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Culturable bacteria in glacial meltwater at 6,350 m on the East Rongbuk Glacier, Mount Everest

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Abstract Culturable bacteria in the glacial meltwater in the ablation zones of glacier at high altitude (6,350 m) on Mt Everest were isolated and identified by 16S rRNA amplification and sequencing. The obtained sequences revealed the presence of members of α, β, and γ-Proteobacteria, Actinobacteria, and Firmicutes, with the Actinobacteria dominant in the studied habitat. All 16S rRNA sequences were similar to previously determined sequences, ranging from 97 to 99% identical values. The strains isolated from meltwater were distinctly different from those recovered from a cryoconite hole and under glacier habitat. The majority of the isolates' nearest neighbors were from the permafrost, dust, soil, plant, and aqueous environments. The Biolog bioassay and growth test under different temperatures suggested that the culturable bacteria in glacial meltwater could be divided into three categories in terms of their survival strategies: *Group I* sensitive to temperature change but versatile in utilization of carbon substrates (capable of utilization of about 70% of the Biolog carbon substrates); *Group II* tolerant to variable temperature and less capable of carbon utilization (less than half of the Biolog carbon species can be used); *Group III* slow in growth and weak in carbon utilization (only a few Biolog carbon substrates can be used).

Keywords Culturable bacteria · Glacial meltwater · Mt Everest

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Introduction

Microorganisms in the glaciers have received increasing attention during the past decade. Diverse bacteria have been recovered from the deep ice core from polar and alpine glaciers, and the results provide important clues to lifestyles that might be encountered on Mars and Europa (Christner et al. 2000, 2001, 2003b; Miteva et al. 2004; Sheridan et al. 2003). A significant number of bacteria beneath glaciers are reported to play important roles in chemical weathering and carbon cycling processes (Cheng and Foght 2007; Foght et al. 2004; Sharp et al. 1999; Skidmore et al. 2000, 2005). Complex microbial communities have been found in the cryoconite holes at the glacial surface. The cryoconite holes are thought to contain the highest level of biodiversity when compared with the other glacial niches (Christner et al. 2003a; Foreman et al. 2007; Margesin et al. 2002; Sawstrom et al. 2002). Not only were the microbial communities investigated, but also the factors influencing these microbes in the glacial environment (Abyzov et al. 1998; Yao et al. 2006). Temperature and nutrients were found to enhance bacterial growth and production in the glacial stream (Battin et al. 2001; Mindl et al. 2007). Although microorganisms were noted in various niches in polar and alpine glaciers in those studies (Cheng and Foght 2007; Christner et al. 2000; Foght et al. 2004; Tung et al. 2006), up to date, there is no report of

culturable bacteria inhabiting in meltwater on the surface of alpine glaciers at high altitude. Here, we report on the culturable bacteria (and their physiological properties) obtained from glacial meltwater at 6,350 m on the East Rongbuk Glacier, Mt Everest.

Glacial meltwater is available in the ablation zones of glaciers when temperatures rise above zero. It is ephemeral and only exists in summer. Microbes inhabiting it have to endure harsh conditions such as low temperature (Xie et al. 2007) and low substrates [dissolved organic carbon is about 0.39 μg L^{-1} (Liu et al. 2006)]. There is a paucity of information concerning the characteristics of bacteria in this extreme aquatic environment. As is known, cultivation and characterization of isolates are of particular importance for providing a more comprehensive view of the diversity of cultured organisms and for assessing the physiological functions of the organisms. In this study, we isolated diverse bacteria from cold oligotrophic glacial meltwater at 6,350 m, and we focus here on presenting evidence for bacterial physiological features in response to two essential environmental determinants, temperature and nutrients, since former studies have reported that bacteria isolated from the glacial environment are psychrophilic or psychrotolerant (Cheng and Foght 2007; Christner et al. 2000; Foght et al. 2004; Miteva et al. 2004; Xiang et al. 2005a). Our objectives were firstly to assess the diversity of cold-adapted heterotrophic culturable bacteria in high-altitude glacial meltwater and secondly to investigate bacterial utilization of nutrients and their flexibility to adapt to different temperatures.

Materials and methods

Sample site

East Rongbuk Glacier is located on the north slope of Mt Everest. The highest altitude of the glacier is 8,000 m, and the average altitude is 6,780 m. The glacier is highly sensitive to climate warming (Ren et al. 2004). Ecosystems within the glacier include snow, ice, ephemeral glacier meltwater, and rock. They are colonized predominantly by microorganisms and no higher plants or animals are present except for a few climbers and yak passing by during short climb periods.

Sample collection

Glacial meltwater samples were collected from a small pond on the surface of the glacier at 6,350 m a.s.l. on the East Rongbuk Glacier on April 26, 2005. The small pond was about 0.5 m^2 in area and less than 0.3 m in depth. There was no visible sediment in the pond. Samples (500 mL) were contained in prewashed and sterilized Nalgene bottles. Extreme care was taken at all times to ensure minimal contamination. Nonparticulating sterile suits, sterile gloves, and masks were worn during the entire sampling process. Samples were kept at about 4°C during transport.

Enrichment and isolation of bacteria

Ten milliliters of glacial meltwater was inoculated into 50 mL of rich organic (RO) liquid medium (Yurkov and Beatty 1998) and incubated aerobically at 4°C until the liquid medium became thick. Then 400-μL aliquots were plated onto RO agar medium. These plates were incubated aerobically at 18°C for 1–2 weeks. Colonies of visibly different morphology and color on the plates were picked and purified by streaking four to five times on new RO agar medium. The purified isolates were then subcultured on RO plates, and the cell cultures were stored at −80°C in a 30% glycerol solution.

Morphological observation by electron microscopy

Cells from 2-day cultures grown in RO liquid medium were observed under a transmission electron microscope (TEM, JEM100CX II) after negatively staining with 1% aqueous uranyl acetate after fixation with 2.5% glutaraldehyde, dehydration in sequential alcohol, and coating with gold. For thin sections, the bacteria were embedded in Epon after fixation with 5% glutaraldehyde and 1% osmium tetroxide.

