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Bacterial diversity in Himalayan glacial ice and its relationship to dust

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Abstract

Concentrations and community diversity of bacteria from 50 segments of a 108.83 m ice core drilled from the East Rongbuk (ER) Glacier (28.03° N, 86.96° E, 6518 m above sea level) on the northeast slope of Mt. Qomolangma (Everest), covering the period

5 950–1963 AD, were investigated by epifluorescence microscope, DGGE and Shannon-Weaver index analysis. There are four general periods of bacterial diversity, corresponding to four phases of dust abundance revealed by Ca^{2+} concentrations. It is indicated that higher bacterial community diversity is associated with warm periods, while lower bacterial community diversity with cold periods. However, a previously

10 suggested positive correlation between bacterial and Ca^{2+} concentrations was not indicated by our observations. In fact, a weakly negative correlation was found between these two parameters. Our results suggest that bacterial community diversity, rather than concentrations, might be a suitable biological proxy for the reconstruction of past climatic and environmental changes preserved in glacial ice.

15 ## 1   Introduction

The relationship between concentrations of microorganisms and dust in glacial ice has been studied for polar regions and the Tibetan Plateau (TP). For instance, Mitskevich et al. (2001) analyzed 10 samples from the Antarctic glacial ice over Lake Vostok. They observed that the maximum diversity of bacterial cells was correlated with organic detri-

20 tus, and was presented in samples with maximum density of microorganisms. By using confocal microscopy, Priscu et al. (1998) identified cyanobacterial cells in the sediment of the ice cover of Lake Bonney, Antarctica. Zhang et al. (2003) suggested that the concentrations of total and culturable microorganisms from 23 samples of the Tibetan Malan ice core were correlated with dust content. By using these samples and δ^{18}O as

25 a proxy for temperature, Yao et al. (2006) further suggested that more microorganisms were associated with cold periods, and fewer with warm periods. It is suggested that

microorganisms in glacial ice depend on the transportation of micro-particles during glacier growth (Zhang et al., 2003). Sediment particles can serve as nutrient-enriched (inorganic and organic) microzones for the establishment of a physiologically and ecologically complex microbial consortium that is capable of contemporaneous photosynthesis, N_2 fixation, and decomposition (Priscu et al., 1998). However, in a shallow ice core from the Himalayan Yala Glacier in the Langtang region of Nepal (Yoshimura et al., 2006), algal biomass in each annual layer from 1984 to 1994 AD was negatively, but not significantly correlated with micro-particle concentrations ($r = -0.345$, $p > 0.2$). Results of samples from the upper 12.5 m of a 22.4 m long ice core drilled from Muztagh Ata in the north-western TP also showed insignificant correlation ($r = 0.104$, $n = 43$) between concentrations of microparticle ($1–30 \mu$m in diameter) and bacteria (Xiang et al., 2006). These imply that, although microparticles may serve as the source and nutrition for bacteria, it is only one of the factors that determines bacterial concentrations in glacial ice. Therefore more efforts are necessary to verify the feasibility of reconstructing past climatic and environmental changes by means of glacial microorganism. In addition, most of the above work suffered from limited samples, and some of the Tibetan ice cores are not well dated. Here we investigate the bacterial concentrations of 50 samples collected along a 108.83 m ice core recovered from the ER Glacier on the northeast slope of Mt. Qomolangma (Everest). Among them, 13 samples were analyzed for bacterial community diversity. We wish to extend (with more reliable statistics) our current understanding of bacterial concentrations and community diversity in the Himalayan glacial ice and to study their relationship with dust content. This work extends our previous results of the seasonal variations of abundance and species diversity of culturable bacteria recovered from Himalayan glacial ice (Zhang et al., 2007a).

2 Materials and Methods

2.1 The ice core

A 108.83 m ice core, diameter 9.4 cm, was drilled to the bedrock on the col of the ER Glacier (Fig. 1) by using an electromechanical drill in a dry hole in September–October 2002. The ER Glacier covers an area of 48.45 km^2 with a length of 14 km. Its equilibrium line of 6250 m above sea level is believed to be the highest among all glaciers on Earth. Repeat surveys with a Sokkia GSS1, a Global Positioning System (GPS), in 1998 and 2002 did not identify horizontal movement at the drilling site. Visible stratigraphy shows no hiatus features, and the ice layers are horizontal. The average annual net accumulation is around 400 mm water equivalent as determined by snow pit and an 80.36 m ice core study (Hou et al., 2002). Bore-hole temperature ranged from a minimum of −9.6 °C at 20 m depth to −8.9 °C at the bedrock. The relatively high annual accumulation and low temperature throughout the glacial ice ensure the preservation of a high resolution environmental and biological record. After drilling, the ice core was transported in a frozen state to a cold room at about −20 °C and was stored there until analysis.

