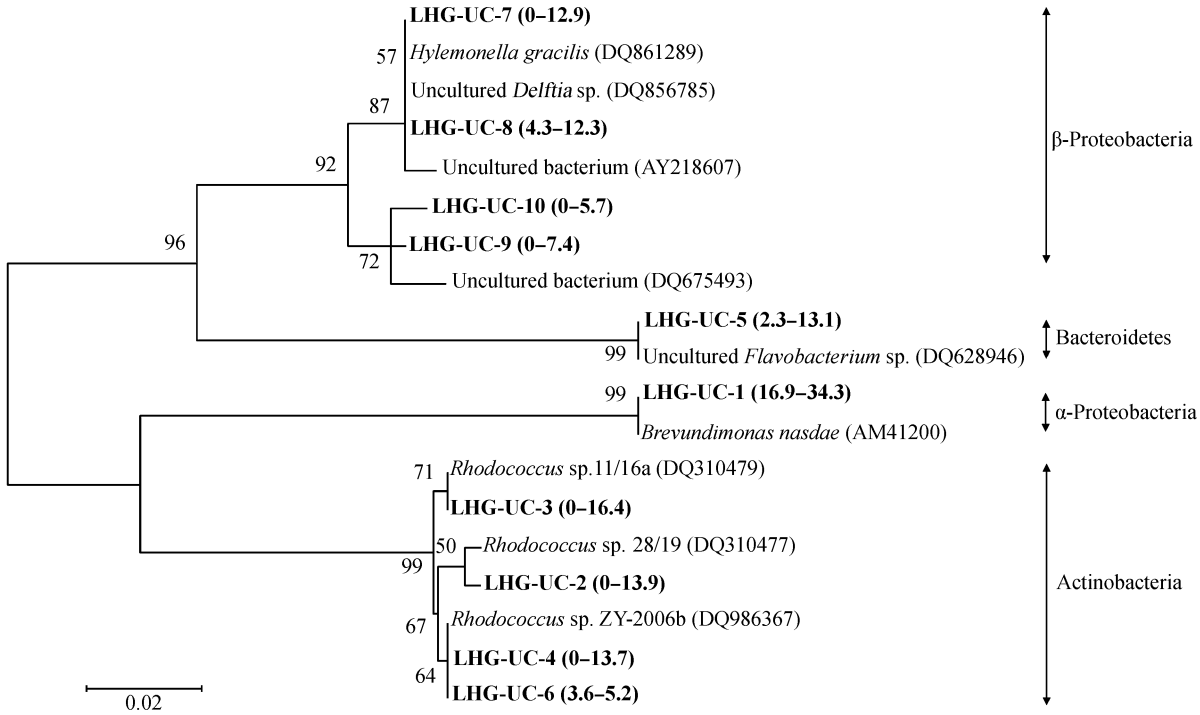

Fig. 4 Neighbor-joining phylogenetic tree based on 16S rDNA sequences from DGGE bands of glacial snow from Laohugou. The numbers of the sequences refer to those in Fig. 2. The numbers in the brackets indicated signal intensity (%) of the corresponding DGGE bands. Only > 50% bootstrap values (1000 replications) were indicated at nodes. Scale bar represents observed number of changes per nucleotide position.

