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Diversity of Bacterial Communities in the Snowcover at Tianshan Number 1 Glacier and its Relation to Climate and Environment

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The bacterial distribution, and its relationship with climate and environment factors were investigated in the snowcover at Tianshan Number 1 Glacier. The results showed that psychrotrophs were the preponderant bacteria in pit samples, though they were not the dominant species in the new fallen snow. The quantity and diversity of the cultivable bacteria decreased with the passage of time, indicating that the bacterial community acclimatized to low temperature by changing its structure. During this time, the peak number of the cultivable bacteria was associated with dirt layers, indicating that the bacterial input came with dust. Concurrently, the quantity and diversity of the cultivable bacteria showed a trend of variation similar to that shown by the δ^{18}O values and the soluble ion concentrations, indicating that the bacterial distribution was related to both temperature and the amount of dust transported onto the glacier. Phylogentic analyses of 16S rRNA indicated that all the isolates fell into six categories: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Cytophaga-Flavobacterium-Bacteroides (CFB) group bacteria, high G+C gram-positive bacteria, and low G+C gram-positive bacteria. In the snow pit, the abundance of the CFB group bacteria (mainly of the genus *Flavobacterium*) decreased from 55.5% to 1.49% with age, and fluctuated similar to the ion concentrations and the δ^{18}O value. Meanwhile the α-Proteobacteria (mainly of the genus *Brevundimonas*) increased from 0.9% to 88.1%, indicating that *Brevundimonas* was the dominant psychrotroph in the study area, whose abundance varied inversely compared to the above-mentioned chemical properties. All the results suggest that bacterial abundance and diversity vary with climate and the physical chemical microenvironment. The pattern of bacterial distribution could be a biological index for the record of climate and environment change in the Tianshan Number 1 Glacier.

Keywords Cultivable bacteria, 16S rRNA, Climate and environment change, Tianshan Number 1 Glacier

INTRODUCTION

Snow permanently covers 35% of the Earth's surface or is present for varying times during the year (Liu et al. 2009a; Miteva 2007). Microbes play important roles in snow ecology, but they have been less studied (Hoham and Duval 2001). Bacterial abundance in snow varied between 10^2 to 10^5 cells/ml in the worldwide (Amato et al. 2007; Carpenter et al. 2000; Garrison et al. 1986; Sattler et al. 2001; Segawa et al. 2005). Carpenter et al. (2000) reported the presence of Thermus-Deinococcus-like organisms and low rates of bacterial DNA and protein synthesis in South Pole snow. Amato et al. (2007) used culture-based methods to isolate 10 bacterial strains and found they belonged to α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, high G+C gram-positive bacteria, and low G+C gram-positive bacteria. Liu et al. (2009a) found α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, high G+C gram-positive bacteria, low G+C gram-positive bacteria, CFB group and other categories distributed in the snow of the four glaciers (Guoqu, Zadang, East Rongbuk and Palong No. 4) at the Tibetan Plateau by using 16S rRNA gene clonelibrary method. Xiang et al. (2009a) found similar results in the snow pits from Kuytun 51 Glacier of Tianshan Mountains.

The microorganisms are blown and deposited (aeolian deposition) with snow onto the surface of glaciers (Liu et al. 2009a, 2009b; Xiang et al. 2009b). Their distribution can be studied to produce records similar to the geochemical and geophysical climate records (insoluble microparticles, soluble ions, and δ^{18}O), which yield different types of climatic information and can record environmental changes (Rozanski et al. 2010; Zhang et al. 2010a; Zheng et al. 2010). The microbial abundance correlated with micro-particle and soluble ion concentrations in a glacier (Yao et al. 2006; Zhang et al. 2006; Zhang et al. 2008),

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given the ambiguous relationship between bacterial concentrations and ice core parameters.

The microbial abundance could also be correlated with the δ^{18}O value in a glacier, which could reflect the past temperature variation (Abyzov et al. 1998; Xiang et al. 2005; Yao et al. 2006; Uetake et al. 2006; Yao et al. 2008). Up to now, the distribution and growth of microorganisms in glaciers (including snow) as related to global climate and environmental changes has been rudimentary (Yao et al. 2006; Xiang et al. 2009b). Thus, studies of bacteria in glaciers (including snow) are important not only biologically but also glaciologically.

Tianshan Number 1 Glacier is located in the Eastern Tianshan Mountains of central Asia, which are surrounded by desert and Gobi (Lee et al. 2003). The climate in this area is a classical continental climate, and wind is an important climatic factor on the upper elevations of the Tianshan Mountain (Williams et al. 1992). Tianshan Number 1 Glacier has been studied intensively from the glaciological point of view since 1959, when the Tianshan Glaciological Station was built.

Because of the availability of extensive glaciological data, this location is a suitable for a study of how microbial distribution and growth relate to both the climatic and other environmental records. The objective of the present research is to evaluate the possible correlation of the distribution of bacteria in the snow and ice with the geochemical and geophysical climatic records on the Tianshan Number 1 Glacier. It identifies the dominant bacteria and discusses the evidence for their origin, evolution with age, and the snow-bacterial succession in relation to climatic and environment changes, so as to determine whether or not the record of bacterial distribution could be a biological index for climate and environment change.

MATERIALS AND METHODS

Sampling Sites and Sampling

The sample sites were located at Tianshan Nunber 1 Glacier (N43°06′, E86°48′), 120 km southwest of Urumchi, China. The top elevation at this glacier was 4486 m. Altogether, 23 samples were collected in October, 2007. Of these, 13 samples were from a 260 cm deep snow pit sampled every 20 cm. The samples from the surface to 100 cm depth represented the snow pack resulting from 2007, 100 cm to 160 cm accumulated during 2006, 180 cm to 240 cm were from 2005, and 260 cm amassed during 2004 (dates given by Tianshan Glaciological Station, Chinese Academy of Sciences, China) (Figure 1). The other 10 samples were collected from sub-surface snow at different altitudes ranging from 3732 m to 4099 m. To collect snow, the surface (about 20 cm in depth) snow was removed using a sterilized spoon and then discarded, and the underlying snow was collected for study.

