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Culturable bacteria in Himalayan ice in response to atmospheric circulation

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Abstract

Only recently has specific attention been given to culturable bacteria in Tibetan glaciers, but their relation to atmospheric circulation is less understood yet. Here we investigate the seasonal variation of culturable bacteria preserved in a Himalayan ice core. High concentration of culturable bacteria in glacial ice deposited during the pre-monsoon season is attributed to the transportation of continental dust stirred up by the frequent dust storms in Northwest China during spring. This is also confirmed by the spatial distribution of culturable bacteria in Tibetan glaciers. Culturable bacteria deposited during monsoon season are more diverse than other seasons because they derive from both marine air masses and local or regional continental sources. We suggest that microorganisms in Himalayan ice can be used to reconstruct atmospheric circulation.

1 Introduction

Bacteria in glacial ice have been studied for polar regions (Dancer et al., 1997; Abyzov et al., 1998; Castello et al., 1999; Willerslev et al., 1999), and the Tibetan plateau (Christner et al., 2000; Zhang et al., 2003; Yao et al., 2006). Abyzov et al. (1998) reported that the amount of bacteria in melt water samples of the Antarctic Vostok ice core was closely correlated with mineral concentrations at the time of deposition. Zhang et al. (2003) and Yao et al. (2006) studied bacteria in Malan ice core recovered from the northern Tibetan Plateau, and suggested that microorganism concentrations tend to be negatively correlated with the temperature at a relatively long timescale and, to some extent, positively correlated with mineral concentrations.

A 40.87 m ice core was recovered from the saddle of East Rongbuk (ER) Glacier (28°01′05″ N, 86°57′52″ E, 6518 m above sea level) in the Himalayas during 2002. This glacier covers a total area of 48.45 km^2 with a length of 14 km. Its equilibrium line of ~6250 m above sea level is among the highest in the world. Borehole temperature is

−9.6°C at 10 m depth, ensuring preservation of a climatic record. At the drilling site, the annual net balance is about 500 mm water equivalent (Kang et al., 2002). The core (diameter 9.4 cm) was recovered using an electromechanical drill in dry hole. Visible stratigraphy showed no hiatus features within the whole core. Ice core was maintained below −5° from the time of drilling until analysis. The 0.57 m ice core from 40.30 m to 40.87 m depth was cut quasi-equally into four sections. Based on seasonal variations of stable oxygen isotope (δ^{18}O) and the annual accumulation rate (Kang et al., 2002), these four samples represent roughly four seasons of one whole year. Here we use these samples to deduce the relationship between concentration, diversity and sources of culturable bacteria and atmospheric circulation.

2 Methodology

2.1 Sampling

For all sampling procedures, plastic tools were exposed to a germicidal UV lamp for ≥30 min and ferric and ceramic tools were autoclaved. Prior to analysis, an outer 1 cm annulus was sliced from each sample with sterile stainless steel scalpels. The inner discs were rinsed in the 4° solution of 2.5% iodine and 0.1% bromo-geramine for 2 min. After finally rinsed with cold deioned water 3 times (melting away about 2 mm of the exterior surfaces), the samples were placed into sterile glass beakers and allowed to melt completely at 4° in the dark.

2.2 Bacterial isolation and count

Samples of about 200 ml melt water from the inner discs were filtered at room temperature (25°) through polycarbonate filters (Whatman) with 0.22 μm pore size, and the particulates collected were resuspended in phosphate-buffered saline. Aliquots of the suspension were spread onto the surface of agar-solidified media PYGV (http://www.

dsmz.de/media/med621.htm) and R2A (http://www.dsmz.de/media/med830.htm) containing low level of nutrients. Afterwards, duplicate plates were incubated aerobically at 4° and 20° for about 30 d and 90 d, respectively. Colonies with different morphologies were checked for purity by streaking on plates of the same medium, and stored in glycerol at −70°.

2.3 DNA extraction

Total DNA of bacteria was extracted using the method described by Zhou (1996). Bacterial colony was resuspended in 13.5 mL DNA extraction buffer (100 mM Tris-HCl (pH 8.0), 100 mM sodium EDTA (pH 8.0), 100 mM sodium phosphate (pH 8.0), 1.5 M NaCl, 1% CTAB), and 100 mL proteinase K (10 mg mL^{-1}) in centrifuge tubes, by horizontally shaking at 225 rpm for 30 min at 37°. Afterwards, 1.5 mL of 20% SDS was added, and the samples were incubated in a 65° water bath for 2 h with gentle end-over-end inversions every 15–20 min. The supernatants were collected after centrifugation at 6000×g for 10 min at room temperature, and transferred into 50 mL centrifuge tubes. Supernatants from the extractions were combined with equal volume of chloroform-isoamyl alcohol (24:1 vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 16 000×g for 20 min at room temperature, washed with cold 70% ethanol, and resuspended in sterile deionized water, to give final volume of 500 mL.

