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Culturable bacteria isolated from snow cores along the 1300 km traverse from Zhongshan Station to Dome A, East Antarctica

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Abstract The abundance and community composition of culturable bacteria in four snow cores along the 1300 km traverse from Zhongshan Station to Dome A, East Antarctica, were investigated through the combination of liquid and solid media and small subunit 16S rRNA sequences. Under aerobic cultivation conditions, the average concentrations of bacterial colonies from each snow core varied from 0.008 to 0.32 CFU mL^{-1}. A total of 37 and 15 isolates with different morphologic characteristics were recovered from solid and liquid media PYGV, respectively. The phylogenetic analysis of 14 representatives with different ARDRA patterns from RFLP showed that all the isolates were affiliated with five phylogenetic groups: *Firmicutes*, *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes*. *Actinobacteria* represented the largest cluster with 43% of strains, and these strains exhibited unique phenotypic properties. The community compositions of culturable bacteria in the four snow cores were distinctly different from each other and the concentrations and community sizes of culturable bacteria along the traverse decreased with increases of latitude, altitude and distance from coast, which likely reflected the different bacterial sources and biogeographies under the different regional climate conditions in the snow cover of East Antarctica.

Keywords Culturable bacteria · Abundance · Community composition · Snow cover · East Antarctica

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Introduction

At present, the cryosphere is important not only as an integral part of the global climate system but also as one of the major habitable ecosystems of the Earth's biosphere and the best analog for the search for extraterrestrial life. Seasonal snow cover is an important component of the cryosphere and can at times cover ca. 35% of the Earth's surface (Jones 1999; Miteva 2007). The eolian varies in content and quantity depending on climate and creates different local snow ecological systems, characterized as dynamic nutrient and microbial reservoirs (Jones 1999). Recent reports suggest that microorganisms impact the abundance and composition of nutrients (Hodson et al. 2008) such that they may shift surface albedo of snow and ice (Thomas and Duval 1995) and impact hydrochemistry (Tranter et al. 2002). In addition, they play important roles in governing redox conditions and Fe, S, N and P cycling (Hodson et al. 2008). Snow algae have been studied extensively at different glaciers (Hoham and Duval 2001; Painter et al. 2001), whereas there is a paucity of information concerning the characteristics of bacteria in snow cover. In the polar region, Carpenter et al. (2000) found 10–20% of all 16S rRNA gene

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sequences obtained from Antarctic snow samples to be related to *Thermus–Deinococcus*-like organisms, known for their high radiation and desiccation resistances, and reported low rates of bacterial DNA and protein synthesis. Amato et al. (2007) reported that bacterial concentrations were about 2×10^4 cells mL^{-1} in snow cover at Spitsbergen, Svalbard, and recovered strains belonging to *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*. Segawa et al. (2005) collected a psychrophilic bacterium, *Cryobacterium psychrophilum*, and two psychrotrophic bacteria, *Variovorax paradoxus* and *Janthinobacterium lividum* in the surface snow from the Tateyama Mountains, Japan. Bacterial abundance ranged from 10^3 to 10^5 cells mL^{-1} in the snow at Mount Sonnblick, Austria and in the Tyrolean Alps (Alfreider et al. 1996; Sattler et al. 2001). Liu et al. (2009) reported that bacterial abundance in the snow from the Tibetan Plateau ranged from 0.68×10^3 to 720×10^3 cells mL^{-1} and detected 15 common genera in the 16S rRNA gene clone library.

Dome A, the highest ice feature in Antarctica, is located near the center of East Antarctica, and is commonly named "the inaccessible pole" due to its most stern climatic condition. To date, several shallow ice cores, snow pits and surface snow samples collected along the traverse from the Zhongshan Station to Dome A, have been investigated for chemical and physical parameters such as annual mean accumulation rate, major ions and microparticle analysis, and isotopic composition (Hou et al. 2007; Ding et al. 2011), but the bacterial features are not yet studied. In 2005, four 1.6 m long snow cores were collected along the traverse at LT886, DT313, DT416 and Dome A at distances of about 280, 920, 1128 and 1251 km from the coast, respectively (Figs. 1, 3). We applied traditional cultivation methods (1) to investigate the abundance, phenotypic properties and community composition of culturable bacteria, and (2) to probe into the spatial distribution of culturable bacteria under the modern climate condition along the traverse in East Antarctica.

Materials and methods

Decontamination

The four snow cores were placed in a UV-sterilized positive-pressure laminar-flow hood in a biologically clean room and manipulated at about −4°C. An annulus (around 16 mm) was cut twice successively from the surface of