All samples were placed into sterile 500-ml polyethylene HDPE containers that had been pushed into the snow without any additional manipulation. Sterile gloves and a mask were worn during the sampling process. The samples in all containers were kept frozen during transport from the glacier to the laboratory.

δ^{18}O, Cations and Anions

The δ^{18}O of the samples was determined using a mass spectrometer (MAT-252), and cations and anions were determined by ion chromatography (Dionex-300 for cations, and Dionex-100 for anions) (Qin et al. 2000) at the State Key Laboratory of Cryospheric Science, Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences.

Cultivation

All samples were thawed at 4°C and the resulting fluid passed through a cellulose-acetate filter membrane (0.2 μm) to remove any mineral particles. The particulates collected on the filter were resuspended in 0.9% NaCl solution by shaking adequately, then the suspensions generated were spread on the surface of culture medium. In a preliminary experiment, bacterial colonies grew better on PYGV medium (DSMZ medium 621, http://www.dsmz.de) than R_2A (DSMZ medium 830, http://www.dsmz.de) and M9 medium (DSMZ medium 382, http://www.dsmz.de). Accordingly, the PYGV medium was used for the rest of the study.

All the samples were cultivated on the PYGV medium at 4°C for three weeks and 25°C for 1 week, respectively. Subsequently, the number of colonies of each different type appearing on a plate were counted, and a colony of each different type was re-streaked on a separate plate.

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

Amplified ribosomal DNA restriction analysis (ARDRA) was used to group the 139 isolates. Genomic DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA genes were amplified with the universal primers 8F (5′-AgAgTTTgATCCTggCTCAg-3′) and 1492R (5′-ggTTACCTTgTTACgACTT-3′) (Zhang et al. 2007).

Amplification reactions were performed in a total volume of 25 μl containing 2 units of Taq DNA polymerase (Fermentas), 1 × Taq buffer, 2 mM $MgCl_2$, 0.2 mM concentration of each dNTP, 10 pmol primer each and approximately 20 ng template DNA. The thermal PCR profile was as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 58°C for 1min, elongation at 72°C for 1.5 min, and the final elongation step was 10 min at 72°C.

ARDRA analysis was performed on the samples using two restriction enzymes (*HaeIII* and *AluI*) at 37°C for 16 hours. The enzymes were inactivated by heating the preparations at 65°C for 15 min, and the reaction products were separated on a 2.5% gel (wt/vol) electrophoresis by using a 100-bp ladder as a marker in TAE buffer containing 0.5 μg of ethidium bromide per ml.

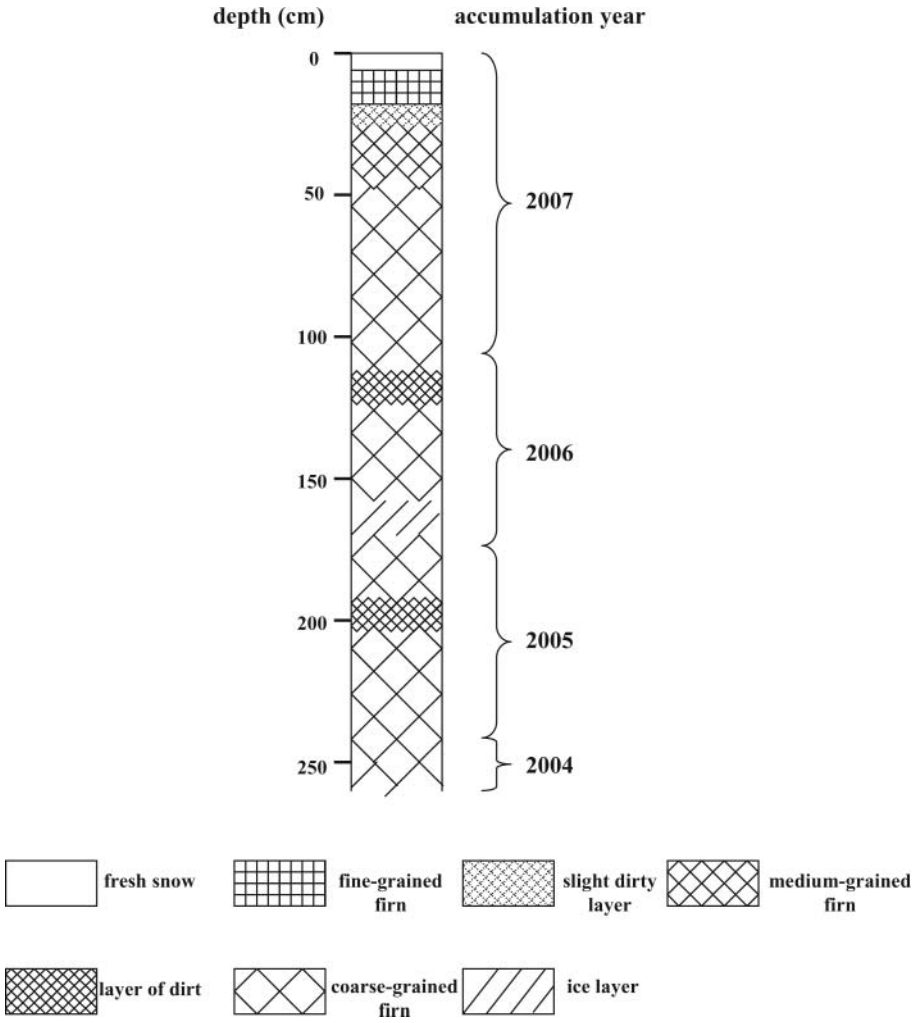

FIG. 1. Stratigraphic profile of the snow pit. The sampling depth, accumulation year and physical traits were mentioned.