2.4 PCR Amplification, Restriction Fragment Length Polymorphism (RFLP) Analysis, sequencing of amplified 16S rDNA and phylogenetic analysis

Isolates' 16S rDNA from glacial culturable bacteria was amplified by PCR using the oligonucleotide primers PB36 (5′-AGAGTTTGATCCTGGCTCAG-3′) and PB38 (5′-CGGTTACCTTG TTACGACTT-3′), corresponding to *Escherichia coli* positions 8–27 and 1492–1509, respectively. PCR was carried out in a final volume of 25 μL using

5 μL template DNA, 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 μM each primer, and 1 U Taq polymerase with 1 concentration of the supplied buffer (MBI). Reactions were performed in the thermocycler (GeneAmp PCR System 2700, Applied Biosystems) with the following cycling parameters: 94° for 1 min, followed by 30 cycles of 94° for 1 min, 58° for 1 min, and 72° for 1.5 min, and a final incubation at 72° for 10 min.

The PCR products were digested with restriction endonuclease *Hae*III and *Hind*6I (MBI) according to the supplier's instructions. Isolates were grouped together on the basis of the restriction fragment patterns.

The 16S rDNA products representing each distinct pattern were further purified and sequenced using a state-of-the-art ABI 3730XL96 capillary sequencer with internally nested primers 27 F, 517 F and 907 F to obtain overlapping sequences.

The single stranded 16S rRNA gene sequences of the bacteria were matched with those from a Blast search of the National Center for Biotechnology Information (NCBI) database. The gene sequences from the bacteria were aligned with reference sequences obtained from GenBank databases by using the Clustal X program. The BioEdit alignment was used in maximum-likelihood and distance analyses utilizing the Mega package (Molecular Evolutionary Genetics Analysis, Version 3.0).

2.5 Analyses of stable oxygen isotope ratios

Stable oxygen isotope ratios ($\delta^{18}O$) were analyzed using a Finnigan MAT-252 mass spectrometer (accuracy 0.05‰) and expressed as the relative deviation of heavy isotope content of Standard Mean Ocean Water (SMOW).

3 Results and discussion

3.1 Spatial distribution of culturable bacteria in glaciers

Concentration of culturable bacteria in ER is between 0–7.0 CFU mL^{-1} (colony forming units per milliliter), while that of other glaciers from the northern Tibetan Plateau

(Guliya, Muztagh Ata and Malan) show a much broad range (Christner et al., 2000; Zhang et al., 2001; Xiang et al., 2005; Fig. 1). Especially, the concentration maximum of culturable bacteria in ER is one order of magnitude lower than that of other Tibetan glaciers. Christner et al. (2000) suggested that glaciers in the Tibetan Plateau, due to its proximity to major biological ecosystems, entrapped higher concentration of culturable bacteria than Antarctic or Greenland ice sheets. Although this is true for Guliya (Christner et al., 2000), Muztagh Ata (Xiang et al., 2005) and Malan glaciers (Zhang et al., 2001; Yao et al., 2006), concentration of culturable bacteria in ER is among the concentration level of the polar region (Fig. 1).

Guliya, Muztagh Ata and Malan glaciers are in a continental climate domain. Impurity within these glaciers is dominated by the influx of continental dust derived from the surrounding arid or semi-arid regions of central Asia. To the contrary, impurity in the Himalayan glaciers is strongly affected by maritime aerosols (Wake et al., 1994). Xiao et al. (2002) showed that the concentration of major ions in the north Tibetan Plateau was 6–30 times higher than that in the south. In fact, microparticle in ER (unpublished data) is two orders of magnitude lower than that in Guliya ice core (Wu et al., 2004). The common spatial distribution of culturable bacteria, major ions and microparticle in the Tibetan glaciers implies that they are influenced by similar environmental factors including dust and moisture sources, monsoon circulation.

3.2 Seasonality of concentration and variety of culturable bacteria

There is a prominent variation of concentration of culturable bacteria recovered from Samples No. I to IV (Fig. 2). The highest concentration is 7.0 CFU mL^{-1} in Sample No. I, while Samples II, III and IV have much lower concentration of culturable bacteria than Sample No. I. We suggest that Sample No. I was deposited during premonsoon season, a period consistent to the highest atmospheric dust loading. The close correlation between concentration of culturable bacteria and dust was also identified in Malan (Zhang et al., 2001; Yao et al., 2006) and Muztagh Ata (Xiang et al., 2005) ice cores.