2.2 Ice core measurements and dating

At first, this core was split axially into two equivalent portions for different measurements. One portion was melted continuously into 3123 samples and analyzed for major soluble ions, hydrogen isotopes (δD), and Bismuth at the Climate Change Institute, University of Maine, USA, and oxygen isotopes (δ^{18}O) at the State Key Laboratory of Cryospheric Science, Chinese Academy of Sciences, China. Ca^{2+} analysis was performed via suppressed ion chromatography (Dionex 4000 series instruments) with a CS12 column, 125 mL loop, which was described in detail by Ivask et al. (2001). The other half core was sampled discontinuously for measurement of gases at Laboratoire de Glaciologie et Géophysique de l'Environnement, France, gas stable isotopes at

Laboratoire des Sciences du Climat et de l'Environnement, France (Hou et al., 2004), trace metals at Korea Polar Research Institute, lead isotopes at Department of Applied Physics, Curtin University of Technology, Australia, and bacteria (presented in this paper) at the State Key Laboratory of Cryospheric Science, Chinese Academy of Sciences, China. A total of 50 samples were collected for bacterial cell concentrations and community diversity, roughly proportionally but discontinuously along this core. The vertical length of these samples was in the range 0.25–0.48 m (Fig. 2)

The ice core was annually dated to 1534 AD to a depth of 98.0 m using seasonal variations of δD and soluble ions, and the timescale was verified by identifying large volcanic horizons from the first high-resolution measurements of Bismuth on an Asian ice core (Kaspari et al., 2007). The bottom 2 m of the ice core was dated to 1498–2055 years BP using methane and isotopic composition of atmospheric O_2 (Hou et al., 2004). Below 98.0 m annual layer counting is not possible due to layer thinning, thus prior to 1534 AD the ice core was dated using a flow model. The details of dating were described by Kaspari et al. (2007; 2008).

2.3 Ice core decontamination

In order to obtain reliable microbiological data, it is essential to eliminate the potential contamination caused during drilling, transportation, storage and sampling. This procedure was established according to Willerslev et al. (1999) and Christner et al. (2005) with minor modification. The ice core was sampled in a class-100 unidirectional flow clean bench, which was located inside a −20°C class-1000 dedicated cold room. Personnel involved wore clean room garbs and polyethylene gloves. At first, the potentially contaminated surface layer was removed (about 2 mm thick) by shaving with a sterile stainless steel scalpel. Afterwards, the operator changed to a new pair of gloves and the second veneer layer was shaved off by using a new scalpel. This procedure was repeated three times and about 6 mm thick veneer layer was removed. Then still in the cold room (temperature was switched to 4°C), the remaining inner core was rinsed with 300 mL sterile high purity water of >18 MΩ to melt away about 2 mm of the exterior sur-

face. The remaining inner core was then placed into a sterile glass beaker for melting completely at 4°C in the dark. At the same time, the scraped surface ice was collected and used as controls. The same procedure was used to process an artificial ice core of ultrapure water as an analytical blank.

2.4 Direct cell counts

Treatment of the samples by SYBR Green-II (Molecular Probes, Inc.) was modified from Yamagishi et al. (2003). Fifty milliliters of each sample were fixed in 2% (vol/vol) glutaraldehyde buffered with phosphate-buffered saline (PBS) and then filtered onto 25 mm, 0.2 μm black track-etch membrane filters (Whatman). Dimethyl sulfoxide (DMSO) was diluted with PBS to 5% (vol/vol) as stock solution for SYBR Green II. Both SYBR Green II and the diluted DMSO were passed through 0.2 μm filters to remove extraneous particles and cells prior to analysis. Filtered samples were incubated with DMSO-buffered 1 g/L SYBR Green II for at least 15 min at 20°C in the dark. Afterwards the filters were mounted onto a glass slide treated with mounting medium (1:1 mixture of PBS diluted glycerin and 0.1% p-phenylenediamine). Cells on the filters were counted using an Olympus BH-2 microscope with a 405 nm argon laser at the final magnification of 1000×. The total number of cocci and rods in 60 fields of view was determined, and the number of cells per mL was calculated by computing the cumulative average cell per field (field of view area at 1000× is 16 741 μm^2).

2.5 DNA extraction

Thirteen samples selected from the above 50 were employed for DNA extraction. The remaining 200–1100 mL melted ice core samples were filtrated through 0.22 μm hydrophilic polyethersulfone membranes (Pall) with a vacuum pump (Ntengwe, 2005). Microorganisms on the membranes were eluted by agitation for 2 min by hand, then by sonication for 2 min with a sonicator (model 14; Branson Ultrasonics Corp) (Uga et al., 2003) and then suspended in 2.0 mL PBS. Half of the suspension was used for extrac-

tion 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).