Assessment of bacterial carbon source utilization

Substrate utilization profiles were tested using Biolog bioassay, which included an array of 96 wells for the oxidation of 95 carbon sources. Sample preparation and analysis was performed following the directions of the manufacturer (Biolog). Each plate was inoculated with a bacterial suspension (150 μL/well) and incubated for 7 days at 18°C. The resulting utilization patterns were read against a substrate blank well at $A_{590\ nm}$ with an automated plate reader (Molecular Devices Corporation, USA).Overall color development in BIOLOG plates was expressed as average well color development (AWCD). AWCD of the plate was calculated using the formula $(C - R)/\{[\sum(C - R)/95]\}$, where R is the value of the control well and C is the values of wells containing sole carbon sources.

Growth curve determination in different temperatures

Growth conditions at 15 and 25°C of five strains were determined in liquid RO medium by measuring $OD_{600\ nm}$

values of cultures every 8 h until their growth reached a stationary phase.

PCR amplification, restriction fragment length polymorphism, and sequencing of amplified 16S rRNA

Genomic DNA of the isolates obtained above was extracted using the chloroform–isoamyl alcohol extraction procedure (Johnson 1981). 16S rRNA genes of these isolates were amplified using the primer set of 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) (Brosius et al. 1978). The reaction mixture (30 μL) consisted of 1 U of La Taq (TaKaRa Co., Dalian, China), 0.2 mM dNTP, 3 μL of 10× Buffer, 0.15 mM of each primer, and 1 μL (ca. 10 ng DNA) of template. The PCR program was as follows: initial incubation at 94°C for 5 min, followed by 33 cycles (94°C for 50 s, 55°C for 1 min, and 72°C for 90 s), and then by a final extension at 72°C for 10 min.

The PCR products were screened for similarity using restriction fragment length polymorphism (RFLP) analysis. Amplified rRNA was digested with the restriction endonucleases *Hha*I and *Afa*I (TaKaRa Co., Japan) according to the supplier's instructions. The digested fragments were visualized on a 3% agarose gel. Isolates were grouped together on the basis of RFLP patterns, and one isolate was chosen from each group for sequencing. Prior to sequencing, PCR products were purified using an agarose gel DNA purification kit (TaKaRa Co., Dalian, China).

Phylogenetic analysis

Near full-length 16S rRNA gene sequences (ca. 1,500 bp) were compared to known sequences in the GenBank (http://www.ncbi.nih.gov) using the BLAST search tool. The closest neighbors were retrieved. The sequences obtained were compared with those in the database, and those displaying similarities >98% with known species were identified as corresponding species. A phylogenetic tree including obtained isolates and their closest relatives was constructed using MEGA software 3.1 (Kumar et al. 2004). Neighbor-joining phylogenies were constructed from dissimilatory distances and pair-wise comparisons with the Jukes–Cantor distance model. Bootstrap analysis of 1,000 replicates was performed.

Nucleotide sequence accession numbers

The nucleotide sequences of partial 16S rRNA genes obtained in this study have been deposited in the GenBank database under the following accession numbers: EU584501–EU584530.

Results

Characterization of isolates

A collection of 97 morphologically distinct isolates was obtained. Most isolates were colorful: one strain was red, 23 strains were pink, 11 strains were orange, 22 strains were yellow, nine stains were creamy-yellow. The other 31 strains were creamy-white (24 strains) or translucent (seven strains). Strains examined by electron microscopy were rod-shaped or cocci (Fig. 1).

The isolates were grouped into 30 RFLP patterns (Table 1). One isolate was selected to represent each RFLP group and was sequenced. All strains had 16S rRNA sequences similar to previously determined sequences, being from 97 to 99% identical to database sequences. They were affiliated with five different classes: α-, β-, γ-Proteobacteria, Actinobacteria, and Firmicutes (Fig. 2). The dominant class was the Actinobacteria, containing 54 strains and accounting for 56% of all the isolates retrieved.

Isolates affiliated with the Actinobacteria fell into the genera *Agrococcus*, *Arthrobacter*, *Cellulomonas*, *Clavibacter*, *Frigoribacterium*, *Microbacterium*, *Rhodococcus*, and *Sanguibacter*. While their closest relatives came from different environments, e.g. ice, soil, marsh, sediment, dust, plant, and animal, most isolates had close phylogenic relationship with psychrophilic or psychrotolerant bacteria. All strains belonging to the genus *Arthrobacter* were phylogenetically related to a strain from permafrost (DQ177479) (Zhang et al. 2007b) (Table 1) and a strain from the Puruganri ice core (DQ227778) (Table 2). Sequence Everest gws-4 represents four strains in the genus *Clavibacter*, which is closely related to the psychrotolerant species from permafrost (DQ173026, similarity 99%) (Bai et al. 2006). In the genus *Frigoribacterium*, Everest gws-26 and Everest gws-13 were related to the psychrophilic species from airborne dust in a cattle barn (AF157479, similarity 99%) (Kampfer et al. 2000) and psychrotolerant species from permafrost (DQ172995, similarity 99%) (Bai et al. 2006). They also were similar to glacial bacteria isolated from Greenland and the Puruganri ice core (Table 2). In the genus *Sanguibacter*, Everest gws-78 was similar to a glacial ice bacterium (AF479343) with an identity value of 99%, and Everest gws-55 and Everest gws-59 were similar to this glacial ice bacterium (AF479343) with an identity value of 96%. The closest relatives of Everest gws-11 (genus *Agrococcus*) and Everest gws-20 (genus *Rhodococcus*) were also from cold environments, deep sea sediment, and frozen compost soil (Groth et al. 1996), and Everest gws-20 was similar to bacteria recovered from the Greenland ice core (Table 2).