Sequence and Phylogenetic Analysis

Based on amplified ribosomal DNA restriction analysis (ARDRA) of isolates, one representative strain of each group was selected for 16S rRNA gene sequence determination. Three primers were utilized for sequencing. They were 27F, 5′-AgAgTTTgATCCTggCTCAg-3′, 517F, 5′-CCAgCAgCCg CggTAAT-3′ and 907F, 5′-AAACTCAAATgAATTgACggg-3′ (Zhang et al. 2007). Almost-complete 16S rDNA nucleotide sequences were determined.

The 16S rRNA gene sequences were aligned against representative reference sequences of the most closely related members, obtained from the National Center for Biotechnology Information (NCBI) database (http://www.nvbi.nlm.nih.gov), by use of the multiple-alignment CLUSTALW 1.81 software package (Thompson et al. 1997). The method of Jukes and Cantor (1969) was used to calculate evolutionary distances. Phylogenetic dendrograms were constructed by the neighbor-joining method (Saitou and Nei 1987), and tree topologies were evaluated by performing bootstrap analysis of 1,000 data sets using of the MEGA4.1 package (Kumar et al. 2001).

Nucleotide Sequence Accession Numbers

The 16S rDNA sequences of the 31 representative strains isolated in this study have been deposited in GenBank database under the following accession numbers: FJ979834- FJ979864.

RESULTS

The Variation of Viable Bacteria Population and Their Relationship to Physical Chemistry Properties

Figure 2 showed the quantities and the diversity of the viable bacteria recovered from the 23 samples. After being incubated at 4°C for 3 weeks and 25°C for 1 week, the number of colony forming untis (CFU) on PYGV varied between 3 and 9,170 CFU/ml at 4°C and 7 to 3,295 CFU/ml at 25°C. The number of cultivable bacteria was significantly higher at the depths

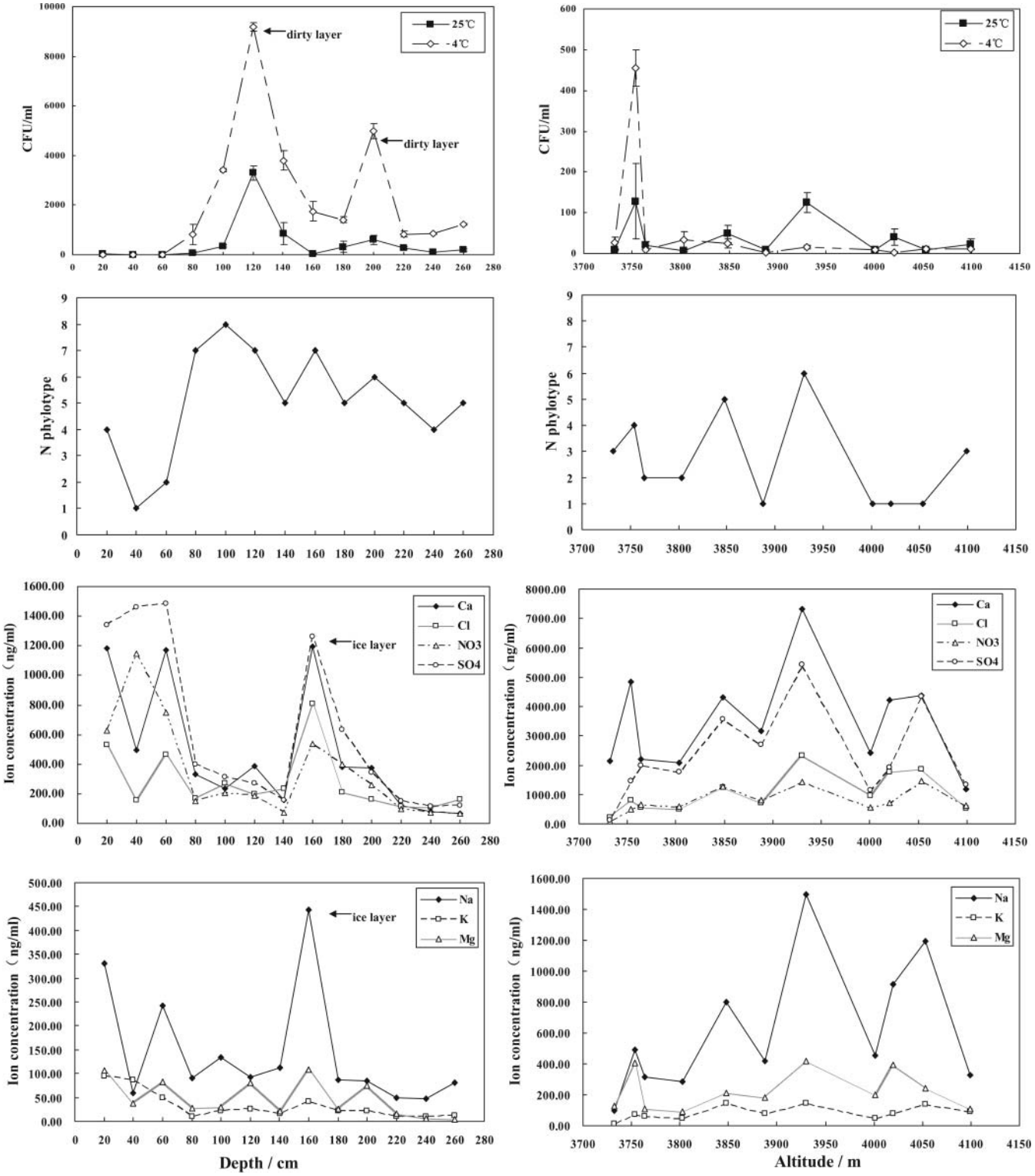

FIG. 2. Results for different depths and altitude. The CFU means number of colony forming untis. N phylotype means number of cultivable bacterial phylotypes.

of 120 cm and 200 cm in the snow pit, which also contained two layers of dirt. The numbers of viable bacteria at 4°C were significantly higher than those at 25°C collected below 60cm depth. However, they were not significantly different in samples collected from 20-cm depths from the surface at various altitudes on the glacier, except for the sample at 3754 m in which the numbers of viable bacteria growing at 25°C were much higher.