2.6 PCR Amplification

Bacterial specific primers, 357F-GC (5′-CGCCCGCCGCGCGCGGCGGGCG GGGCGGGGGCACGGGGGG-CCTACGGGAGGCAGCAG-3′) and 518R (5′-ATTACCGCGGCTGCTGG-3′) were used to amplify the V3 hypervariable region (Crump et al., 2003) of the 16S rDNA gene by PCR with the following conditions: 50 μg genomic DNA solution, 0.5 μM each primer, 1×PCR buffer (Promega), 1.5 mM MgCl$_2$ (Promega), 0.2 mM each deoxyribonucleotide triphosphate, 0.5 U Taq DNA Polymerase (Promega), and sterile deionized water to a final volume of 25 μL. Negative controls, with 1 μL of sterile deionized water as template, were included in all sets of PCR to provide a contamination check. The amplification program consisted of 94 °C for 5 min; 30 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s; and a final extension step of 72°C for 8 min. All the PCR products were analyzed on 30 g/L agarose gels in 1×TAE buffer, purified with QIA quick PCR mini kit (QIAGEN, Germany) and quantified by UV spectrophotometer (UV 7501, Techcomp).

2.7 Denaturing gradient gel electrophoresis (DGGE)

Two hundred and fifty ng of purified amplicons were used in DGGE analysis in 1× TAE buffer with 80 g/L polyacrylamide gels containing a linear denaturant gradient from 25% to 60% of urea (Merck) and formamide (Saarchem) (100% denaturant contains 7 M urea and 40% formamide (vol/vol)) in a D-Code system (Bio-Rad, Hercules, CA, USA) according to McCaig et al. (2001). Gels were run at a constant temperature of 60°C, with 100 V for 14 h. After completion of electrophoresis, gels were fixed overnight (10% ethanol (vol/vol), 0.5% glacial acetic acid (vol/vol), 89.5% H$_2$O (vol/vol)), then incubated by shaking in fresh silver staining solution (2 g/L silver nitrate) for 20 min,

followed by incubation in fresh developing solution (15 g/L NaOH, 0.5% formaldehyde (vol/vol)) until bands appeared. The gels were then fixed for 10 min in 7.5 g/L Na$_2$CO$_3$, and preserved in ethanol-glycerol preservative (25% ethanol (vol/vol), 10% glycerol (vol/vol), 65% H$_2$O (vol/vol)) for at least 15 min (McCaig et al., 2001). Images of each gel were documented with ImageScanner (Amersham Pharmacia Biotech). To ensure the reproducibility and reliability of the DGGE banding patterns, three independent PCR amplification and subsequent DGGE analysis were performed in independent trials. DGGE banding patterns were exactly the same for each replicate (Fig. 3).

2.8 Precautions and controls

DNA extraction was carried out in a fully equipped and physically isolated clean laboratory dedicated solely to ancient DNA work with separate ventilation systems, nightly exposure of surfaces to UV irradiation, and weekly cleaning of surface with bleach. The PCR setup was in a flow clean bench, exposed to UV light during the night. Both extraction and pre-PCR work were carried out with their own sets of lab clothes, lab tools, and unopened reagents (Lydolph et al., 2005). Tools for pre-PCR work were washed in 5% sodium hypochlorite (Willerslev et al., 1999) and exposed to UV light overnight. Full-body suits, facemasks, and sterile surgical gloves were used. Mock extraction and PCR controls were made to check for possible contamination by extraneous DNA (Willerslev et al., 1999).

2.9 Statistical analysis of DGGE profiles

Bacterial community diversity was calculated from the DGGE band profiles. The Shannon-Weaver index of general diversity (H') was analyzed with the software of Labworks 4.0 (UVP Bioimaging system) based on the number and relative intensity of the bands in a gel lane and was calculated according to the equation (Shannon and Weaver, 1963)

$$H' = -\sum (P_i \times \ln(P_i)) \tag{1}$$

where P_i is the relative intensity of the bands in a gel lane and is calculated as

$$P_i = n_i / N \tag{2}$$

where n_i is the height of a peak or the relative intensity of the bands in a gel lane as the result of Labworks 4.0. N is the sum of all peak heights in the densitometric curve.

3   Results and discussion

3.1   Decontamination criteria

To make sure that each inner ice core sample was free of contamination, we analyzed separately each veneer layer and inner core of half of the 50 samples to monitor changes of bacterial concentrations from the surface to the center. Figure 4a shows an example for the change in bacterial concentrations from surface to inner section of the ice core for the depth interval 59.46–59.76 m, indicating apparent contamination for the most outer veneer layer. Bacterial concentrations reduced to a stable low level from the third veneer layer to the center. Thus the low value of the inner core represents an authentic bacterial concentration result.

A control core constructed with Milli-Q-purified water was processed by methods identical with those of the samples. Bacterial concentrations were 14, 11, 9 and 6 cells/mL from surface to inner, respectively (Fig. 4b), and they are not significantly different from each other (using Chi-Square test: $p=0.334>0.05$). Moreover, the bacterial concentration in each layer of the control core was, at least, one order of magnitude lower than those measured in the ice core samples. Thus the potential contribution to the contamination during the process is the existence of a small DNA segment after autoclaving of the redistilled water, and therefore negligible.

All the mock extraction and PCR controls made to test for false-positive results were negative, demonstrating the authenticity of our DGGE data.