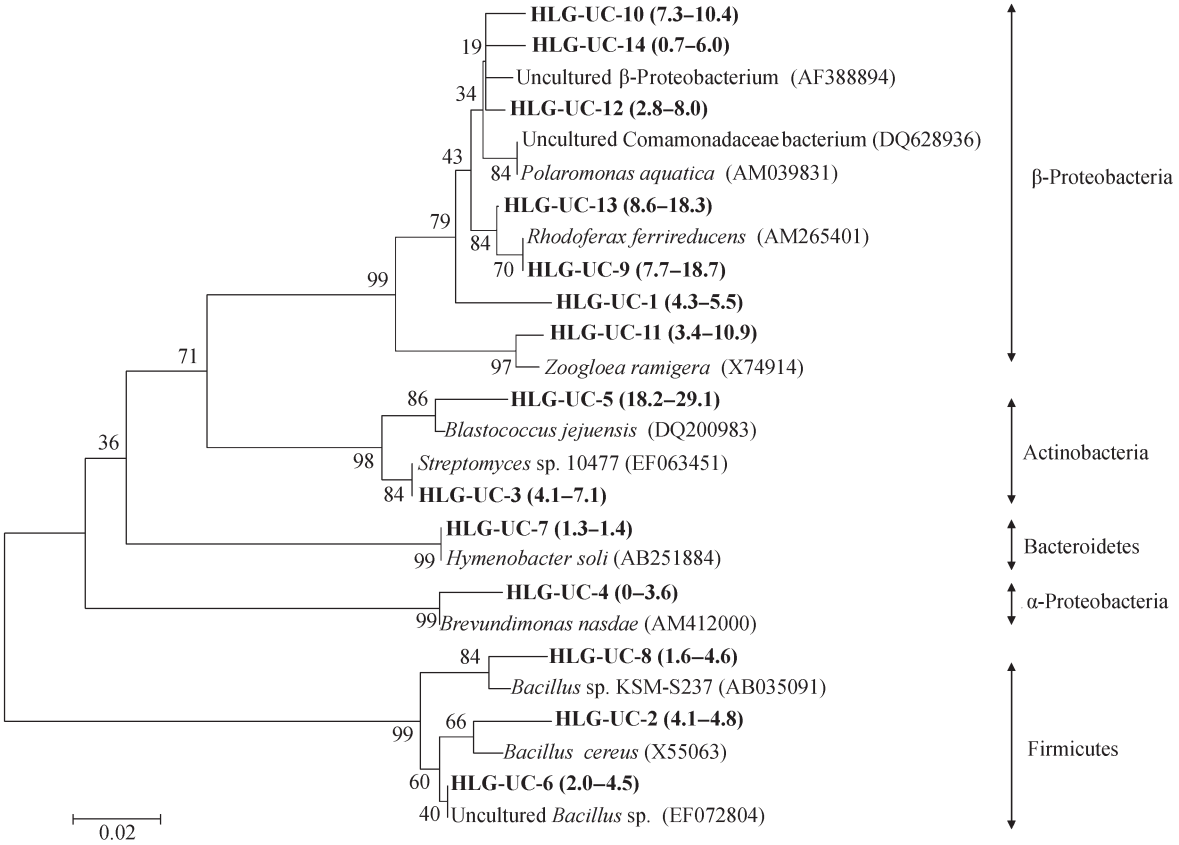

Fig. 5 Neighbor-joining phylogenetic tree based on 16S rDNA sequences from DGGE bands of glacial snow from Hailuogou. The numbers of the sequences refer to those in Fig. 2. The numbers in the brackets indicated signal intensity (%) of the corresponding DGGE bands. Only > 50% bootstrap values (1000 replications) were indicated at nodes. Scale bar represents observed number of changes per nucleotide position.

uncultured *Flavobacterium* sp. (DQ628946).

2.4 Phylogenetic diversity of 16S rRNA sequences from DGGE bands of glacial snow from Hailuogou

The bacterial species represented in glacial snow from Hailuogou were Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes (Fig. 5). The Proteobacteria were distributed in β- and α-subgroups. *Blastococcus* sp. belonging to Actinobacteria was the most abundant.

Regarding Firmicutes, a total of three sequences were close to *Bacillus* sp. HLG-UC-2 showed 100% sequence identity to *Bacillus thuringiensis* (DQ078742) and 94% sequence identity to *Bacillus cereus* (X55063). HLG-UC-6 exhibited 100% similarity to uncultured *Bacillus* sp. (EF072804). HLG-UC-8 was related to *Bacillus* sp. KSM-S237 (AB035091), with 96% sequence similarity, implying that it was probably a new taxon in Firmicutes.

Considering α-Proteobacteria, one sequence was affiliated with it. HLG-UC-4 exhibited a similarity of 99% with *Brevundimonas nasdae* (AM412000). Seven sequences were clustered into β-Proteobacteria, forming three groups. HLG-UC-11 formed one and was 97% similarity to *Zoogloea ramigera* (X74914). HLG-UC-1 formed another one and was 96% identity to *Rhodoferax ferrireducens* (AM265401). The above two sequences were possibly new taxons within β-Proteobacteria phylum. Five sequences formed the third cluster of the Comamonadaceae family. HLG-UC-9 was 99% similarity with *Rhodoferax ferrireducens* (AM265401). HLG-UC-13 showed 99% identity with uncultured Comamonadaceae bacterium (DQ628936), but did not group together with it. HLG-UC-12 exhibited a similarity of 97% with uncultured Comamonadaceae bacterium (DQ628936). HLG-UC-10 and HLG-UC-14 were related to uncultured β-Proteobacterium (AF388894), with 96% and 95% sequence similarity, respectively. HLG-UC-10, HLG-UC-12 and HLG-UC-14 possibly indicated new taxons within β-Proteobacteria phylum.