Altogether, 31 representatives with diverse ARDRA patterns were selected for 16S rDNA sequencing and phylogenetic analysis. The numbers of the individual strains in different samples ranged from 1 to 8 (Figure 2). All the bacteria recovered at 4°C could also grow at 25°C during the isolation and purification procedures. This means they were psychrotrophs, not psychrophiles, according to the definition by Morita (1975).

Seven kinds of ions were determined, with the Mg^{2+} and K^{+} showing lower abundance compared with Na^{+}, Ca^{2+}, Cl^{-}, NO_3^{-} and SO_4^{2-} ions (Figure 2). The ion concentrations varied in the same way as the number of cultivable bacteria and the diversity of phylogenetic types at different altitudes of the glacier. The sample site at 4099m was the observation site of the Upper Tian Shan Glaciological Station, where the human activity was more frequent than at the other sites, so we eliminated its data when calculating the relationship between the ion concentration and the quantities of the viable bacteria. The quantities of the viable bacteria recovered at 25°C were positively correlated with Mg^{2+} ($r = 0.81$, $P < 0.01$) and Ca^{2+} ($r = 0.82$, $P < 0.01$), and the diversity of phylogenic types was positively correlated with Ca^{2+} ($r = 0.64$, $P < 0.05$).

Figure 3 shows the relationship between the quantities and the diversity of the viable bacteria, the average value of $\delta^{18}O$ and the ion concentration for each accumulation year. The quantities of the viable bacteria decreased from 2006 back to low numbers in 2004. The low numbers in 2007 may have been due to a fresh snowfall, in which case the bacteria would not have had sufficient time to adapt to the environment. The diversity of phylogenic types, the $\delta^{18}O$ values and the ion concentrations all tended to decrease with age.

ARDRA and Phylogenetic Analyses

Through amplified ribosomal DNA restriction analysis (ARDRA), all 139 isolates were clustered into 31 groups. After comparing 16S rDNA sequence of the representative strain from every group, the recovered population fell into six groups: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Cytophaga-Flavobacterium-Bacteroides (CFB) group bacteria, high G+C gram-positive bacteria, low G+C gram-positive bacteria (Figures 4–Figure 6). They were closely related to other cold environment bacteria, as revealed by phylogenetic tree constructions (Figures 4–Figures 6).

The most abundant and diverse isolates belonged to the CFB group, the *Flavobacterium* in particular, as a dominant genus in this group. The 16S rDNA sequence phylogenetic analysis of seven isolates related to the *Flavobacterium* confirmed that these isolates presented a more dispersed relationship with several species, namely, *Flavobacterium degerlachei, Flavobacterium frigoris, Flavobacterium succinicans, Flavobacterium omnivorum, Flavobacterium johnsoniae*. The tsz28 stain of this genus had 96.6% sequence similarity with its closest related species. Four other isolates were affiliatd with genus *Hymenobacter* and *Pedobacter*, but had less than 97.0% sequence similarity with their closest related species. The low similarity of rDNA sequences indicated that some of these strains belonged to new taxa.

Six studied isolates were affiliated with the α-Proteobacteria group and belonged to 5 genera: *Brevundimonas, Caulobacter, Paracoccus, Roseomonas* and *Sphingomonas*. Three isolates belonged to two genera of the *β-Proteobacteria* group, and were closely related to the *Polaromonas naphthal* (CP000529), *Polaromonas ginsengisoli* (AB245355), and *Duganella zoogloeoides* (AB495151) with more than 97.7% sequence similarity. Four isolates belonged to two genera of the γ-Proteobacteria group, and were closely related to the *Pseudomonas meridiana* (AJ537602) *Pseudomonas antarctica* (FM213380) and *Psychrobacter aquaticus* (AJ830007) with more than 99.3% sequence similarity.

Of the 31 studied isolates, four belonged to the high G+C gram-positive group and related to the 2 genera: *Arthrobacter, Curtobacterium*. The tsz10, tsz11, tsz36 and tsz25 stains showed 99.5%, 99.0%, 99.5% and 98.8% sequence similarity, respectively, with *Arthrobacter sulfonivorans* (FM955888), *Arthrobacter citreus* (AM237346), *Arthrobacter agilis* (AJ577725), and *Curtobacterium flaccumfaciens* (EU977762). Of the 31 isolates whose 16S rDNA we sequenced, only one belonged to the low G+C gram positive group. The tsz14 strain had 98.1% sequence similarity with *Planococcus psychrotoleratus* (AF324659).

The CFB group bacteria abundance was similar in the 2007 layer (55.5%) and the 2006 layer (57.1%), and decreased with age, with a similar decreasing trend in ion concentrations and the $\delta^{18}O$ value. The α-Proteobacteria abundance increased from 0.9% to 88.1%, thus being negatively correlated with the concentrations of ions and the $\delta^{18}O$ values. The correlation of abundance of β-Proteobacteria, γ-Proteobacteria and high G+C gram-positive bacteria with the ions and $\delta^{18}O$ was not significant, whereas the low G+C gram-positive bacteria was not found in the snow pits (Figure 3).