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3.2   Bacterial concentrations

Figure 5 shows the change of bacterial concentrations along the 108.83 m ice core, with a fluctuation of 0.02×10^3–6.4×10^3 cells/mL (mean=0.71×10^3 cells/mL, $SD=0.95 \times 10^3$ cells/mL, $n=50$). Because Ca^{2+} can be regarded as a good proxy for atmospheric dust transport in Himalayan snow and ice (Kang et al., 2002; Kaspari et al., 2007), we compared the bacterial concentrations with Ca^{2+} concentrations corresponding to the same ice core sections as shown in Fig. 5. It is worth pointing out that Ca^{2+} and bacterial samples were sampled at different resolution (~ 0.04 m per sample for Ca^{2+} and 0.25–0.48 m per sample for bacteria), so all Ca^{2+} concentrations corresponding to the depth range of each bacterial sample were firstly averaged. Among the bacterial samples, four samples were with a length 0.25 m (corresponding to 7–11 Ca^{2+} samples for each of these four samples), eleven samples with a length 0.45 m (corresponding to 10–19 Ca^{2+} samples), and one sample with a length 0.48 m (corresponding to 16 Ca^{2+} samples). Paired t-test was performed for the Ca^{2+} averages between 0.25 m and 0.48 m samples, resulting in insignificant statistical consequences ($p > 0.05$). As to paired t-test for the Ca^{2+} averages between the four 0.25 m and the eleven 0.45 m samples, 36 out of the total 44 pairs (82%) suggest insignificant statistical consequences ($p > 0.05$). These statistical results imply that comparison between the Ca^{2+} averages in different depth range is statistically significant.

The Ca^{2+} concentrations fluctuate in the range 52.18-745.69 ppb (mean=155.77 ppb, $SD=118.93$ ppb, $n=50$). Figure 5 showed obvious difference of distribution trend between the bacterial and Ca^{2+} concentrations. This is further confirmed by their weakly negative correlation ($r=-0.135$, $P=0.351$) (Fig. 6).

Although Yao et al. (2006) suggested that more microorganisms were associated generally with dust layers in the Malan ice core, they did not perform a correlation between the bacterial concentrations and insoluble particles. Our further analysis from their reported data (Yao et al., 2006) of 6 dirty layers for mineral measurement showed a weakly negative correlation between these two parameters ($r=-0.022$, $p=0.967$,

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$n=6$). Moreover, a weakly negative correlation ($r=-0.031$, $p=0.896$, $n=20$) was also found between the bacterial and Ca^{2+} concentrations of 20 samples collected from an 83.45 m Puruogangri ice core from the reported data by Zhang (2006). For all the ice core drilling sites (Fig. 1) in the Himalaya (ER and Yala glaciers), the central TP (Malan and Puruogangri glaciers), or the northern TP (Muztagh Ata Glacier), the correlation between bacterial concentrations/biomass and micro-particle concentrations is insignificant. Therefore, there might be other factors, together with micro-particles serving as a nutrition source for bacteria, that determines bacterial concentrations in glacial ice. For instance, algal biomass in annual layers of a shallow ice core from the Yala Glacier was found to be correlated significantly with two indices of snow-cover thickness, which determines the intensity of light available for algal growth, as well as the amount and period of availability of snow meltwater (Yoshimura et al., 2006). These results demonstrate that not only the relatively perplexing source of microorganisms, but also the complex growth mechanisms of bacterial community can be promoted by different nutrilites or environmental conditions in glaciers (Segawa et al., 2005). Given the ambiguous relationship between bacterial concentrations and ice core parameters, more efforts are needed for the reconstruction of past climatic and environmental changes by means of microbiological concentrations.

3.3 Bacterial community diversity with DGGE and H' index analysis

Among the 50 samples for DNA work, the extraction was successful only for the 13 samples that were not evenly spaced along the core (Fig. 2). The H' index, reflecting the structural diversity of bacterial community, fluctuates in the range of 0–0.75 (mean=0.41, SD=0.19, $n=13$), and the Ca^{2+} average is 132.65 ppb (SD=78.87 ppb, $n=13$) in response to these 13 samples (Fig. 7). Considering Ca^{2+} as a proxy for the atmospheric dust transport to our study site, there are two high dust periods from 32.11 to 37.89 m (1947–1958 AD) and 59.46 to 75.22 m (1752–1847 AD), two low dust periods from 43.15 to 55.64 m (1868–1932 AD) and 83.88 to 106.96 m (1054–1686 AD). Accordingly, bacterial community diversity shows a similar trend to the above four periods

(Fig. 7). A significantly positive correlation ($r=0.486$, $p=0.092$, $n=13$) is also observed between the two parameters (Fig. 8), which is consistent with that of the Puruogangri ice core ($r=0.71$, $P<0.01$, $n=13$) (Zhang et al., 2006).