There were 18 isolates affiliated with the α-Proteobacteria belonging to the genera *Afipia* and *Brevundimonas*.

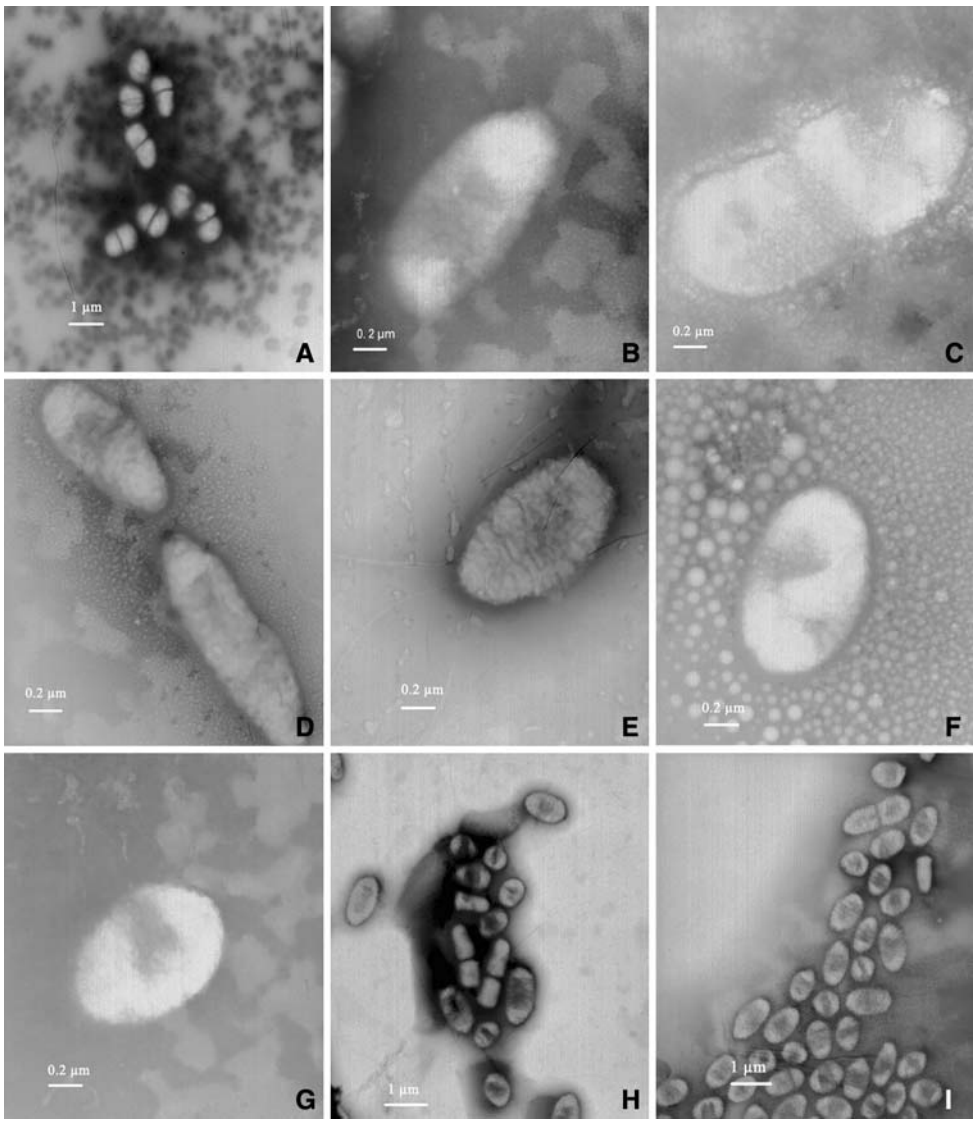

Fig. 1 TEM images of the nine strains isolated from glacial meltwater at 6,350 m on Mt Everest: **a** Everest gws-48, *Acinetobacter* sp.; **b** Everest gws-111, *Microbacterium* sp.; **c** Everest gws-25, *Arthrobacter* sp.; **d** Everest gws-110, *Brevundimonas* sp.; **e** Everest gws-44, *Cellulomonas* sp.; **f** Everest gws-100, *Clavibacter* sp.; **g** Everest gws-26, *Frigoribacterium* sp.; **h** Everest gws-78, *Sanguibacter* sp.; **i** Everest gws-74, *Janthionbacterium* sp

All of them had a close phylogenetic relationship to bacteria isolated from glaciers (Table 2). Meanwhile, two isolates in the genus *Afipia* were similar to a strain from a high mountain lake (AJ864853, similarity 98%). Among 16 isolates in the genus *Brevundimonas*, the nearest neighbor of 15 isolates was a psychrotolerant strain from the Tibetan Plateau tundra (DQ177489, similarity 99%). The other one was close to a strain from oligotrophic water from Mediterranean Sea water (AJ244705, similarity 99%)(Fritz et al. 2005).

Three isolates affiliated with the β-Proteobacteria all belonged to the genus *Janthinobacterium* and were closely related to a psychrophilic strain from the glacial meltwater of Hamta glacier in the Indian Himalayas (AJ846273, similarity 99%).

Twenty-one isolates in the γ-Proteobacteria belonged to the genera *Acinetobacter* (14 strains), *Aeromonas* (one strain), *Moraxella* (five strains), and *Stenotrophomonas* (one strain). Their nearest neighbors were aquatic and soil bacteria (Table 1). Four sequences representing 12 isolates were similar to glacier bacteria with identified values from 97 to 99% (Table 2).

Only one isolate belonged to the Firmicutes class, and fell into the genus *Bacillus*. Its closest relative was a bacterium in soil (DQ267829, 99%) and in the Guliya ice core (AF479335, 99%).