Eleven genera were identified in the samples from the snow pit, with *Brevundimonas, Caulobacter, Duganella, Pseudomonas and Flavobacterium* being the main genera. The *Brevundimonas* increased from 0% in the newer samples (2007) to 69.3% in the aged sample (2004), with the same trend showing in the case of α-Proteobacteria. The abundance of *Flavobacterium* showed the same decreasing trend as did the CFB group of bacteria (Figure 3).

DISCUSSION

Cold environments that dominate the biosphere (Satyanarayana et al. 2005), can be divided into two categories:

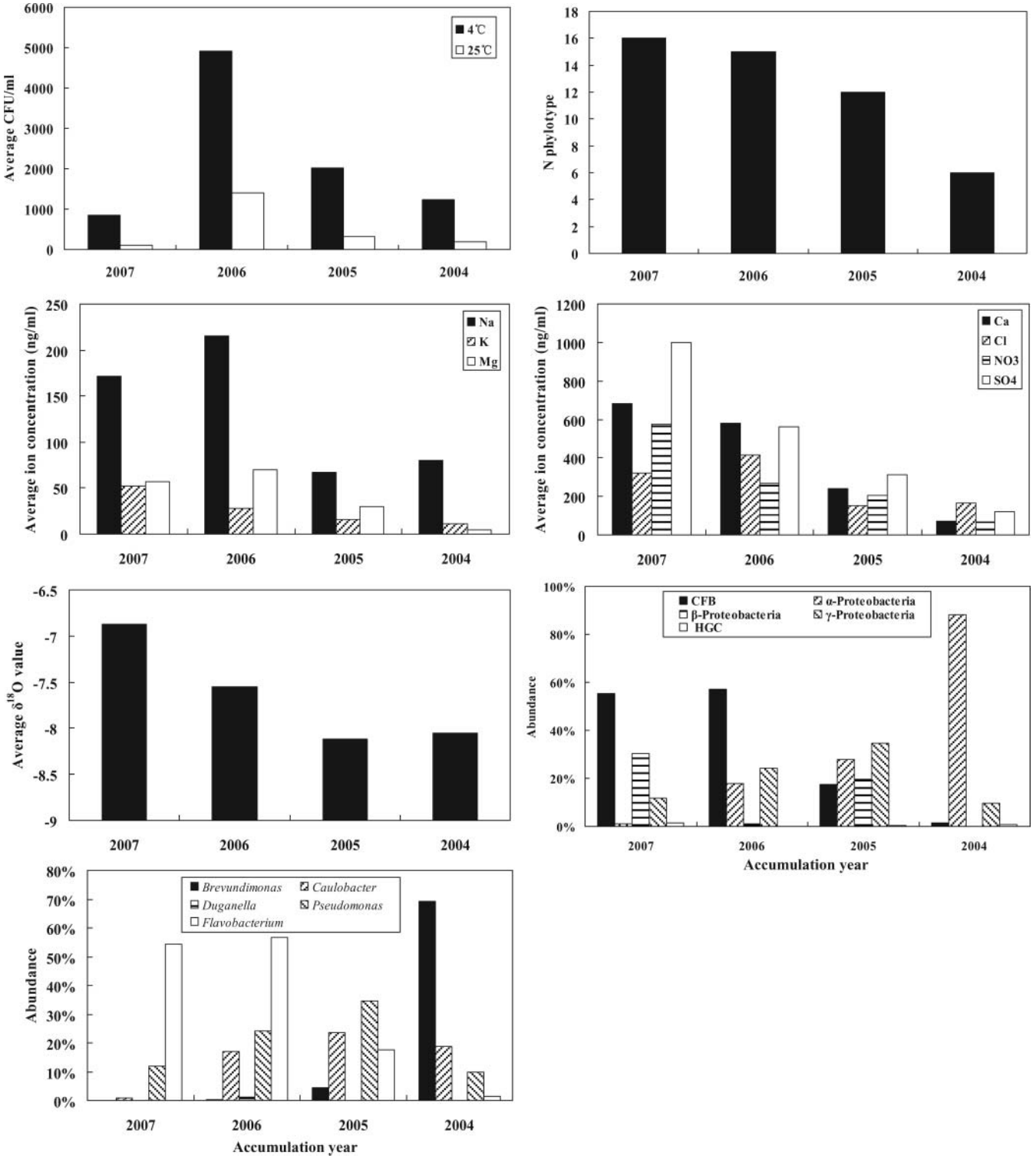

FIG. 3. Results for different accumulation years. The average CFU means average number of colony forming units. N phylotype means number of cultivable bacterial phylotypes. The abundance means the bacterial community abundance.