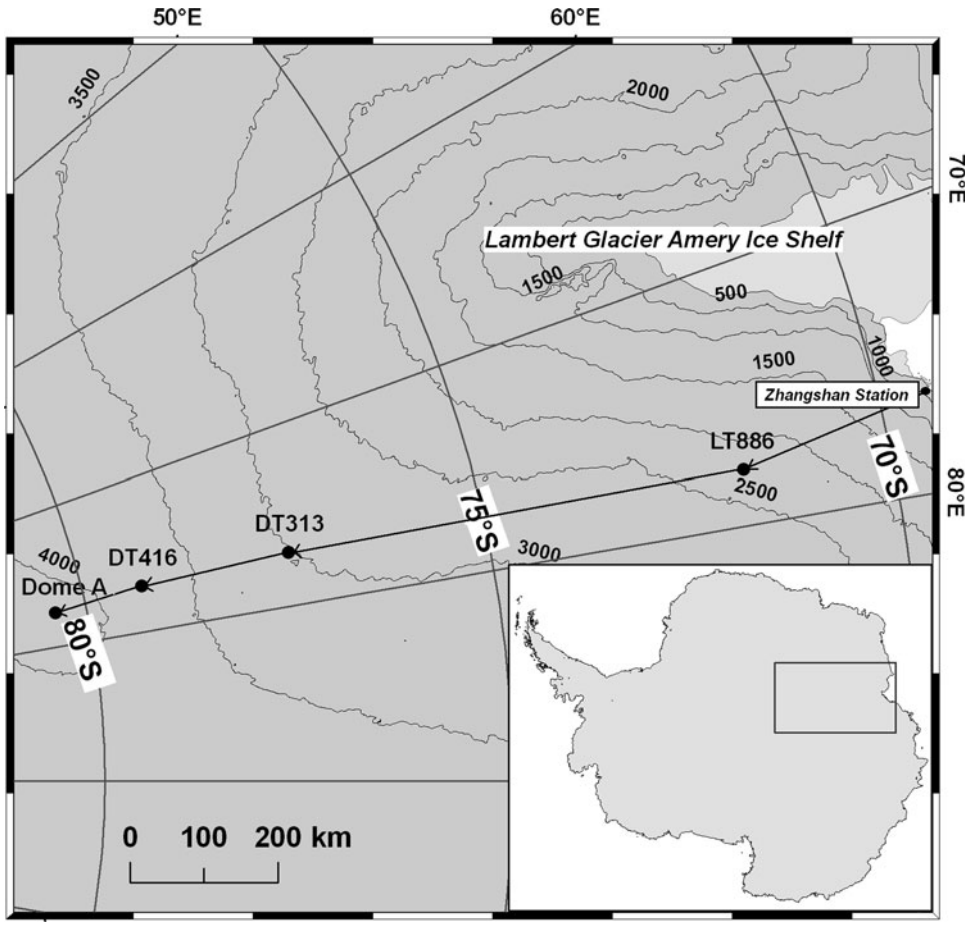

Fig. 1 Map showing the Zhongshan-Dome A traverse route and locations of sampling sites

each snow core with two clean and sterilized sawtooth knives. Each decontaminated snow core was placed into a sterile glass beaker and allowed to melt completely at 4°C in the dark.

Bacterial isolation and count

Meltwater samples (300–500 mL) from each snow core were filtered with 0.22 μm polycarbonate filters (Whatman). The particulates collected from each sample were resuspended in 0.6 mL phosphate buffered saline (Zhang et al. 2007a), and 0.4 mL of each suspension was spread onto the surface of solid media PYGV (http://www.genebank.go.kr/gp/resourceInfoSearch/microbe/media_pop_view.jsp?sMediaSn=164&sMediaName=PYGV%20Agar) containing low levels of nutrients. The duplicate plates were incubated aerobically at both 4 and 20°C. The concentrations of cultured bacteria were estimated by counting the average colony formation units (CFU) per milliliter on each plate colonies. The remaining suspension from each sample was pipetted into 50 mL flask filled with 20 mL liquid media PYGV, and the flasks were shaken at 80 rpm and 9°C. Two replicate 10 mL samples of meltwater from each snow core were pipetted into 150 mL flasks with 40 mL liquid media PYGV. Half of the duplicate flasks were shaken at 100 rpm and 20°C, and the other half were incubated at 9°C. After aerobic enrichment, 0.1 mL inocula from each turbid flask were plated onto the corresponding solid medium for isolation of colonies. The colonies with different morphologic characteristics were checked for purity by streaking on plates of the same medium, and stored in glycerol at −70°C.

DNA extraction, PCR amplification, restriction fragment length polymorphism (RFLP) analysis, and sequencing of amplified 16S rRNA

Genomic DNA of the isolates was extracted according to the method described by Zhang et al. (2007a). The universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) were used for polymerase chain reaction (PCR) amplification of 16S rRNA in a 25 μl system with Taq DNA polymerase (MBI) and standard amplification conditions (Miteva et al. 2004). Amplified rRNA restriction analysis (ARDRA) was performed with *Alu*I and *Hae*III (MBI) digestion. The 16S rRNA fragments representing each distinct ARDRA pattern were further purified with PCR purification columns and sequenced with bacterial universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′), 517F (5′-CCAGCAGCCGCGGTAAT-3′) and 907F (5′-AAACTCAAATGAATTGACGGG-3′) to obtain overlapping sequences.

Phenotypic characterization

Gram staining, cell morphology observations, presence of pigment, oxidase and catalase tests and oxidative or fermentative acid production from carbohydrates test were performed on the 13 strains according to previously reported methods (Maugeri et al. 1996).

Phylogenetic analysis

All the sequences were checked for the presence of chimeric sequences using the CHECK_CHIMERA program (available at http://rdp.cme.msu.edu/). For further phylogenetic analysis, the single-stranded 16S rRNA gene sequences of the 14 isolates were matched with those in the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov) by BLAST searching. The similar reference sequences were downloaded and aligned with the isolate sequences using Clustal X 1.8 program (http://bips.u-strasbg.fr/fr/documentation/clustalX/). Phylogenetic relationships of the sequences were constructed using MEGA 4.1 software (Kumar et al. 2008).