Patterns of carbon substrates utilization

Nine strains (three from the α, β, and γ subgroups of Proteobacteria, one from the Firmicutes phylum, and five from five genera of the Actinobacteria) were chosen to assess their ability to oxidize carbon sources. After 3 days of incubation, seven strains had used 17–58 of the 95

Table 1 Taxonomic affiliations of 30 RFLP groups, determined by phylogenetic analysis of 16S rRNA

Everest gws group	Number of isolates	Colony color	16S rRNA analysis			
			GenBank accession no.	Division	Phylogenetically nearest taxon (GenBank accession no.; source)	Identity (%)
91	2	Pink	EU584530	α-Protobacteria	*Afipia* sp.(AJ864853; biofilm on granite stone from high mountain lake)	98
110	10	Creamy-white	EU584506	α-Protobacteria	*Brevundimonas* sp. (DQ177489; Qinghai–Tibet Plateau permafrost)	99
42	5	Pink	EU584516	α-Protobacteria	*Brevundimonas* sp. (DQ177489; Qinghai–Tibet Plateau permafrost)	99
106	1	Translucent	EU584502	α-Protobacteria	*Brevundimonas mediterranea* (AJ244705; water from the Mediterranean Sea)	99
74	3	Yellow	EU584527	β-Protobacteria	*Janthinobacterium* sp. (AJ846273; water from Hamta glacier, Himalayas India)	99
48	7	Orange	EU584520	γ-Protobacteria	*Acinetobacter* sp. (EF494199; phenol-degrading strain, unspecified source)	99
28	7	Yellow	EU584513	γ-Protobacteria	*Acinetobacter* sp. (EF494199; phenol-degrading strain, unspecified source)	99
80	1	Creamy-white	EU584529	γ-Protobacteria	*Aeromonas punctata* (X60409; unspecified source)	99
71	3	Pink	EU584526	γ-Protobacteria	*Moraxella osloensis* (AJ505859; lake water)	99
63	1	Yellow	EU584525	γ-Protobacteria	*Moraxella osloensis* (AJ505859; lake water)	99
54	1	Red	EU584522	γ-Protobacteria	*Moraxella osloensis* (AJ505859; lake water)	99
46	1	Creamy-white	EU584518	γ-Protobacteria	*Stenotrophomonas maltophilia* (AB008509; soil)	99
30	1	Creamy-white	EU584514	Firmicutes	*Bacillus megaterium* (DQ267829; soil)	99
11	1	Creamy-white	EU584505	Actinobacteria	*Agrococcus jenensis* (X92492; frozen compost soil)	98
39	1	Yellow	EU584515	Actinobacteria	*Arthrobacter gandavensis* (AM237357; barnyard dust)	99
17	2	Yellow	EU584517	Actinobacteria	*Arthrobacter gandavensis* (AJ491108; veterinary origin)	99
25	3	Yellow	EU584511	Actinobacteria	*Arthrobacter* sp. (DQ177479; Qinghai–Tibet Plateau permafrost)	99
107	1	Creamy-yellow	EU584503	Actinobacteria	*Arthrobacter luteolus* (DQ486130; unspecified source)	98
44	1	Creamy-white	EU584517	Actinobacteria	*Cellulomonas* sp. (EU303279; rhizosphere)	98
100	4	Pink	EU584501	Actinobacteria	*Clavibacter* sp. (DQ173026; permafrost in Tianshan Mountains, China)	99
26	1	Creamy-white	EU584512	Actinobacteria	*Frigoribacterium* sp.(AF157479; airborne dust in a cattle barn)	99
13	6	Creamy-white	EU584508	Actinobacteria	*Frigoribacterium* sp. TSBY-26 (DQ172995; permafrost in Tianshan Mountains, China)	98
109	1	Creamy-white	EU584504	Actinobacteria	*Microbacterium aurum* (Y17229; unspecified source)	98
111	8	Creamy-yellow	EU584507	Actinobacteria	*Microbacterium* sp. PHD-5 (DQ227343;phenol-degrading strain)	99
50	9	Pink	EU584521	Actinobacteria	*Microbacterium* sp. OS-6 (AJ296094; coastal marsh)	99
47	3	Yellow	EU584519	Actinobacteria	*Microbacterium* sp. (AJ244679; unspecified source)	98
20	4	Orange	EU584510	Actinobacteria	*Rhodococcus* sp. (AY582940; deep-sea sediment)	99
59	2	Yellow	EU584524	Actinobacteria	*Sanguibacter* sp. (DQ339590; alpine subnival plants)	99
55	6	Translucent	EU584523	Actinobacteria	*Sanguibacter* sp. (DQ339590; alpine subnival plants)	99
78	1	Creamy-white	EU584528	Actinobacteria	Glacial ice bacterium G200-C18 (AF479343; the Guliya ice core, Tibet)	99

Each group is represented by one isolate whose 16S rRNA sequence was used to determine the taxonomic affiliation of the group