From the results of Restriction Fragment Length Polymorphism (RFLP), Sample No. II that was deposited during monsoon season has the highest variety of culturable bacteria among the four samples (Fig. 2). This is because culturable bacteria deposited during monsoon season originate from either marine air masses and/or continental air fluxes, whereas culturable bacteria deposited during the other seasons are from only continental environment. However, it's worthy pointing out that the samples may not correspond exactly to their specific seasons due to coarse sampling resolution. For instance, Sample No. I may include partly culturable bacteria deposited during monsoon season, resulting in its relatively higher variety of culturable bacteria than Samples III and IV. Such a seasonality of culturable bacteria is also prominent for major ions of an 80 m ice core recovered from ER (Kang et al., 2002).

Stable oxygen isotopes (δ^{18}O) in Himalayan precipitation show enrichment in winter and depletion in monsoon season (Tian et al., 2001). Thus Sample No. II with the lowest δ^{18}O (−20.651‰) is confirmed to be deposited in monsoon season (Fig. 2), while Sample No. IV with the highest δ^{18}O (−13.604‰) was deposited in winter. Accordingly, Sample No. I was deposited roughly during premonsoon and Sample No. III deposited during postmonsoon season.

3.3 Seasonality of culturable bacteria sources

16S rDNA sequences of culturable bacteria (Fig. 3) shows that near half of culturable bacteria recovered from Sample No. II (zf-IIPht1 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=54303761&dopt=GenBank), zf-IIRht1 (Groudieva et al., 2004), zf-IIRht10 (Brinkmeyer et al., 2003), zf-IIRht12 (Christner et al., 2001), zf-IIRht16 (Tiago, et al., 2004), zf-IIRlt1 (Takami et al., 1997), zf-IIRlt3 (Dai et al., 2005)) are associated with marine environment, confirming their sources of monsoon air masses. Other culturable bacteria recovered from Sample No. II (e.g. zf-IIPht24 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=40456390&dopt=GenBank), zf-IIRht4 (Chin et al., 1999), zf-IIRht17 (Sander et al., 2001), zf-IIRlt12 (http://www.ncbi.nlm.nih.gov/entrez/

query.fcgi?cmd=Retrieve&db=Nucleotide&list_uids=62866400&dopt=GenBank)) are related to soil or other natural habitats, indicating their local or regional sources. This is consistent with the wet deposition dominated by marine air masses and dry deposition dominated by local air masses during monsoon seasons in the Himalayan region. Only two culturable bacteria (zf-IVRht8 and zf-IVRht11) were recovered from Sample No. IV deposited in winter (Fig. 3). Both are clustered with the genus *Methylobacterium*, ubiquitous in terrestrial habitats including soil, dust, etc. (Green, 1997), implying that sources of these two bacteria are associated solely with continental environment. Therefore, sources of culturable bacteria recovered from ER are consistent with the climate in the Himalaya being dominated by the Indian Monsoon in summer and by westerly cyclonic activities in winter (Thompson et al., 2000).

4 Conclusions

The concentrations of culturable bacteria in ER is among the concentration level of the polar region that are two orders of magnitude lower than that of the other Tibetan ice cores. This is coincident with the different environmental conditions between the southern and northern part of the Tibetan plateau. In ER, larger amount of culturable bacteria were deposited in spring, when much dust is transported into the ice. But a greater diversity of culturable bacteria was deposited in summer, when bacterial sources were both the deposition of Indian monsoon air masses and the regional or local mineral aerosol. Therefore, we suggest a fingerprint of atmospheric circulation in the microorganisms isolated from Himalayan glacier ice.

Our work expands knowledge of spatial and temporal microbes in the Tibetan glaciers, but in the absence of sufficient samples, a more thorough study is required to better understand the correlation between microorganisms and their living environment.

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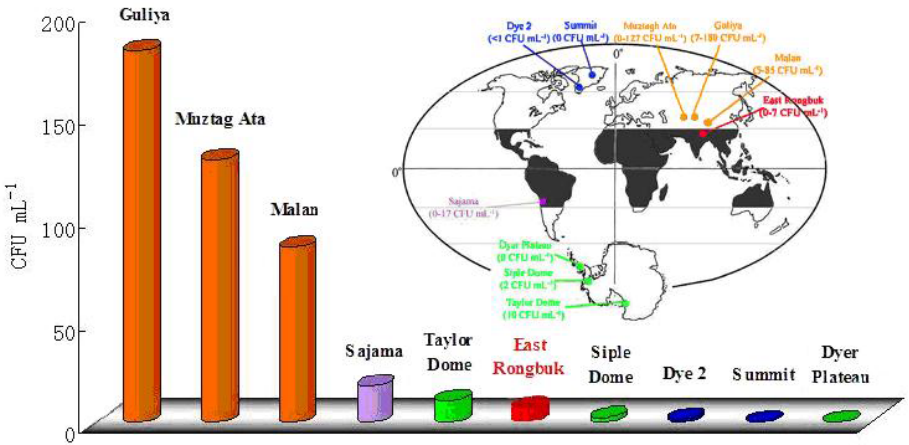

Fig. 1. Concentration of culturable bacteria recovered from ER (red) compared with other Tibetan (orange), Sajama, Bolivia (purple), Arctic (blue) and Antarctic (green) ice cores. Inset shows the location of sampling sites (modified from Christner et al., 2000).