Nucleotide sequence accession number

The 16S rRNA gene sequence data reported in the present paper have been submitted to the GenBank nucleotide sequence databases under accession numbers: MC10-5 (HM196763), MC10-6 (HM196764), MC10-12 (HM196765), MC10-14 (HM196766), MC10-17 (HM196767), MC10-21 (HM196768), MC10-F2 (HM196769), MC7-1 (HM196770), MC8-1 (HM196771), MC5-1 (FJ932656), MC5-5 (FJ932658), MC5-F2 (FJ93260), MC5-F4 (FJ932663), MC7-F1 (FJ932666).

Results

Isolated strains

After 3 months of aerobic incubation, a total of 89 visible colonies were detected on the PYGV plates. However, there were some plates on which no colonies were observed. All the colonies on plates were classified into 10 bacterial strains through sequencing. Among them, strain MC5-5 was recovered at 4°C and strain MC5-1 was recovered at both 4 and 20°C. The other eight strains were recovered at 20°C. After 2 months of aerobic enrichment, four strains were recovered from liquid media PYGV. Strain MC10-F2 and strain MC7-F1 were enriched from flasks inoculated with suspensions at 9°C. Strain MC5-F2 and strain MC5-F4 were enriched from flasks containing meltwater at 20 and 9°C, respectively (Table 1).

Table 1 Characteristics of bacteria strains along the traverse from Zhongshan Station to Dome A and the cultivation conditions

Snow core	Strain	Colony description	Nearest phylogenetic neighbor (origin; GeneBank accession no.)	Identity (%)	Cultivation condition
LT886	MC10-5	Cream; large; mucoid	*Rhizobium cellulosilyticum* strain ALA10B2 (sawdust; DQ855276)	99.4	Suspension plating at 20°C
	MC10-6	Orange; medium; smooth	*Kaistella flava* strain YIM 47657T (soil; AM421015)	98.9	Suspension plating at 20°C
	MC10-12	Yellow; small; mucoid	*Microbacterium* sp. CTDE4 (deep sea sediment; GQ169066)	99.9	Suspension plating at 20°C
	MC10-14	Laurel-green; small; mucoid	*Dermacoccus* sp. BSi20643 (Arctic sea ice; EU330343)	99.6	Suspension plating at 20°C
	MC10-17	Dark yellow; medium; smooth	Uncultured bacterium clone 1H3C_20 (subseafloor sediment; JN229885)	98.0	Suspension plating at 20°C
	MC10-21	Yellow; medium; raised	*Microbacterium* sp. CTDE4 (deep sea sediment; GQ169066)	96.2	Suspension plating at 20°C
	MC10-F2	Pale yellow; small; mucoid	*Kocuria rosea* (Arctic Ocean marine sediments; DQ060382)	99.9	Suspension enrichment at 9°C, 80 rpm
DT313	MC5-1	White; large; matte	*Paenibacillus vortex* strain V453 (other samples; HQ005270)	99.7	Suspension plating at 4 and 20°C
	MC5-5	Orange-yellow; medium; mucoid	*Clavibacter michiganensis* (arid and semiarid environments; DQ507208)	99.7	Suspension plating at 4°C
	MC5-F2	Orange-red; small; raised	*Stenotrophomonas rhizophila* strain Asd M1-7 (Midtre Lovenbreen glacier sediment; FM955853)	99.9	Meltwater enrichment at 20°C, 100 rpm
	MC5-F4	Cream; small; smooth	*Brevundimonas vesicularis* strain Asd M7-3 (Midtre Lovenbreen glacier sediment; FM955876)	99.9	Meltwater enrichment at 9°C, 80 rpm
DT416	MC7-1	Orange; medium; smooth	*Kytococcus sedentarius* DSM 20547 (marine; CP001686)	99.6	Suspension plating at 20°C
	MC7-F1	Purple; small; raised	*Sphingomonadaceae* bacterium PB59 (terrestrial and freshwater environments; AB220107)	99.6	Suspension enrichment at 9°C, 80 rpm
Dome A	MC8-1	Yellow; medium; mucoid	Uncultured bacterium clone EDW07B001_137 (Texas state well; HM066326)	99.8	Suspension plating at 20°C

The strains tested showed unique morphological traits, enzyme production and fermentable carbohydrates profiles (Tables 1, 2). The majority of the strains formed pigmented colonies. The colonies of six strains presented pale yellow, yellow, orange-yellow and dark yellow and laurel-green colonies were rare. Four strains had deeper orange to purple pigmentation and few strains formed white and cream colonies. Almost 86% of the strains formed small or medium colonies, which were mostly smooth or mucoid and the remaining were raised. Only one strain produced matte colonies. Strain MC7-1 had potentially useful oxydase and urease, whereas its closest relative (*Kytococcus sedentarius* DSM 20547) had been reported not to (Sims et al. 2009). Strain MC10-14 had no catalase activity, but its most similar strains (*Dermacoccus* sp. BSi20643) had been reported to have catalase activity (Stackebrandt et al. 1995). The sequences of strain MC5-F4 and MC8-1 were very similar, but the former could utilize gelatin, mannose and xylose, while strain MC8-1 could not.

16S rRNA-based phylogenetic relationships

Among all the initially recovered colonies by liquid and solid cultivations, 52 isolates with visually different morphologic characteristics were subcultured to obtain pure colonies. To avoid sequencing several identical 16S rRNA samples, the PCR products of the 52 representative bacterial isolates were digested with restriction endonucleases and grouped into 14 different ARDRA patterns. The sequences obtained were affiliated with five phylogenetic groups: *Actinobacteria*, *Firmicutes*, *Alphaproteobacteria*, *Gammaproteobacteria* and *Bacteroidetes* representing the bacterial genera or families *Rhizobium*, *Microbacterium*, *Dermacoccus*, *Kocuria*, *Paenibacillus*, *Clavibacter*, *Stenotrophomonas*, *Sphingomonadaceae*, *Kytococcus*, *Brevundimonas* and *Kaistella* (Fig. 2).