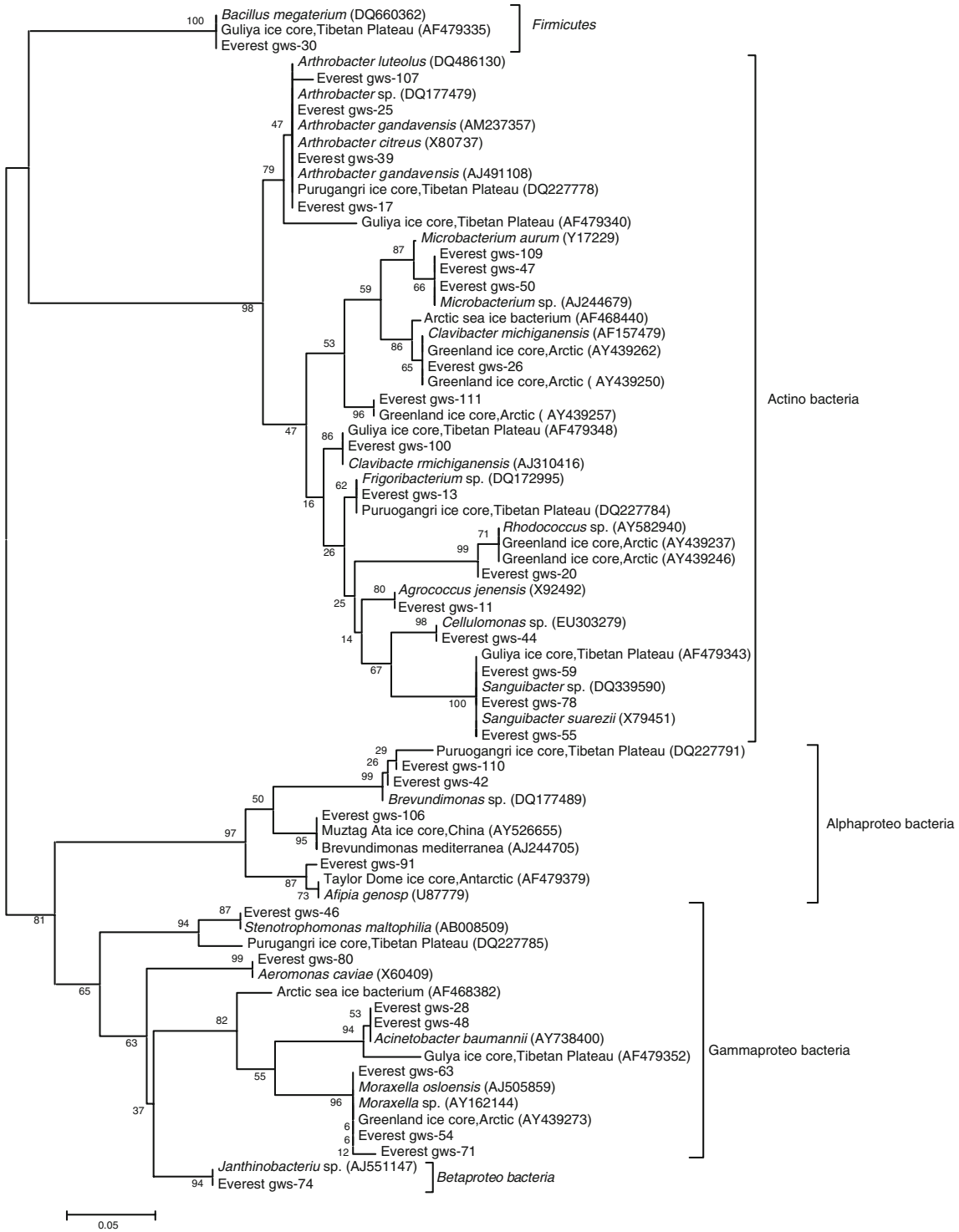

Fig. 2 Neighbor-joining tree showing the phylogenetic relationships between culturable bacterial 16S rRNA gene sequences from glacial meltwater at 6,350 m on Mt Everest and closely related sequences from the GenBank database

substrates, but the other two did not use any substrates. After 7 days of incubation, three strains grew on more than 60 of the 95 different substrates, three strains grew on 40–43 substrates, one strain grew on 32 substrates, one strain only grew on citric acid and formic acid, and the other one did not use any substrate (Table 3). Of the 95 substrates tested, 26 were not used by any of the nine strains tested (Table 3).

Table 2 Isolates in glacial meltwater with close phylogenetic relationship with strains in glacial ice

Everest gws group	Division	Isolates in ice core from a series of glaciers		Identity (%)
		GenBank no.	Name and location of ice cores	
91	α-Proteobacteria	AF479379	Taylor Dome ice core, Antarctic	98
110		DQ227791	Puruogangri ice core, Tibetan Plateau	98
42		DQ227791		98
106		AY526655	Muztag Ata ice core, China	99
48	γ-Proteobacteria	AF479380	Taylor Dome ice core, Antarctic	97
71		AY439273	Greenland ice core, Arctic	98
63		AY439273	Greenland ice core, Arctic	99
54		AY439273	Greenland ice core, Arctic	99
30	Firmicutes	AF479335	Guliya ice core, Tibetan Plateau	99
107	Actinobacteria	DQ227778	Puruogangri ice core, Tibetan Plateau	97
39		DQ227778		97
25		DQ227778		99
100		AF479348	Guliya ice core, Tibetan Plateau	99
13		DQ227784	Puruogangri ice core, Tibetan Plateau	97
26		AY439250	Greenland ice core, Arctic	99
		AY439262	Greenland ice core, Arctic	99
111		AY439239	Greenland ice core, Arctic	99
		AY439257	Greenland ice core,Arctic	98
20		AY439237	Greenland ice core, Arctic	98
		AY439246	Greenland ice core, Arctic	99
78		AF479343	Guliya ice core, Tibetan Plateau	99

The nine strains were classified into three groups based on their profiles of carbon substrates utilization (Fig. 3). Group I included Everest gws-25, Everest gws-74, and Everest gws-78. They grew rapidly and utilized about 70% of the carbon substrates. Group II contained four strains (Everest gws-26, Everest gws-44, Everest gws-48, and Everest gws-110), which grew slowly and utilized fewer carbon substrates within 48 h. However, they utilized nearly half of the carbon substrates provided by the end of incubation. Everest gws-30 and Everest gws-111 were in Group III. They barely utilized any of the carbon substrates provided.

Growth condition at 15 and 25°C

Based on the result of carbon substrates utilization, five strains were chosen for growth at 15 and 25°C (Fig. 4). Everest gws-74, Everest gws-25, Everest gws-44, and Everest gws-48 grew rapidly at 25°C and reached their logarithmic phase after 8 h of inoculation. However, the growth rates of these four strains at 15°C were different from each other. The first two strains did not start to grow after 32 h of incubation, while the others started to grow rapidly after 16 h of inoculation. Everest gws-111 did not grow at 25 or 15°C until 40 or 64 h after inoculation, respectively.