In the Himalaya and southern TP, the periods of high dust concentrations generally coincide with warm climate, while that of low dust concentrations with cold climate (Wang et al., 2006). Dust storm outbreaks over the TP are associated with the movement of westerlies (Xu et al., 2007). During the cold periods of Little Ice Age (LIA), the westerlies were enhanced resulting in higher than normal precipitation and large seasonal snow cover on central Asia, thus the dust concentrations decreased. However, during the relatively warm periods of LIA, the intensity of westerlies weakened resulting in low precipitation in the winter and spring seasons and small seasonal snow cover (Xu et al., 2007[1]). As a consequence, the dust concentrations increased, resulting in desiccation reduction and protection of microorganisms necessary for their survival (Priscu et al., 1998). The rise of air temperature not only increases the outbreak of the dust storm which may possibly influence the physiological state of microbes, but also provides a favorable condition for bacterial production, e.g. favorable temperature and sufficient light. Our previous research also demonstrated that bacterial diversity is higher in the temperate glacier of Hailuogou than in the ER glacier (Zhang et al., 2007b). The common distribution of cryoconite holes in temperate glaciers of the mid-latitudes (McIntyre, 1984; Margesin et al., 2002) and sediments in small patches on the glacier surface (Fountain et al., 2004) indicated that both temperature and sediment are crucial for cold weather critters. Our result that bacterial diversity is higher during warm periods further illuminates the complicated environments for glacial microorganisms.

[1]Xu, J., Hou, S., Qin, D., et. al.: A 108.83 m ice core record of atmospheric dust deposition in Mt. Qomolangma (Everest), central Himalaya, Quaternary Res., submitted, 2007.

4   Conclusions

The quantitative distribution of bacterial population preserved in a Himalayan glacier displays a dissimilar variation trend to Ca^{2+} concentrations, and a weakly negative correlation was observed between them. However, the Shannon-Weaver index showed a certain similarity with Ca^{2+} concentrations. Therefore, dust concentrations might be more important in determining bacterial community diversity than bacterial concentrations in the Himalayan glaciers. Because only 13 samples were employed for deducing such a relationship between Ca^{2+} concentrations and bacterial community diversity, further detailed investigation with more samples are needed to better understand the distribution mechanism of bacterial abundance and community diversity in the glacial ice. This is crucial for the suitability of glacial microorganisms as a potential new indicator of past climatic and environmental changes.

References

Christner, B. C., Mikucki, J. A., Foreman, C. M., Denson, J., and Priscu, J. C.: Glacial ice cores: a model system for developing extraterrestrial decontamination protocols, Icarus, 174, 572–584, 2005.

Crump, B. C., Kling, G. W., Bahr, M., and Hobbie, J. E.: Bacterioplankton community shifts in an Arctic lake correlate with seasonal changes in organic matter source, Appl. Environ. Microbiol., 69(4), 2253–2268, 2003.

Fountain, A. G., Tranter, M., Nylen, T. H., Lewis, K. J., and Mueller D. R.: Evolution of cryoconite holes and their contribution to meltwater runoff fromglaciers in theMcMurdo DryValleys, Antarctica, J. Glaciol., 50(168), 35–45, 2004.

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Hou, S., Qin, D., Yao, T., Zhang, D., and Chen, T.: Recent change of the ice core accumulation rates on the Qinghai-Tibetan Plateau, Chinese Sci. Bull., 47(20), 1746–1749, 2002.

Hou, S., Jouzel, J., Chappellaz, J., Qin, D., Masson-Delmotte, V., von Grafenstein, U., Landais, A., and Caillon, N.: Age of Himalayan bottom ice cores, J. Glaciol., 50(170), 467–468, 2004.

Ivask, J., Pentchuk, J., and Vaikmäe, R.: Ion chromatographic determination of major anions and cations in Antarctic ice, P. Estonian Acad. Sci. Chem., 50(1), 46–51, 2001.

Kang, S., Mayewski, P. A., Qin, D., Yan, Y., Hou, S. Zhang, D., Ren, J., and Kruetz, K.: Glaciochemical records from a Mt. Everest ice core: relationship to atmospheric circulation over Asia, Atmos. Environ., 36(21), 3351–3361, 2002.

Kaspari, S., Hooke, R., Mayewski, P. A., Kang, S., Hou, S., and Qin, D.: Snow accumulation rate on Mt. Everest: synchroneity with sites across the Tibetan Plateau on 50–100 year timescales, J. Glaciol., 54(185), 343–352, 2008.

Kaspari, S., Mayewski, P., Kang, S., Sneed, S., Hou, S. Hooke, R., Kreutz, K., Introne, D., Handley, M., Maasch, K., Qin, D., and Ren, J.: Reduction in northward incursions of the South Asian monsoon since 1400 AD inferred from a Mt. Everest ice core, Geophys. Res. Lett., 34, L16701, doi:10.1029/2007GL030440, 2007.

Lydolph, M. C., Jacobsen, J., Arctander, P., Thomas, M., Gilbert, P., Gilichinsky, D. A., Hansen, A. J., Willerslev, E., and Lange, L.: Beringian paleoecology inferred from permafrost–preserved fungal DNA, Appl. Environ. Microbiol., 71(2), 1012–1017, 2005.

Margesin, R., Zacke, G., and Schinner, F.: Characterization of heterotrophic microorganisms in alpine glacier cryoconite, Arct. Antarct. Alp. Res., 34(1), 88–93, 2002.