Actinobacteria was the dominant group with 43% of all strains and fell into five major lineages. Two strains were clustered in the genus *Microbacterium*. Strain MC10-12

Table 2 Phenotypic properties of bacteria strains along the traverse from Zhongshan Station to Dome A

Strain	MC10-5	MC10-6	MC10-12	MC10-14	MC10-17	MC10-21	MC10-F2	MC5-1	MC5-5	MC5-F4	MC7-1	MC7-F7	MC8-1
Gram staining	−	+	+	+	−	+	+	+	+	−	+	−	−
Cell morphology	r	r	c	c	c	c	c	r	r	r	c	r	r
Indole production	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	−	−	−	−	−	+	+	−	−	−	−	−	−
Catalase	−	+	+	−	+	+	+	−	−	+	+	+	+
Oxydase	+	+	−	−	+	+	−	+	+	−	+	−	−
Citrate	−	−	−	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−	−	−	−
Hydrolysis Urea	+	+	−	+	−	−	−	−	−	−	+	+	−
Gelatin	−	+	+	+	−	−	−	+	−	+	−	+	−
Acid produced aerobically from O–F Glucose	F	−	F	O	−	F	F	F	F	F	F	−	F
Arabinose	+	−	−	−	−	−	+	+	−	−	−	−	−
Mannose	+	−	+	−	−	+	+	+	+	+	−	−	−
Mannitol	+	Nd	+	−	−	+	+	+	+	−	−	−	−
Sucrose	+	Nd	+	−	−	−	−	+	−	−	−	−	−
Fructose	+	+	+	−	−	+	+	+	+	−	−	−	−
Galactose	+	−	+	−	−	+	−	+	+	−	−	−	−
Xylose	+	−	−	−	−	−	+	+	+	+	−	+	−
Lactose	+	−	+	−	−	+	−	+	+	−	−	−	−
Rhamnose	+	−	−	−	−	−	+	+	−	−	−	−	−
Maltose monohydrate	+	+	+	−	−	+	+	+	+	+	−	−	+

O oxidation, *F* fermentation, *Nd* not determined, *c* cocci, *r* rod

and strain MC10-21 had 99.9 and 96.2% sequence similarities, respectively, to *Microbacterium* sp. CTDE4 (GQ169066) from deep sea sediment (Fig. 2; Table 1). Strain MC10-14 had 99.6% sequence similarity to *Dermacoccus* sp. BSi20643 (EU330343) from Arctic sea ice. Strain MC10-F2 showed 99.9% sequence similarity to *Kocuria rosea* (DQ060382) from Arctic Ocean marine sediments. Strain MC5-5 showed 99.7% sequence similarity to *Clavibacter michiganensis* (DQ507208) from arid and semiarid environments. Strain MC7-1 shared 99.6% sequence similarity to *Kytococcus sedentarius* (CP001686) from marine.

Another predominant group was *Proteobacteria* which classified into alpha and gamma subdivisions. Five strains belonged to *Alphaproteobacteria* and two strains of these were affiliated with the genus *Brevundimonas*. Strain MC5-F4 shared 99.9% sequence similarity to *Brevundimonas vesicularis* (FM955876) from Midtre Lovenbreen glacier sediment and strain MC8-1 shared 99.8% sequence similarity to uncultured bacterium clone (HM066326) from Texas State well. Strain MC10-5 and strain MC7-F1 of *Alphaproteobacteria* shared 99.4 and 99.6% sequence similarities, to *Rhizobium cellulosilyticum* (DQ855276) from sawdust and *Sphingomonadaceae* bacterium (AB220107) from terrestrial and freshwater, respectively. Only one strain (strain MC5-F2) belonged to *Gammaproteobacteria* and had *Stenotrophomonas rhizophila* (FM955853) from Midtre Lovenbreen glacier sediment, as the nearest neighbor in lineage with 99.9% sequence similarity.

Firmicutes and *Bacteroidetes* both had only one strain. Strain MC10-6 belonged to *Flavobacteria*, with 98.9% sequence similarity to *Kaistella flava* (AM421015) from soil. Strain MC5-1 showed 99.7% sequence similarity to *Paenibacillus vortex* (HQ005270) from other samples. From the work of Stackebrandt and Ebers (2006), it was clear that a cutoff of 98.7% 16S rRNA gene homology was appropriate for species differentiation within a genus. Strain MC10-21 and strain MC10-17 possibly represented new species in virtue of their highest similarities (96.2 and 98.0%) to *Microbacterium* sp. CTDE4 (GQ169066) and uncultured bacterium clone 1H3C_20 (JN229885), respectively.