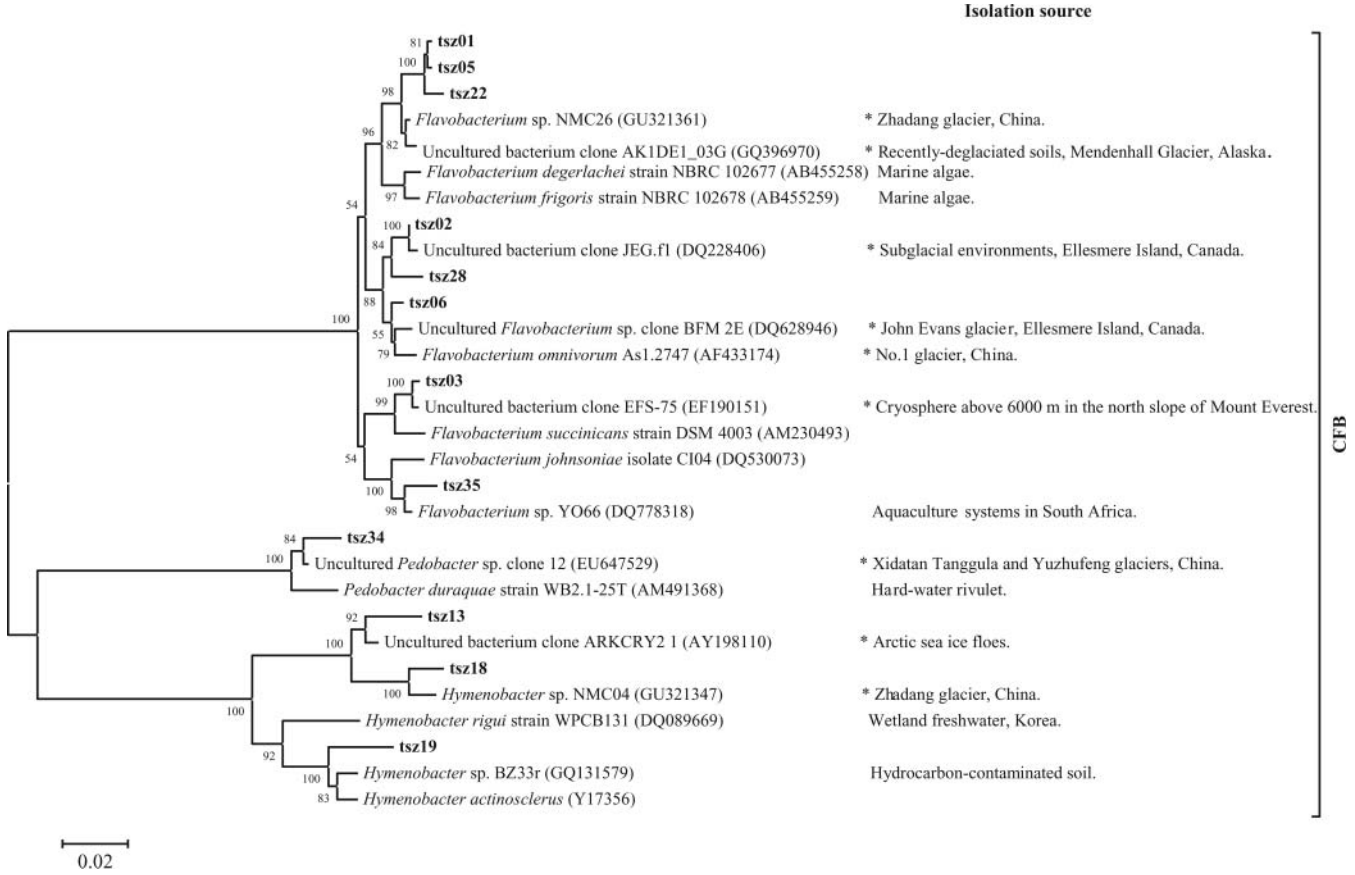

FIG. 4. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of Cytophaga-Flavobacterium-Bacteroides (CFB) group isolates from the snowcover of Tianshan Number 1 glacier and some of their closest phylogenetic relatives. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. The isolation source column lists the environments that the closest phylogenetic relatives come from. *indicates the most closely related members were obtained from similar cold environments.

psychrophilic (permanently cold) and psychrotrophic (seasonally cold or where temperature fluxes into mesophilic range) environments (Morita, 1975). In any cold environment, many of the isolated bacteria have been found to be psychrotrophs (Satyanarayana et al. 2005). Their adaptation to low temperature is dependent on a number of survival strategies such as the ability to modulate membrane fluidity, ability to carry out biochemical reactions at low temperatures, ability to regulate gene expression at low temperatures, and capability to sense temperature (Ray et al. 1998; Satyanarayana et al. 2005). In our study, the abundance of cultivable bacteria cultured at 4°C was significantly higher than for those samples cultivated at 25°C and collected below 60 cm depth (Figure 2). Meanwhile, the quantity and diversity of the cultivable bacteria decreased with age (Figure 3). These phenomena indicate that the psychrotrophs may become dominant with the passage of time though they were not the dominant bacteria in freshly fallen snow. The bacteria apparently acclimatized to the low-temperature environment by changing community structure.

The bacterial population in the snowcover of Tianshan Number 1 Glacier consisted of six groups: α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, CFB group bacteria, high G+C bacteria, and low G+C bacteria. When comparing our 16S rRNA sequences to entries in GenBank, many of the most closely related were reported from similar cold environments, such as glaciers and frozen ground (Figure 4–Figure 6). The consistent isolation of related microbes from such cold environments suggests that these species may indeed have features that confer resistance to freezing and extended survival under frozen conditions.

On the whole, gram-negative bacteria are more often reported than gram-positive bacteria from cold environments, such as in polar and glacier samples (Amoto et al. 2007). The α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria and the CFB group bacteria were the most commonly reported microorganisms in cold environment (D'Amico et al. 2006). They were found in pack ice at both the north and south poles using cultivation or molecular methods (Brinkmeyer et al. 2003; Groudieva et al. 2004), and were dominant in the bacterial flora of the winter cover (snow and ice) and pelagic zone of a midlatitude high mountain lake (Alfreider et al. 1996).