McCaig, A. E., Glover, L. A., and Prosser, J. I.: Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns, Appl. Environ. Microbiol., 67(10), 4554–4559, 2001.

McIntyre, N. F.: Cryoconite hole thermodynamics, Can. J. Earth Sci., 21(2), 152–156, 1984.

Mitskevich, I. N., Poglazova, M. N., Abyzov, S. S., Barkov, N. I., Bobin, N. E., and Ivanov, M. V.: Microorganisms found in the basal horizons of the Antarctic glacier above Lake Vostok, Dokl. Biol. Sci., 381(3), 420–423, 2001.

Ntengwe, F. W.: An overview of industrial wastewater treatment and analysis as means of preventing pollution of surface and underground water bodies – the case of Nkana Mine in Zambia, Phys. Chem. Earth, 30, 726–734, 2005.

Priscu, J. C., Fritsen, C. H., Adams, E. E., Giovannoni, S. J., Paerl, H. W., McKay, C. P., Doran,

3446

P. T., Gordon, D. A., Lanoil, B. D., and Pinckney, J. L.: Perennial Antarctic lake ice: an oasis for life in a polar desert, Science, 280(5372), 2095–2098, 1998.

Segawa, T., Miyamoto, K., Ushida, K., Agata, K., Okada, N., and Koshima, S.: Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama mountains, Japan, analyzed by 16S rDNA gene sequencing and Real-Time PCR, Appl. Environ. Microbiol., 71(1), 123–130, 2005.

Shannon, C. E. and Weaver, W.: A mathematical theory of communication, 2nd ed,, Illinois Univ. Press, Urbana, USA, 226 pp., 1963.

Uga, S., Oda, T., Kimura, K., Kimura, D., Koesdarto, S., Margono, S., Nuvit, K., and Apakupakul, N.: Detection of microorganisms in tap water in Indonesia and Thailand, Jpn. J. Trop. Med. Hyg., 31, 87–91, 2003.

Wang, N., Thompson, L. G., and Davis, M. E.: Variations of atmospheric dust loading in the southern and northern Tibetan Plateau over the last millennium recorded in ice cores, Quat. Sci., 26 (5), 752–761, 2006 (in Chinese).

Willerslev, E., Hansen, A. J., Christensen, B., Steffensen, J. P., and Arctander, P.: Diversity of Holocene life forms in fossil glacier ice, P. Natl. Acad. Sci. USA, 96, 8017–8021, 1999.

Xiang, S., Yao, T., Wu, G., Chen, Y., Shang, T., Pu, L., and An, L.: Deposition on properties of bacterial populations in the Muztag Ata ice core, Quat. Sci., 26(2), 185–191, 2006 (in Chinese).

Xu, J., Hou, S., Qin, D., Ren, J., and Ming, J.: Dust storm activity over the Tibetan Plateau recorded by a shallow ice core from the north slope of Mt. Qomolangma (Everest), Tibet-Himal region, Geophys. Res. Lett., 34, L17504, doi:10.1029/2007GL030853, 2007.

Yamagishi, T., Hishinuma, T., and Kataoka, H.: Bicarbonate enhances synchronous division of the giant nuclei of sporophytes in Bryopsis plumose, J. Plant. Res., 116, 295–300, 2003.

Yao, T., Xiang, S., Zhang, X., Wang, N., and Wang, Y.: Microorganisms in the Malan ice core and their relation to climatic and environmental changes, Global Biogeochem. Cy., 20, GB1004, doi:10.1029/2004GB002424, 2006.

Yoshimura, Y., Kohshima, S., Takeuchi, N., Seko, K., and Fujita, K.: Snow algae in a Himalayan ice core: new environmental markers for ice core analyses and their correlation with summer mass balance, Ann. Glaciol., 43, 148–153, 2006.

Zhang, S., Hou, S., Ma, X., Qin, D., and Chen, T.: Culturable Bacteria in the East Rongbuk Glacier, Mt. Qomolangma (Everest) in response to atmospheric circulation, Biogeosciences, 4, 1–9, 2007a,

http://www.biogeosciences.net/4/1/2007/.

Zhang, S., Hou, S., Qin, X., Wang, Y., and Du, W.: Relation between bacteria in glacial snow with climate and environment in the Tibetan Plateau, Res. Environ. Sci., 20(5), 39–44, 2007b, (in Chinese).

Zhang, X.: Diversity of microorganisms entrapped in Puruogangri ice core and its relation to environment, Ph.D. thesis, Cold and Arid Reg. Environ. Eng. Res. Inst., Chin. Acad. of Sci., Lanzhou, China, 2006.

Zhang, X., Yao, T., An, L., Tian, L., and Xu, S.: 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, 2006.

Zhang, X., Ma, X., Yao, T., and Zhang, G.: Diversity of 16S rDNA and environmental factor influencing bacteria in Malan ice core, Chinese Sci. Bull., 48(11), 1146–1151, 2003.