Spatial distribution of cultured bacteria

The average concentrations of cultured bacteria isolated from snow core LT886, DT313, DT416 and Dome A were 0.320, 0.160, 0.017 and 0.008 CFU mL^{-1}, respectively. Members of *Alphaproteobacteria* were found in all snow cores (Fig. 2; Table 1). The isolates in the *Brevundimonas*

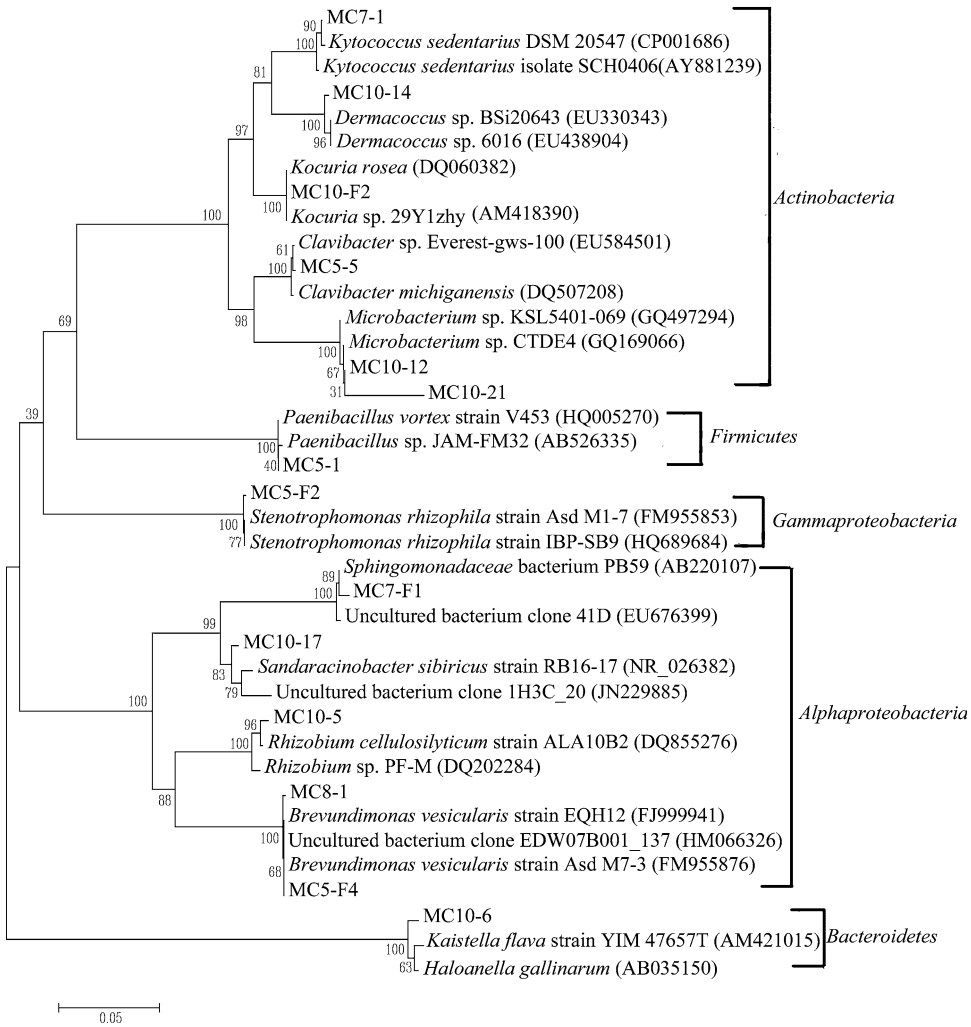

Fig. 2 The phylogenetic relationships of isolates from the Antarctic snow cores and their nearest relatives based on GenBank 16S rRNA sequences, bootstrap values of >50% (of 100 iterations) were obtained by maximum parsimony analysis for bootstrap sampling of 100; *scale bars* indicates *p* distance

genus were recovered from snow core DT313 and Dome A. No strains were distributed in all snow cores. Seven strains isolated from snow core LT886 were affiliated with the bacterial genera *Rhizobium*, *Kaistella*, *Microbacterium*, *Dermacoccus*, and *Kocuria*. Four strains isolated from snow core DT313 were affiliated with *Paenibacillus*, *Clavibacter*, *Stenotrophomonas* and *Brevundimonas*. Two strains isolated from snow core DT416 were affiliated with *Kytococcus* and *Sphingomonadaceae*. Only one strain from snow core Dome A was affiliated with *Brevundimonas*.

Discussion

Cultivation and isolation of bacteria

Two-sevenths of all the strains were enriched with melt-water from snow cores and this enrichment was, therefore, an effective complementary method for the recovery of isolates from the Antarctic snow cover. Amato et al. (2007) and Elster et al. (2007) had successfully increased the number and diversity of recovered isolates with such methods of amendment. Many bacteria might have been damaged or persist in a dormant or very low metabolic state in the harsh environment and the incubation in liquid media allowed them to recover the ability to form colonies.

Comparison between bacteria in the Antarctic ice sheet and other glaciers

The concentration and phylogenetic diversity of cultured bacteria in Antarctic snow cover were distinctly lower than that in non polar glaciers likely due to different microbial sources (Zhang et al. 2007a). Low-latitude, high-altitude glaciers are usually close to exposed soils and tropical and subtropical ecosystems, while the Antarctic ice sheet is surrounded by the Southern Ocean and remote to the terrestrial environment. A low biomass and biodiversity of air-borne microorganisms have been detected at Halley station, Antarctica (Pearce et al. 2010). The advection of moisture toward inland Antarctica is mostly provided by midtroposphere airflow from the subtropics, partially

replenished by intense vertical mixing associated with midlatitude cyclonic activity (Masson-Delmotte et al. 2008). The main terrestrial sources of the Antarctica ice sheet are large Australian desert and South America (Marshall 1996; Grousset et al. 1992). The phylogenetic diversity of cultured bacteria in Antarctic snow cover was almost equivalent to that of Lake Vostok accretion ice and Arctic snow cover (Amato et al. 2007; D'Elia et al. 2008), while it was lower than that of Greenland ice core, probably resulting from the contribution of soil particles in the Greenland ice core (Miteva et al. 2004). The low concentration and phylogenetic diversity of cultured bacteria were possibly also influenced by the use of 0.22 μm filters which could not retain ultrasmall cells such as those recently detected in other ice cores (Loveland-Curtze et al. 2009).