Discussion

Comparison between culturable bacteria from Mt Everest glacial meltwater and other glacial niches

Diverse culturable bacteria were obtained from glacial meltwater on Mt Everest. They had common features with other glacier bacteria. First, 68% of the isolates formed highly pigmented colonies, consistent with the presence of pigments providing protection from solar irradiation in the glacier environment (Christner et al. 2000; Foght et al. 2004). Second, half of the isolates grouped phylogenetically with psychrophilic or psychrotolerant isolates from cold environment (Cheng and Foght 2007; Christner et al. 2003a; Foght et al. 2004). It indicated that cold environments choose similar bacteria (Miteva et al. 2004; Priscu and Christner 2003).

However, culturable bacteria component in meltwater was distinct from those recovered from a cryoconite hole and under glacier niches. Bacterial isolates from cryoconite hole sediment at Taylor G, McMurdo Dry Valleys of Antarctica, were members of the Cytophagales or Actinobacteria lineages or were β-Proteobacteria (Christner et al. 2003a).Cultured bacteria beneath the John Evans glacier in the Canadian high Arctic comprised Bacteroidetes (predominantly Flavobacterium) and β-Proteobacteria

Table 3 Carbon substrate utilization patterns of nine strains affiliated with different groups

	Everest gws-								
	25	74	78	26	44	110	48	111	30
α-Cyclodextrin	−	−	−	−	−	−	−	−	−
Dextrin	+	+	+	+	+	+	+	−	−
Glycogen	+	+	+	−	−	−	−	−	−
Tween 40	+	+	+	+	+	+	+	−	−
Tween 80	+	+	+	+	+	+	+	−	−
N-Acetyl-D-galactosamine	+	+	+	+	+	+	−	−	−
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+	−	−
Adonitol	−	−	−	−	−	−	−	−	−
L-Arabinose	−	−	−	−	−	−	+	−	−
D-Arabitol	−	−	−	−	−	−	−	−	−
D-cellobiose	+	+	+	+	+	+	+	−	−
i-Erythritol	−	−	−	−	−	−	−	−	−
D-Fructose	+	+	+	+	+	+	+	−	−
L-Fucose	−	−	−	−	−	−	−	−	−
D-Galactose	+	−	+	−	−	−	−	−	−
Gentiobiose	+	+	+	+	+	+	+	−	−
α-D-Glucose	+	+	+	+	+	+	+	−	−
m-Inositol	−	−	−	−	−	−	−	−	−
α-D-Lactose	+	+	+	+	−	+	−	−	−
Lactulose	+	+	+	+	+	+	−	−	−
Maltose	+	+	+	+	+	+	+	−	−
D-Mannitol	+	+	+	−	−	−	−	−	−
D-Mannose	+	+	+	+	+	+	+	−	−
D-Melibiose	+	+	+	+	+	+	+	−	−
β-Methyl-D-glucoside	+	+	+	+	+	+	−	−	−
D-Psicose	+	+	+	+	−	−	+	−	−
D-Raffinose	−	−	−	−	−	−	−	−	−
L-Rhamnose	+	+	+	−	−	−	−	−	−
D-Sorbitol	−	+	−	−	+	−	+	−	−
Sucrose	+	+	+	+	+	+	+	−	−
D-Trehalose	+	+	+	+	+	+	+	−	−
Turanose	+	+	+	+	−	+	+	−	−
Xylitol	−	−	−	−	−	−	−	−	−
Methyl pyruvate	+	+	+	+	+	+	+	−	−
Mono-methyl-succinate	+	+	+	+	+	+	−	−	−
Acetic acid	+	+	+	−	+	+	+	−	−
cis-Aconitic acid	+	+	+	+	+	+	−	−	−
Citric acid	+	+	+	+	+	+	−	+	−
Formic acid	+	+	+	+	+	+	−	+	−
D-Galactonic acid lactone	−	−	−	−	−	−	−	−	−
D-Galacturonic acid	−	−	−	−	−	−	−	−	−
D-Gluconic acid	−	−	−	−	−	−	+	−	−
D-Glucosaminic acid	−	−	−	−	−	−	−	−	−
D-Glucuronic acid	−	−	−	−	−	−	−	−	−
α-Hydroxy butyric acid	+	+	+	+	+	−	+	−	−
β-Hydroxy butyric acid	+	+	+	+	+	+	−	−	−
γ-Hydroxy butyric acid	−	+	−	−	−	−	−	−	−
p-Hydroxy phenylacetic acid	−	−	−	−	−	−	+	−	−
Itaconic acid	−	−	−	−	−	−	−	−	−