Zhou, J., Bruns, M. A., and Tiedje, J. M.: DNA recovery from soils of diverse composition, Appl. Environ. Microbiol., 62(2), 361–322, 1996.
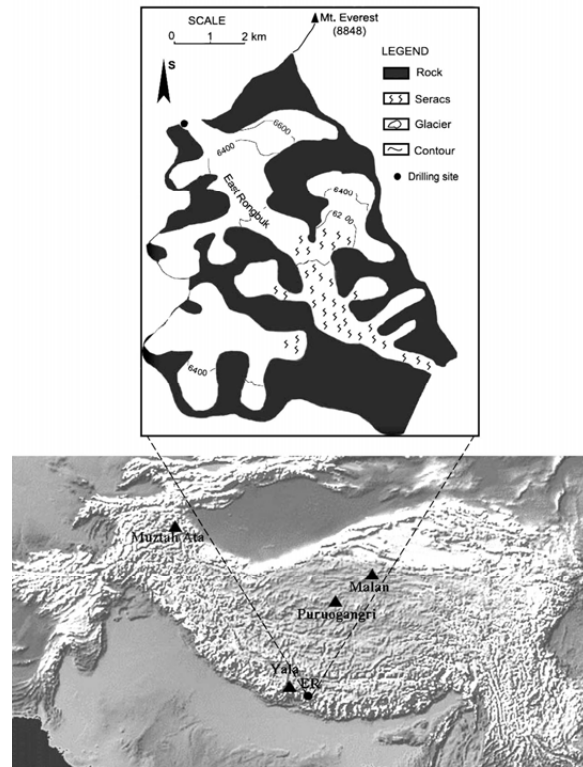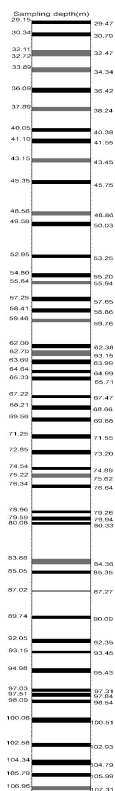
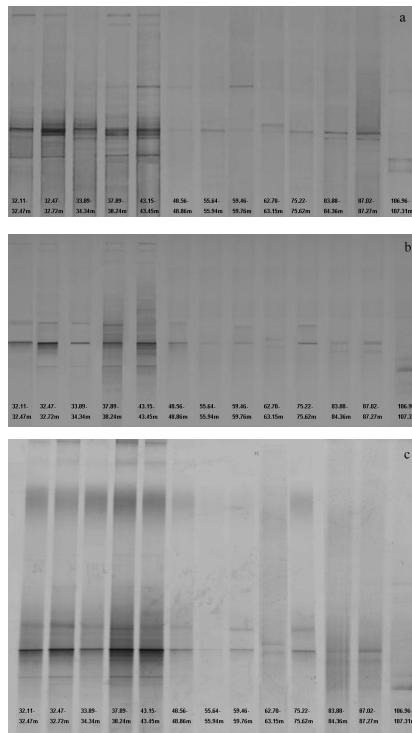**Fig. 1.** Location map of ice core drilling site.

Fig. 2. The sampling depth for bacterial cell concentrations (a total of 50 samples in black and gray) and community diversity (13 among the 50 samples in gray).