Almost all genera determined in Antarctic snow cover were also detected in other glacial environments. For instance, isolates of *Kocuria* and *Brevundimonas* have been recovered from East Rongbuk, Puruogangri and Muztag Ata ice cores from the Tibetan Plateau (Zhang et al. 2007a, b; Xiang et al. 2005). Members of *Dermacoccus*, *Clavibacter* and *Paenibacillus* have been isolated from Vostok ice core (Christner et al. 2000; D'Elia et al. 2008). Members of *Stenotrophomonas* and *Microbacterium* have been obtained from Greenland ice core and many members of *Sphingomonadaceae* have been found from Sajama (Bolivia), Taylor Dome, Siple (Antarctic) and the Guliya glaciers on the Tibetan Plateau (Christner et al. 2000; Miteva et al. 2004; Sheridan et al. 2003). Members of *Rhizobium* have been detected in 16S rRNA gene clone library of the Palong No. 4 glacier on the Tibetan Plateau (Liu et al. 2009). These findings indicate that the occurrence of related phylotypes in geographically diverse cold environments is possibly due to similar strategies to survive freezing and remain active at low temperatures (Priscu and Christner 2004). However, Members of *Kaistella* and *Kytococcus* were not detected in other glaciers. *Kytococus sedentarius* is known for the production of oligoketide antibiotics and *Kaistella flava* produces a lipophilic polysaccharide (Sims et al. 2009; Gargiulo et al. 2008). These two special metabolites may enhance bacterial resistances to extremely harsh environments.

Based on bacterial 16S rRNA sequences, 14 determined sequences were dominated by *Actinobacteria*. Amato et al. (2007) documented that isolates from the Arctic snow cover were dominated by *Alphaproteobacteria* and Liu et al. (2009) found that *Gammaproteobacteria* dominated in the 16S rRNA gene clone library for the snow cover on the Tibetan Plateau Glaciers. The distinction among niches and different methods employed in various studies might be responsible for the differences in bacterial communities.

The feature of pigmented colony dominance in cultured bacteria from Antarctic snow cover was also detected in other cold habitats (Zhang et al. 2007b; Miteva et al. 2004). Pigments might be a result of low temperature and UV-tolerant strategy on surface snow that the pigments could absorb UV light and detoxify superoxide and free radicals (Dillon et al. 2003). Fong et al. (2001) found a correlation between the production of carotenoids and the cold adaptation of microorganisms, possibly due to increased rigidity of the membranes and Newsham (2003) reported that UV-B radiation arising from stratospheric ozone depletion influenced the pigmentation of Antarctic moss. The colonies of strain MC7-F1 were purple with 99.6% sequence similarity to aerobic phototrophic bacteria and might synthesize substrates to maintain life under the oligotrophic habitat. Relatively low proportions of autotrophic bacteria were also reported in the Antarctic Dry Valleys (Moodley 2004).

The fact that the 16S rRNA gene sequences of cultured bacteria from Antarctic snow cover closely related to that of other habitats, but were physiologically distinct populations, were probably attributed to differences in the ecological conditions of bacteria survival habitats. Zeng et al. (2010) identified three bacterial strains isolated from the Arctic and the Antarctic sea waters belonging to the same species, but with different phenotypic properties. Klassen and Foght (2011) found that horizontal gene transfers and phenotypic variation emerged among five new species of *Hymenobacter*, even between apparently closely related strains, and might reflect evolutionary trajectories resulting from dormancy during interment in glacial ice.

Variation in the cultured bacteria along the traverse

The concentrations and community sizes of cultured bacteria along the traverse decreased evidently with increases of latitude, altitude and distance from coast (Fig. 3). A negative relationship was found between bacterial diversity and latitude for fell-field soils, but no such pattern was observed for vegetated sites, across a range of Antarctic terrestrial habitats (Yergeau et al. 2007). Lawley et al. (2004) reported that the microeukaryote diversity across a range of Antarctic terrestrial sites revealed no clear pattern of decreasing diversity with latitude. These results indicated that the interrelation between bacterial diversity and latitude might be dependent on characteristics of spatial scale and taxonomic hierarchy. Along the Zhongshan Station to Dome A traverse, it is thought that, near the coast, the oceanic climate affected bacterial sources and they were derived from short-distance marine masses. The continental climate mainly affected the Antarctic inland ice sheet, with the bacterial sources derived from the remote continent with long-distance transportation. It is also known that the humidity and temperature decline on the upper layers of the East Antarctic from coast to inland. Dome A is the most arid region and the "pole of cold" on Earth (Ding et al. 2011). Therefore the coastal environment