Table 3 continued

	Everest gws-								
	25	74	78	26	44	110	48	111	30
α-Keto butyric acid	+	+	+	+	+	−	+	−	−
α-Keto glutaric acid	+	+	+	+	+	+	−	−	−
α-Keto valeric acid	+	+	+	−	−	−	−	−	−
D,L-Lactic acid	+	+	+	+	+	+	+	−	−
Malonic acid	+	+	+	+	+	+	+	−	−
Propionic acid	+	+	+	+	+	+	−	−	−
Quinic acid	−	−	−	−	−	−	−	−	−
D-Saccharic acid	−	−	−	−	−	−	−	−	−
Sebacic acid	−	−	−	−	−	−	−	−	−
Succinic acid	+	+	+	+	+	+	−	−	−
Bromo succinic acid	+	+	+	−	+	+	−	−	−
Succinamic acid	−	−	−	−	−	−	−	−	−
Glucuronamide	−	−	−	−	−	−	−	−	−
L-Alaninamide	+	+	+	+	+	+	−	−	−
D-Alanine	+	+	+	+	+	+	+	−	−
L-Alanine	+	+	+	+	+	+	−	−	−
L-Alanyl-glycine	+	+	+	+	+	+	+	−	−
L-Asparagine	+	+	+	−	+	−	−	−	−
L-Aspartic acid	+	+	+	+	+	+	+	−	−
L-Glutamic acid	+	+	+	−	−	+	−	−	−
Glycyl-L-aspartic acid	+	+	+	+	+	+	−	−	−
Glycyl-L-glutamic acid	+	+	+	+	+	+	−	−	−
L-Histidine	+	+	+	−	−	+	−	−	−
Hydroxy-L-proline	−	−	−	−	−	−	−	−	−
L-Leucine	+	+	+	+	+	+	−	−	−
L-Ornithine	+	+	+	−	−	−	−	−	−
L-Phenylalanine	+	+	+	−	−	−	−	−	−
L-proline	+	+	+	+	+	+	−	−	−
L-Pyroglutamic acid	−	−	−	−	−	−	−	−	−
D-Serine	+	+	+	−	−	−	−	−	−
L-Serine	+	+	+	+	+	+	−	−	−
L-Threonine	+	+	+	+	+	−	−	−	−
D,L-Carnitine	−	−	−	−	−	−	−	−	−
γ-Amino butyric acid	−	−	−	−	−	−	−	−	−
Urocanic acid	−	−	−	−	−	−	+	−	−
Inosine	+	+	+	−	+	−	+	−	−
Uridine	+	+	+	−	+	−	−	−	−
Thymidine	+	+	+	−	−	−	−	−	−
Phenyethylamine	−	−	−	−	−	−	−	−	−
Putrescine	−	−	−	−	−	−	−	−	−
2-Aminoethanol	−	−	−	−	−	−	−	−	−
2,3-Butanediol	+	+	+	−	−	−	+	−	−
Glycerol	−	−	−	−	−	−	+	−	−
D,L-α-Glycerol phosphate	−	−	−	−	−	−	−	−	−
Glucose-1-phosphate	−	+	−	−	−	−	−	−	−
Glucose-6-phosphate	−	+	−	−	−	−	−	−	−

Everest gws-25, *Arthrobacter* sp.; Everest gws-74, *Janthionbacterium* sp.; Everest gws-78, *Sanguibacter* sp.; Everest gws-26, *Frigoribacterium* sp.; Everest gws-44, *Cellulomonas* sp.; Everest gws-110, *Brevundimonas* sp.; Everest gws-48, *Acinetobacter* sp.; Everest gws-111, *Microbacterium* sp.; Everest gws-30, *Bacillus* sp

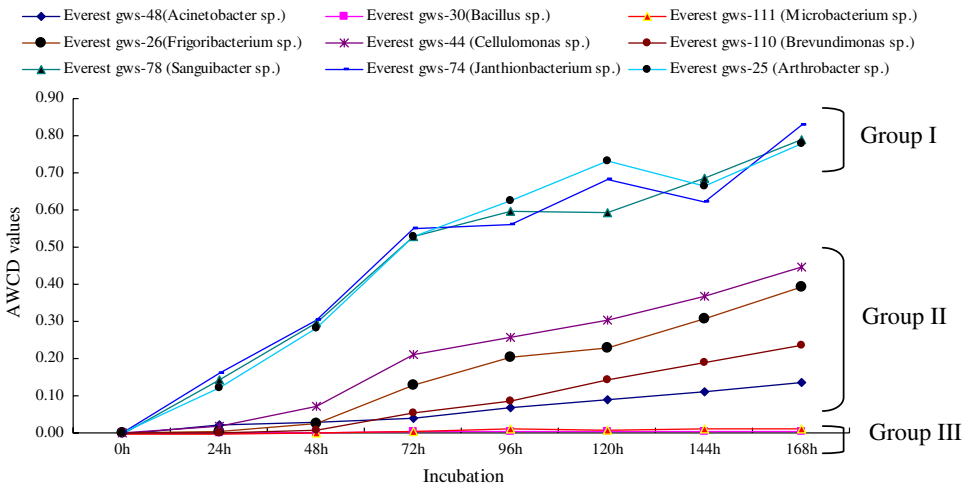

Fig. 3 AWCD values of nine strains during inoculation. AWCD represents overall color development in BIOLOG plates and is calculated using the formula $(C - R)/\{[\sum(C - R)/95]\}$, where R is the value of the control well and C is the values of wells containing sole carbon sources

(particularly *Comamonadaceae*), while Actinobacteria, α-, and γ-Proteobacteria were not detected as clones (Cheng and Foght 2007). Bacteria in subglacial sediments from the Franz Josef and Fox glaciers located in the New Zealand were also dominated by the Bacteroidetes and β-Proteobacteria group, with a few belonging to Actinobacteria, α-Proteobacteria, and Firmicutes (Foght et al. 2004). Subglacier environment are permanent dark (Cheng and Foght 2007; Mikucki and Priscu 2007), and the habitat of cryoconite sediment was different from glacial meltwater (Christner et al. 2003a). The distinction between niches may be the major factor responsible for the differences between bacterial communities, and the different incubation methods and substrate employed in various studies could impact as well.

Bacteria in glacial meltwater showed more similarities to those in deep glacial ice core. Actinobacteria bacteria were both dominant in glacial meltwater and ice cores drilled from the Greenland ice sheet (Miteva et al. 2004), the Muztag Ata glacier in China (Xiang et al. 2005b), and the Puruogangri glacier on the Tibetan Plateau (Zhang et al. 2008). Eighteen of the 30 sequences representing about 62% of the isolates in glacial meltwater were similar to glacier ice bacteria from the Arctic, Antarctic, Tibetan Plateau, and Pamirs Plateau in China with similarity values higher than 97% (Table 2). However, it is noted that in the same glacier and at similar sampling sites isolates recovered from meltwater are different from those from ice (Zhang et al. 2007c). Zhang et al. (2007c) recovered bacteria from ice in the East Rongbuk Glacier. The sampling site for an ice core was about 2 km away from and 200 m higher than the site where the meltwater was sampled. Three common genera existed in both the ice and meltwater, e.g. the genus *Acinetobacter* in the γ-Proteobacteria, the genus *Arthrobacter* in the Actinobacteria, and the genus *Bacillus* in the Firmicutes. The similarities between sequences affiliated to these genera in the two habitats were 93–96%. Variations in the incubation methods and substrate used could cause this difference. The different environment may be the reason as well. We will do more study in this region in the future.