Fig. 3. Three replicates of DGGE profiles of PCR product amplified from 16S rRNA (V3) gene of the ER ice core samples. Numbers at the bottom of the DGGE profiles are their corresponding depth range.
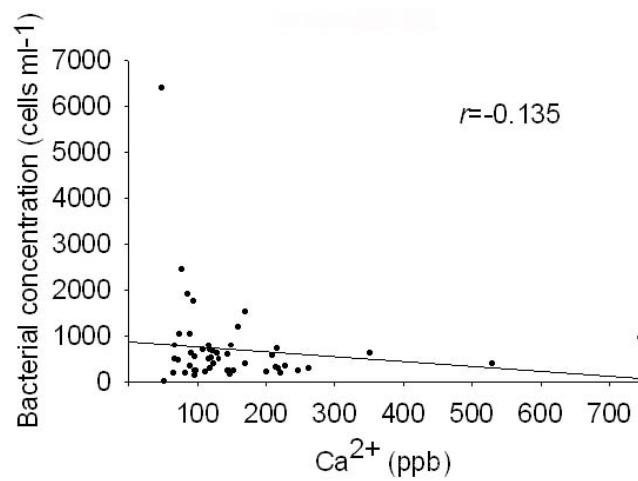
3451
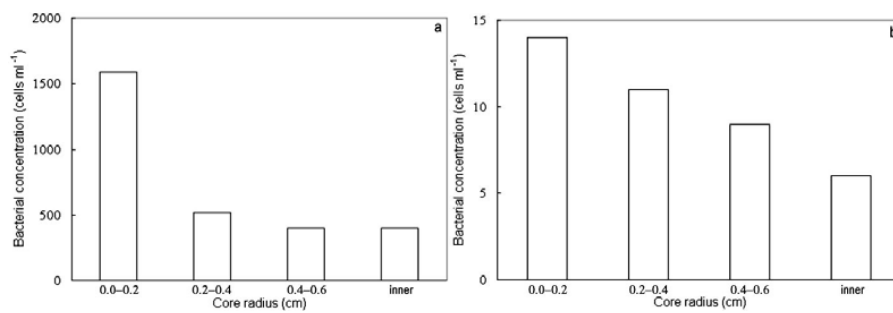
Fig. 4. Change in bacterial concentrations from surface to inner core of the samples. **(a)** example of ice core section for the depth interval 59.46–59.76 m; **(b)** artificial ice sample.
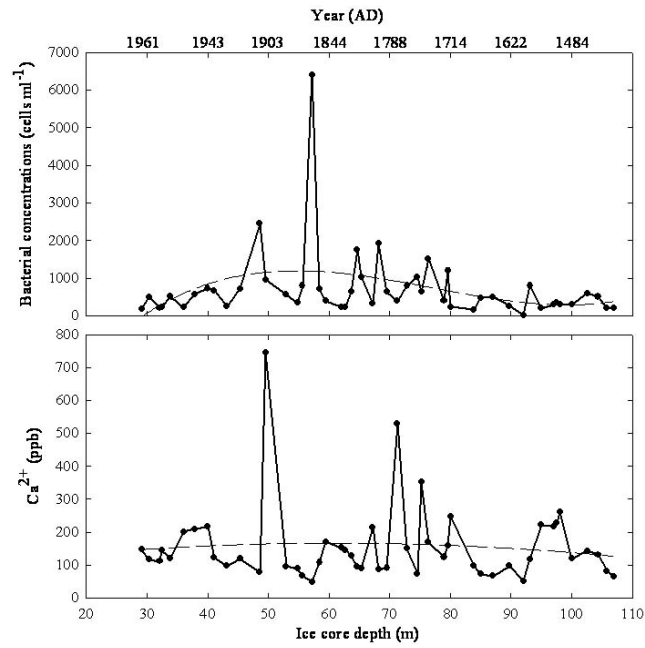
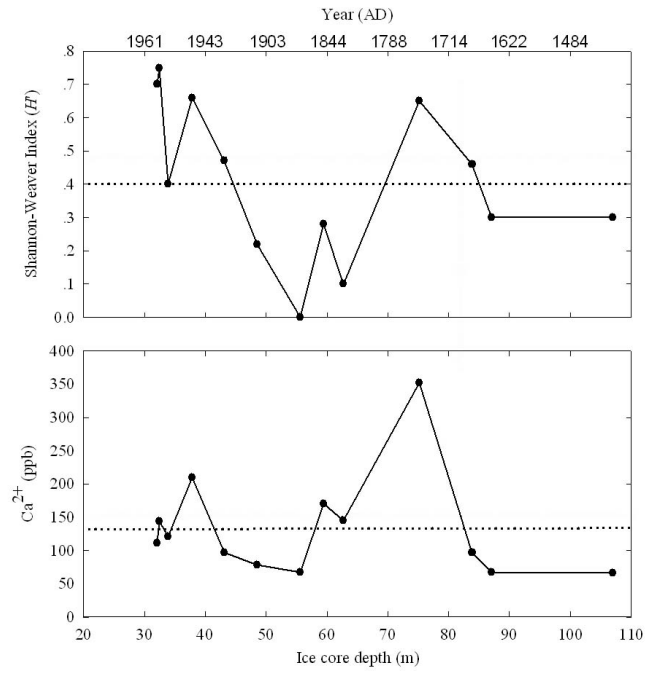**Fig. 5.** Profiles of bacterial and Ca^{2+} concentrations along the ER ice core. Dashed lines through the data sets indicate the third-order regression.
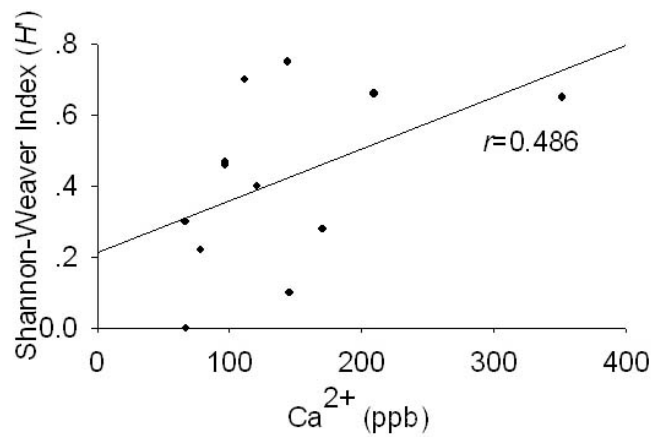
Fig. 6. The linear correlation between the bacterial and Ca^{2+} concentrations of the ER ice core samples.

Fig. 7. Vertical distribution of Shannon-Weaver index and Ca^{2+} concentrations along the ER ice core. Dotted lines through the data sets indicate their mean values.

Fig. 8. The linear correlation between Ca^{2+} concentrations and bacterial community diversity of the ER ice core samples.