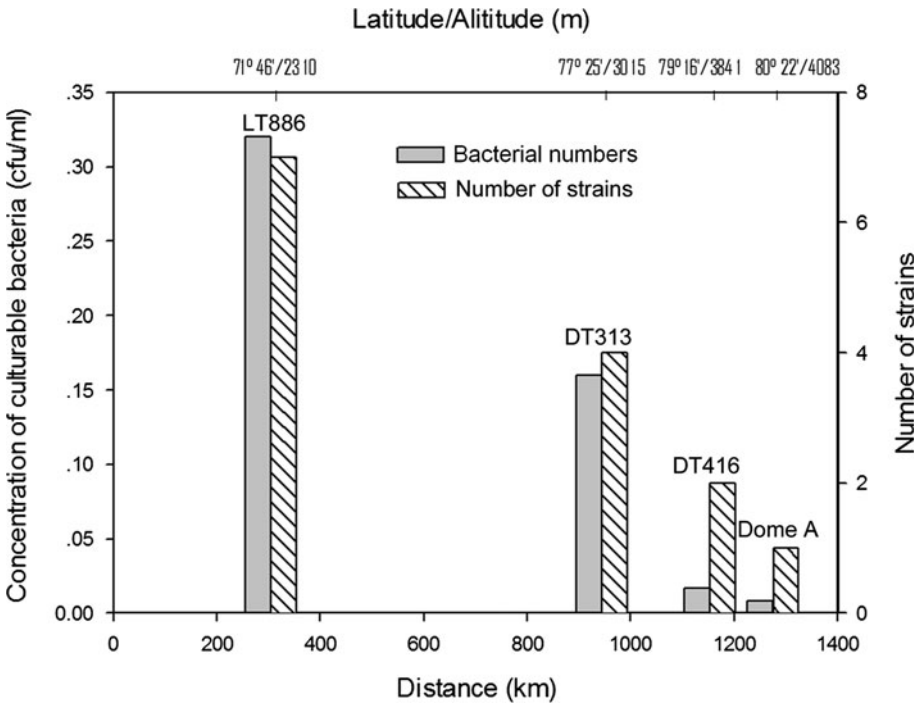

Fig. 3 The variation of bacterial concentration and number of strains recovered from different snow cores from different latitudes, altitudes and distances to Zhongshan Station, East Antarctica

was more favorable for bacteria survival than inland. This was also supported by the decreased density of aerial heterotrophic microflora with increasing distance from the coast in Antarctica aerosol samples (Wynn-Williams 1991; Elster et al. 2007).

The community compositions of cultured bacteria in four snow cores were different and no strain was present in two snow cores. The layered distribution of bacteria in ice cores presumably reflected the diverse bacterial sources and inhabiting niches under different past climate conditions (Miteva et al. 2004; Zhang et al. 2007b). Segawa et al. (2010) suggested that each bacterial phylotype was adapted to a distinct set of conditions on the glacier. LT886, DT313, DT416 and Dome A sampling sites were more than 100 km apart and subjected to different climatic environments. Given this explanation, the community distribution of bacteria recovered from the four snow cores might reflect the different bacterial sources and biogeographies under the different climate conditions in the upper snow layers of East Antarctica. Further studies are necessary to define the local scope and understand the characteristics of the different biogeographies.

Conclusion

The concentrations of cultured bacteria in the four snow cores along the traverse from Zhongshan Station to Dome A, East Antarctica ranged from 0.008–0.320 CFU mL^{-1}, and were lower than that in ice cores from mountain glaciers. This was likely attributed to the remote sources of airborne microorganisms in the Antarctica ice sheet compared with other glaciers. A total of 14 partial 16S rRNA gene fragments were sequenced into five groups: *Actinobacteria* (two *Microbacterium* sp., one *Dermacoccus* sp., one *Kocuria* sp., one *Clavibacter* sp. and one *Kytococcus* sp.), *Alphaproteobacteria* (two *Brevundimonas* sp., one *Rhizobium* sp. and one *Sphingomonadaceae* bacterium), *Gammaproteobacteria* (one *Stenotrophomonas* sp.), *Firmicutes* (one *Paenibacillus* sp.) and *Bacteroidetes* (one *Kaistella* sp.). *Actinobacteria* was dominant with 43% of all strains. Members of all genera obtained from the four snow cores had already been detected in other glaciers except *Kaistella* and *Kytococcus*, which suggested bacteria similar strategies to survive freezing and remain active at low temperatures. Some strains from snow cores had high genetic homogeneity with some from different ecological niches, whereas their physiological characteristics were different.

Along the traverse, the abundance and diversity of cultured bacteria in the four snow cores decreased with the increases of latitude, altitude and distance from coast. The community compositions were also distinctly different, which indicated the different bacterial sources and the different biogeographies under the different regional climate conditions in the upper snow layers. This study presents the first results of culturable bacteria corresponding to spatial variation in East Antarctica. A more profound investigation is required to better understand the correlation between

microorganisms and the complex regional climatic conditions in East Antarctica.

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References

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

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

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

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

D'Elia T, Veerapaneni R, Roger SO (2008) Isolation of microbes from Lake Vostok accretion ice. Appl Environ Microbiol 74:4962–4965

Dillon JG, Miller SR, Castenholz RW (2003) UV-acclimation responses in natural populations of cyanobacteria (*Calothrix* sp.). Environ Microbiol 5:473–483

Ding M, Xiao C, Li Y, Ren J, Hou S, Jin B, Sun B (2011) Spatial variability of surface mass balance along a traverse route from Zhongshan station to Dome A, Antarctica. J Glaciol 57:658–666

Elster J, Delmas RJ, Petit J-R, Řeháková K (2007) Composition of microbial communities in aerosol, snow and ice samples from remote glaciated areas (Antarctica, Alps, Andes). Biogeosci Discuss 4:1779–1813

Fong NJC, Burgess ML, Barrow KD, Glenn DR (2001) Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilisin* response to thermal and salt stress. Appl Microbiol Biotechnol 56:750–756

Gargiulo V, De Castro C, Lanzetta R, Jiang Y, Xu LH, Molinaro A, Parrilli M, Jiang CL (2008) Structural elucidation of the capsular polysaccharide isolated from *Kaistella flava*. Carbohydr Res 343:2401–2405