One bacterial sequence was affiliated with Bacteroidetes. HLG-UC-7 exhibited a similarity of 98% with uncultured *Hymenobacter soli* (AB251884).

Regarding Actinobacteria, two sequences forming two clusters were affiliated with it. HLG-UC-5 within one cluster was related to *Blastococcus jejuensis* (DQ200983), with 98% sequence similarity. HLG-UC-3 within the other cluster showed 99% sequence identity with *Streptomyces* sp. 10477 (EF063451).

3 Discussion and conclusions

The bacterial concentration in ER Glacier was close to that in the surface snow and firn (200–5000 cells/mL) from the South Pole (Carpenter et al., 2000). The result was consistent to our previous study in which bacterial concentration in ER Glacier is among the level of polar regions (Zhang et al., 2007a). Meanwhile, bacterial concentrations

in Hailuogou Glacier and Laohugou Glacier yielded similarity to that of glacial snow from Guoqu (450×10^3 cells/mL, Liu et al., 2009), Puruogangri ice core (94.7×10^3 cells/mL, Zhang et al., 2008b), snow pack of Spitzberg, Svalbard (2×10^4–2×10^5 cells/mL, Amato et al., 2007), and mountain snow in the Tateyama Mountains, Toyama Prefecture, Japan (84×10^3 cells/mL, Segawa et al., 2005).

The results of the collection of phylogenetically diverse 16S rRNA sequences from the three glacial snow of the Tibetan Plateau made it possible to compare them with other bacteria from geographically different glaciers. Among the snow bacteria, some genera showed widespread distribution in glaciers of cold regions. *Brevundimonas* sp. had been recovered from three ice cores of Guliya (Christner et al., 2000), Muztagh Ata (Xiang et al., 2005) and Puruogangri (Zhang et al., 2008b). *Flavobacterium* was isolated from Muztagh Ata (Xiang et al., 2005), Guliya (Christner et al., 2000), Malan (Zhang et al., 2003), Puruogangri (Zhang et al., 2008b) ice cores and mountain snow from Tateyama Mountains (Segawa et al., 2005). *Hymenobacter* was recovered from Puruogangri ice core (Zhang et al., 2008b). *Bacillus* was frequently recovered from three ice cores of Malan (Zhang et al., 2003), Guliya (Christner et al., 2000), Puruogangri (Zhang et al., 2008b) and glacial snow of South Pole (Carpenter et al., 2000). *Polaromonas* was isolated from Malan ice core (Zhang et al., 2003), two subglacial environments of Bench Glacier and John Evans Glacier (Skidmore et al., 2005), glacial snow of South Pole (Carpenter et al., 2000). *Rhodoferax* was recovered from ice cores of Puruogangri (Zhang et al., 2008b) and Malan (Zhang et al., 2003). *Streptomyces* was isolated from Malan ice core (Zhang et al., 2003). The phenomena of widely distributed bacteria in glaciers from different regions implies that the similar selective mechanism occurs across the cold regions (Zhang et al., 2007b), and that these bacteria have certain abilities to endure the harsh living conditions such as low temperature, low nutrients, high light, and UV irradiation (Liu et al., 2007). However, a few sequences showed relatively low identity with above described species, suggesting that the three glacial snow on the Tibetan Plateau provided a specific ecologic niche that accommodates an original microbial assemblage.

The remaining five genera of *Hylemonella*, *Delftia*, *Zoogloea*, *Blastococcus* and *Rhodococcus* were only recovered from our studied samples. They were probably endemism of the glaciers. The results revealed the distinctive differences among bacterial communities from the 3 glacial habitats in high altitude regions. The notable distinction was most likely caused by the sampling sites with different local environments (Liu et al., 2009) or the change in niche from snow to ice, consequently, differences in selection pressure on the bacterial community (Liu et al., 2007). However, due to the short 16S rRNA sequences, it is difficult to determine the exact phylogenetic assignments of some band sequences obtained. For instance, ER-UC-7 was 174 bp, it was associated with both *Rhodococcus* sp. ZY-2006b (DQ986367) and *Lechevalieria xinjiangensis* (DQ898283), with 99% sequence identity. Therefore, further studies are necessary to identify the isolates, and present their characteristics, such as their phylogenetic relatedness to known microbes. In addition, the factors leading to the spatial differences of bacterial community are still necessary to be explored in studied future work.

In conclusion, seven genera were widespread on glacial regions and five were unique in studied sampling sites. Some unculturable and novel bacteria also existed. Our study pointed out the important diversity of bacterial communities from the three glaciers in the Tibetan Plateau.

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