Xiang et al. (2005), Segawa et al. (2005) and Liu et al. (2009b) obtained similar results from the snowcover of the Tateyama Mountains and two glaciers on the Tibetan Plateau by building

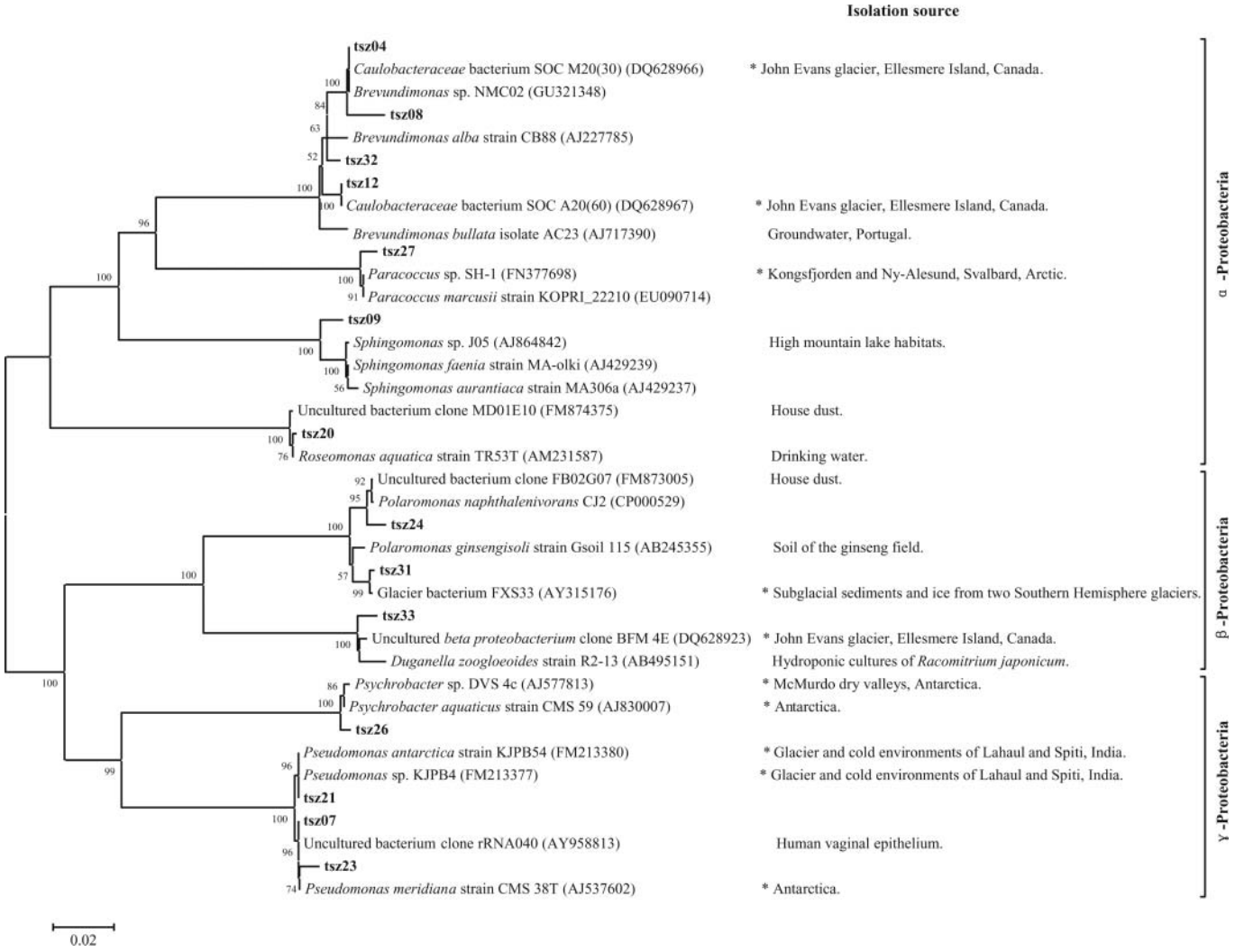

FIG. 5. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of the gram-negative isolates from the snowcover of Tianshan Number 1 glacier and some of their closest phylogenetic relatives. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. The isolation source column lists the environments that the closest phylogenetic relatives come from. *indicates the most closely related members were obtained from similar cold environments.

a 16S rRNA gene clone library. However, some different results have been previously reported. Amato et al. (2007) cultured bacteria from the snowcover at Spitzberg, but found no CFB group bacteria. Cheng and Foght (2007) found no low G+C bacteria in the cultivable bacteria community from the John Evans Glacier. Those differences may be the result of environmental change, and could be potentially valuable as indicators of environmental change.

Brevundimonas and *Flavobacterium* have been found widely distributed in various glaciers, such as the East Rongbuk Glacier (Liu et al. 2009b; Zhang et al. 2010b), Muztagata Glacier (Xiang et al. 2005), the Puruogangri Glacier (Zhang et al. 2008), the Roopkund Glacier (Pradhan et al. 2010), the Malan Glacier (Zhang et al. 2001; Xiang et al. 2004), the Midre Lovén and Kongsvegen Glaciers of Ny-Ålesund (Amato et al. 2007; Schütte et al. 2010), and the Greenland Glacier (Sheridan et al. 2003; Miteva et al. 2004).

In our study, *Flavobacterium* predominated in the surface snow (54.3%) but decreased with age, and had similar trends of abundance to the ion concentrations and the $\delta^{18}O$ values. *Brevundimonas* increased and predominated in the bottom of snowpit (69.3%), demonstrating a negative correlation with physical chemistry properties, and could be the representative *psychrotrophs* in the study area. Ion concentration and $\delta^{18}O$ values have been used in the glaciological literature as indicators of past climatic and environmental change, of regional temperature change, of changes in monsoon and dust signals, emissions from combustion of fossil fuels, and of impacts of agricultural activity and the burning of biomass (Duan et al. 2007; Kaspari et al. 2007; Rozanski et al. 2010; Zheng et al. 2010).