Grousset FE, Biscaye PE, Revel M, Petit JR, Pye K, Joussaume S, Jouzel J (1992) Antarctic (Dome C) ice-core dust at 18 k.y. B.P.: Isotopic constraints on origins. Earth Planet Sci Lett 111:175–182

Hodson A, Anesio AM, Tranter M, Fountain A, Osborn M, Priscu J, Laybourn-Parry J, Sattler B (2008) Glacial ecosystems. Ecol Monogr 78:41–67

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

Hou SG, Li YS, Xiao CD, Ren JW (2007) Recent accumulation rate at Dome A, Antarctica. Chin Sci Bull 52:428–431

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

Klassen JL, Foght JM (2011) Characterization of *Hymenobacter* isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. Extremophiles 15:45–57

Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9:299–306

Lawley B, Ripley S, Bridge P, Convey P (2004) Molecular analysis of geographic patterns of eukaryotic diversity in Antarctic soils. Appl Environ Microbiol 70:5963–5972

Liu Y, Yao T, Jiao N, Kang S, Xu B, Zeng Y, Huang S, Li X (2009) Bacterial diversity in the snow over Tibetan Plateau Glaciers. Extremophile 13:411–423

Loveland-Curtze J, Miteva V, Brenchley J (2009) Novel ultramicrobacterial isolates from a deep Greenland ice core represent a proposed new species, Chryseobacterium greenlandense sp. nov. Extremophiles 14:61–69

Marshall WA (1996) Biological particles over Antarctica. Nature 383:680

Masson-Delmotte V, Hou S, Ekaykin A, Jouzel J, Aristarain A, Bernardo RT, Bromwich D, Cattani O, Delmotte M, Falourd S, Frezzotti M, Gallée H, Genoni L, Isaksson E, Landais A, Helsen MM, Hoffmann G, Lopez J, Morgan V, Motoyama H, Noone D, Oerter H, Petit JR, Royer A, Uemura R, Schmidt GA, Schlosser E, Simões JC, Steig EJ, Stenni B, Stievenard M, van den Broeke MR, van de Wal RSW, van de Berg WJ, Vimeux F, White JWC (2008) A review of Antarctic surface snow isotopic composition: observations, atmospheric circulation, and isotopic modeling. J Climate 21:3359–3387

Maugeri TL, Gugliandolo C, Bruni V (1996) Heterotrophic bacteria in the Ross Sea (Terra Nova Bay, Antarctica). New Microbiol 19:67–76

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

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

Moodley K (2004) Microbial diversity of Antarctic Dry Valley mineral soil. http://etd.uwc.ac.za/usrfiles/modules/etd/docs/etd_init_2494_1176881817.pdf

Newsham KK (2003) UV-B radiation arising from stratospheric ozone depletion influences the pigmentation of the Antarctic moss *Andreaea regularis*. Oecologia 135:327–331

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

Pearce DA, Hughes KA, Lachlan-Cope T, Harangozo SA, Jones AE (2010) Biodiversity of air-borne microorganisms at Halley station, Antarctica. Extremophiles 14:145–159

Priscu JC, Christner BC (2004) Earth's icy biosphere. In: Bull AT (eds) Microbial diversity and bioprospecting. American Society for Microbiology, Washington, pp 130–145

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

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

Segawa T, Takeuchi N, Ushida K, Kanda H, Kohshima S (2010) Altitudinal changes in a bacterial community on Gulkana glacier in Alaska. Microbes Environ 25:171–182

Sheridan PP, Miteva VI, Brenchley JE (2003) Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. Appl Environ Microbiol 69:2153–2160

Sims D et al (2009) Complete genome sequence of *Kytococcus sedentarius* type strain (541^T). Stand Genomic Sci 1:12–20

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155

Stackebrandt E, Koch C, Gvozdiak O, Schumann P (1995) Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. Int J Syst Bacteriol 45:682–692

Thomas WH, Duval B (1995) Sierra Nevada, California, U.S.A., snow algae: snow albedo changes, algal-bacterial interrelationships, and ultraviolet radiation effects. Arct Alp Res 27:389–399

Tranter M, Sharp MJ, Lamb HR, Brown GH, Hubbard BP, Willis IC (2002) Geochemical weathering at the bed of Haut Glacier d'Arolla, Switzerland—a new model. Hydrol Process 16:959–993

Wynn-Williams DD (1991) Aerobiology and colonization in Antarctica—the BIOTAS Programme. Grana 30:380–393

Xiang S, Yao T, An L, Xu B, Wang J (2005) 16S rRNA sequences and differences in bacteria isolated from the Muztag Ata glacier at increasing depths. Appl Environ Microbiol 71:4619–4627

Yergeau E, Newsham KK, Pearce DA, Kowalchuk GA (2007) Patterns of bacterial diversity across a range of Antarctic terrestrial habitats. Environ Microbiol 9:2670–2682

Zeng Y, Zheng T, Yu Y, Chen B, He J (2010) Relationships between Arctic and Antarctic *Shewanella* strains evaluated by a polyphasic taxonomic approach. Polar Biol 33:531–541

Zhang S, Hou S, Ma X, Qin D, Chen T (2007a) Culturable bacteria in Himalayan glacial ice in response to atmospheric circulation. Biogeosciences 4:1–9

Zhang XF, Yao TD, Tian LD, Xu SJ, An LZ (2007b) Phylogenetic and physiological diversity of bacteria isolated from Puruogangri ice core. Microb Ecol 55:476–488