The majority of the isolates from strains closely related to meltwater were from nonglacial environment (Table 1). More than half of the isolates' nearest neighbors were from the permafrost, and most of these from the permafrost in the Tibetan Plateau (Bai et al. 2006; Zhang et al. 2007a, b). The other isolates were related to bacteria from dust, soil, plant, water, and cattle (Kampfer et al. 2000). It is suggested that the bacteria in meltwater may have originated from the glacier or from surrounding environment. Most of them were introduced and harbored in this habitat in the same way as the bacteria enclosed in the glacial ice are transported by wind or snow from nearby aquatic and terrestrial sources attached to dust or organic particles (Christner et al. 2000; Miteva et al. 2004; Miteva and Brenchley 2005).

Possible strategies of the glacial-meltwater-bacteria for oligotrophic and variable temperature conditions

When bacteria are transported into a glacier from outside, they have to face two critical challenges: oligotrophy and variable temperature. Based on the analysis of the Biolog bioassay of carbon substrate utilization and growth tests under different temperatures, the culturable bacteria in glacial meltwater could be divided into three categories in terms of their survival strategies. Some strains such as Everest gws-74 and Everest gws-25, which were affiliated with the genera *Arthrobacter* and *Janthinobacterium*, were sensitive to temperature change but versatile in their utilization of carbon substrates, while other strains such as Everest gws-44 and Everest gws-48 belonging to the genera *Cellulomonas* and *Acinetobacter* were incapable of utilization of a variety of carbon species but tolerant to variable temperature. In contrast, Everest gws-111 in the

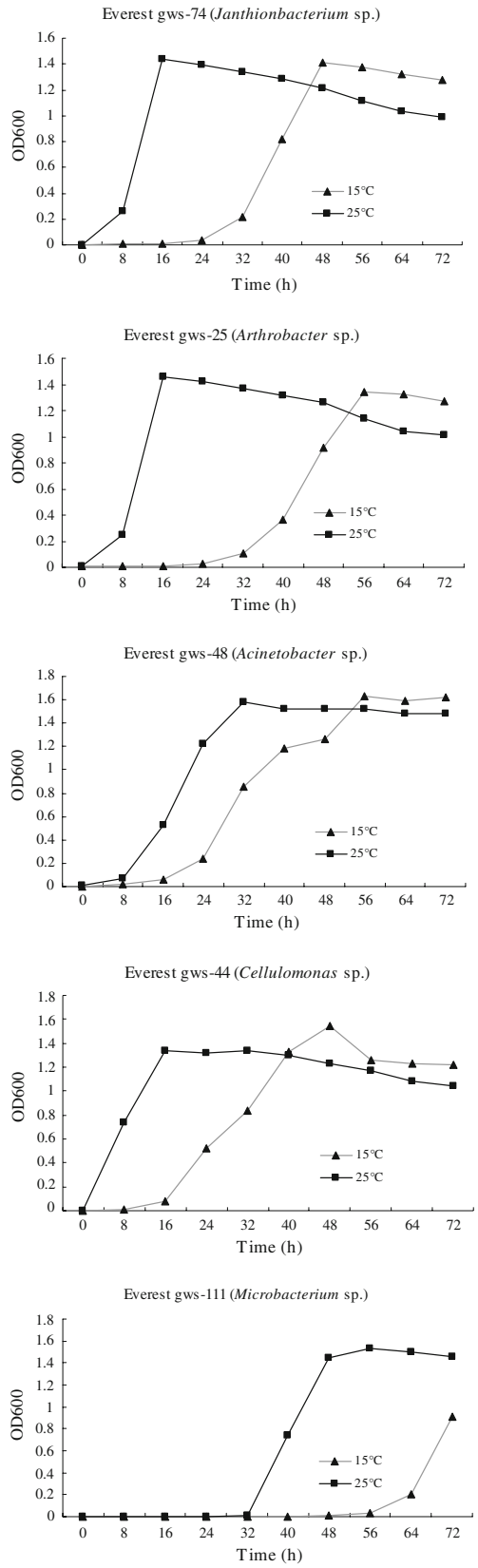

Fig. 4 Growth of the isolates in liquid RO medium at 15 and 25°C observed during exponential and stationary growth stages. *OD* optical density

genus *Microbacterium* hardly utilized a carbon substrate and grew slowly in low temperature. Its metabolism seemed to be weak and slow in the cold environment.

It has been reported that predominant soil *Arthrobacter* isolates use a wide range of diverse organic substrates as their sole or principal sources of carbon and energy (Balows et al. 1992a, b). *Janthinobacterium* is common in soil and water environments in temperate climates. They can grow rapidly on a medium containing citrate and ammonia as the sole carbon and nitrogen source (Balows et al. 1992a, b). The two genera are most commonly identified in glacial ice (Miteva 2007). These facts validated the findings in our studies that the versatility of *Arthrobacter* and *Janthinobacterium* in utilization of carbon sources allowed them to survive successfully in the harsh environment of glacial meltwater.

Conclusions

In summary, diverse culturable bacteria were recovered from an extreme aquatic environment, the glacial meltwater at 6,350 m on the highest mountain of the world. The isolates were distinctly different from those harbored in Antarctic hole and under glacier habitats, but were phylogenetically related to bacteria in deep glacial ice. The majority of the isolates' nearest neighbors were from the permafrost, dust, soil, plant, and aqueous environments. Our bioassay observations suggest that the culturable bacteria in glacial meltwater may have multiple origins beyond the glacier itself. A variety of bacteria can survive in the meltwater environments with different survival strategies. Some strains being sensitive to temperature change could grow rapidly on diverse carbon substrates, while other strains were not good at utilization of multiple carbon species but tolerant to variable temperature, and some strains could only metabolize slowly in this harsh environment.

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