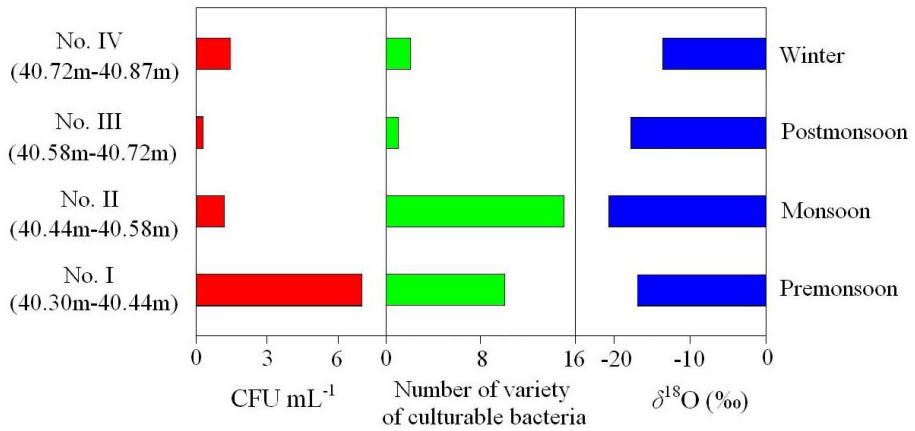

Fig. 2. Seasonal variation of concentration of culturable bacteria (CFU mL^{-1}), number of culturable bacteria varieties and $\delta^{18}O$ (‰).

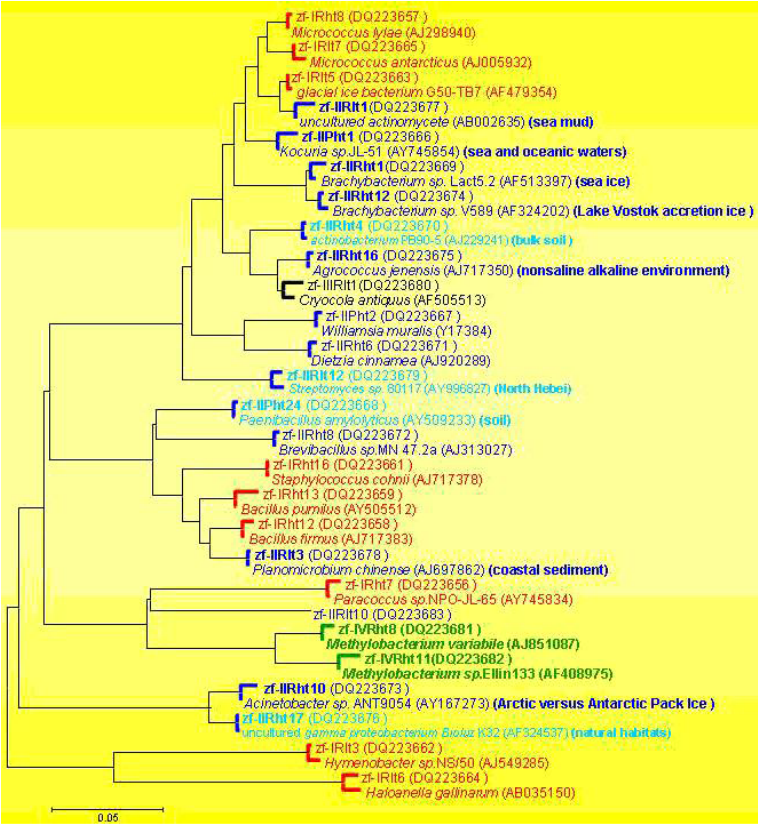

Fig. 3. Maximum Parsimony phylogeny of culturable bacterial 16S rDNA sequences amplified from the four samples. The culturable bacteria were nominated as: R and P refer to isolates recovered from culture medium R2A and PYGV, respectively. I, II, III and IV correspond to isolates recovered from Sample No. I (red), No. II (blue), No. III (black) and No. IV (green), respectively. ht and lt correspond to isolates recovered from 20° and 4°, respectively. Previously unpublished sequences are deposited in GenBank under accession numbers DQ223656–DQ223683, except DQ223659.