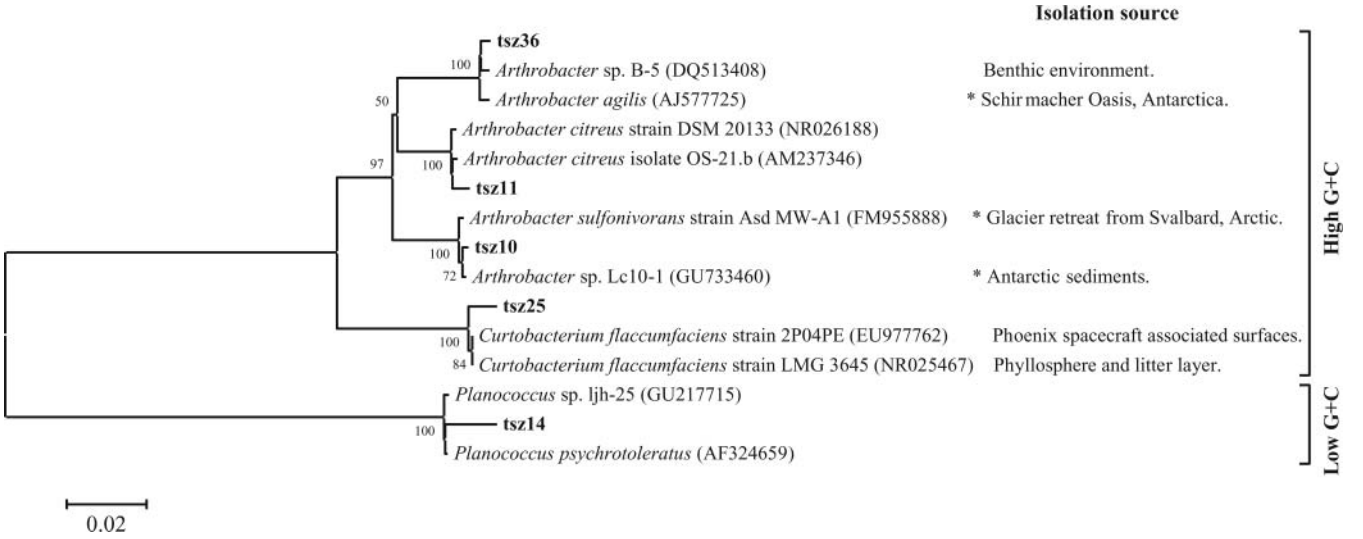

FIG. 6. Phylogenetic dendrogram based on a comparison of the 16S rDNA sequences of the gram-positive isolates from the snowcover of Tianshan Number 1 glacier and some of their closest phylogenetic relatives. The numbers on the tree indicate the percentages of bootstrap sampling derived from 1000 replications. The isolation source column lists the environments that the closest phylogenetic relatives come from. *indicates the most closely related members were obtained from similar cold environments.

In previous study of this area, it was found that the ice core records of annually averaged $\delta^{18}O$ were positively correlated with contemporaneous air temperature (Hou et al. 1999), and the $\delta^{18}O$ value of precipitation was also positively correlated with contemporaneous air temperature by long time observation (Yao et al. 1999) or mathematical model setting (Zhang et al. 2003). Both genuses could synthesize carotenoid, which made them adapt to cold environments (Nishida et al. 2005; Rählert et al. 2009).

For the *Brevundimonas*, it corresponds to the least nutritionally versatile bacteria, and occurs typically in freshwater and marine habitats with low nutrient levels (Tayeb et al. 2008). The ion concentration, such as Ca^{2+}, which was one of the abundant ion in the study area, could be typical nutrition source for microorganism's growth (Uroz et al. 2009). The No.1 glacier located in arid region where surrounded by desert and gobi. When the temperature raised, the dust events occurred and increased ion concentration consequently (Wu et al. 2010).

Based on those, we speculated that caused the *Brevundimonas* negatively correlated with $\delta^{18}O$ value and ion concentration, and the exact role need further study to clarify. The close connection between the shifting bacterial community structure and ion concentrations and $\delta^{18}O$ values in our study indicated that the shifting bacteria community structure could be a new index for environmental and climatic change, especially the changes associated with the predominant genera *Flavobacterium* and *Brevandimonas.*

In the snow pit, decreasing trends of the number of cultivable bacteria and the diversity of phylogenic types were observed from 2007 back to 2004. However, the number of cultivable bacteria was low in the layer deposited in 2007 (Figure 3). This may have been because the bacteria had just arrived on the surface of the glacier, and did not have sufficient time to multiply. The number of cultivable bacteria and diversity of phylogenic types decreased mainly because of the natural selection with age, but may also be connected with the ion concentrations and $\delta^{18}O$ values of the snow (Figure 3).

In our study, the number of cultivable bacteria was significantly higher in the layer at the 120 cm and 200 cm depth in the pit, where the layers of dirt existed. Christner et al. (2000) and Xiang et al. (2005) also reported similar results. Layers of dirt in a glacier could reflected past variations of dust event (Wang 2005). Bacteria became attached to aeolian dust, which was then deposited on the surface of glaciers (Abyzov et al. 1998; Yao et al. 2006). Therefore, a high concentration of bacteria could therefore indicate the presence of a layer of dirt in a glacier and have the same climatic meaning as a layer of dirt itself.

The abundance of cultivable bacteria and diversity of phylogenic types in the surface snow samples positively correlated with the ion concentrations, but showed no obvious relationship with altitude itself (Figure 2), perhaps because of the effect of blowing and drifting snow. The blowing and drifting snow results in the redistribution of material, causing a blurring of any relationship with altitude (Kang et al. 2002). Evidence for this was also found in our study. The sample from 3754 m was collected near the margin of the glacier, which faced in the same direction as the site from which the sample at 3732 m was collected, but this direction was different from the sites at other elevations from which samples were collected. The katabatic winds from the front of the glacier blow directly on to this slope, and may have carried some bacteria in its aeolian load from the frozen soil at this site. This may have made the

abundance of bacteria significantly higher than in other surface snow samples.

In conclusion, the quantity and diversity of the cultivable bacteria showed a close relationship to the soluble ion concentrations and the $\delta^{18}O$ values, and the bacterial community composition shifted with those geochemical and geophysical factors. All the results indicated that distribution of the bacteria could be used as a biological index for climate and environment change.

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