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ORIGINAL PAPER

Microbial diversity in the snow, a moraine lake and a stream in Himalayan glacier

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Abstract The microbial diversity and abundance in surface snow at different altitudes (5300 and 5504 m above sea level), a moraine lake and a glacial stream in the Yala Glacier on the southern slope of the Himalayas were investigated through a 16S rRNA gene clone library and flow cytometry approaches. Cell abundance in different habitats changed from 1.1×10^4 to 25×10^4 cells mL^{-1}, with the highest abundance in the moraine lake and the lowest abundance in the snow at 5504 m. Microbial communities in the snow were significantly different from those in the moraine lake and stream, although they were similar within snow and within the aquatic habitats. The two snow libraries were both dominated by Cyanobacteria, which accounted for about half of the total, followed by the Alphaproteobacteria and Firmicutes. The moraine lake and stream libraries were dominated by the Bacteroidetes and Betaproteobacteria, followed by the Actinobacteria. The results indicated that snow and water were highly diverse systems even in the same glacier. Microbial communities in the snow on the Yala Glacier were distinctly different from those in the East Rongbuk Glacier on the northern slope of Himalayas. However, microbes in the moraine lakes at two glaciers had similar community features. The snow habitat was easily affected by various environmental factors, while the aquatic habitats were comparatively stable in different glaciers.

Keywords Microbes · Snow · Moraine lake · Glacial stream · Himalayan glaciers

Introduction

The concept of glacial ecology has been developed in recent years (Hodson et al. 2008). Snow and meltwater are not only two important parts in glacier energy and mass balance but also two major habitats of microbes. Compelling evidence indicates that microbes have a significant effect upon the dynamics, composition and abundance of nutrients in meltwater (Hodson et al. 2008; Tranter et al. 2002). Studies on microbes living in snow, moraine lakes and streams have been carried out on a number of glaciers (Amato et al. 2007b; Carpenter et al. 2000; Hoham and Duval 2001; Liu et al. 2006b, 2009; Segawa et al. 2005; Stibal et al. 2006; Xiang et al. 2009; Xiong et al. 1999; Yoshimura et al. 1997). The results show that the snow bacterial communities varied in different regions (Xiang et al. 2009). In moraine lakes and meltwater, low temperature at high altitude is considered to be critical for

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component dominancy, and nutrient availability plays a role in regulating population structure at the same altitude (Liu et al. 2006a). Bacterial abundance and production are found to increase significantly in glacial runoff and aquatic habitats along a 500-m transect from the ablation area down to a series of proglacial lakes in the forefield of a Svalbard glacier (Mindl et al. 2007). Although detailed studies had been carried out on the microbes in snow, moraine lakes and streams, these studies have taken place separately and in different glaciers of different regions. Information concerning the microbes in these three habitats and their variation in one glacier are very sparse now. Except for the report of Larose et al. (2010) that bacterial communities in snow and meltwater in Arctic glaciers are significantly different, to the best of our knowledge, no related study of mountain glaciers has taken place. However, such information is the basis for any understanding of the glacial ecosystem.

We chose glaciers in the Himalayas, which are at high altitude and far away from the influence of human activity, as our study region. First, we investigated the microbial abundance and diversity in surface snow, a moraine lake and a glacial stream in the Yala Glacier, which is located on the southern slope of the Himalayas, in order to explore their microbial features and their relationship with the environment in one glacier. Next, we compared snow and aquatic microbes in the Yala Glacier with those in the East Rongbuk Glacier on the northern slope of the Himalayas (Fig. 1), in order to study the microbial communities' variations in different glaciers in the region.

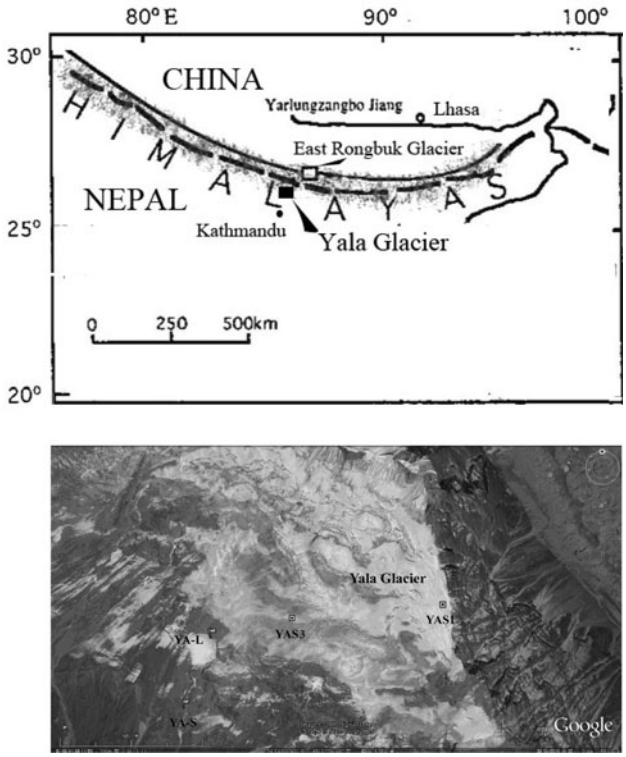

Fig. 1 Location map of the Yala Glacier and the sample sites

Methods

Description of sampling site

The Yala Glacier in Langtang Valley is a plateau-shaped small glacier without rock debris (Fig. 1). The highest and lowest altitudes are 5749 and 5049 m above sea level (a.s.l.), and the area of the glacier is 2.5 km^2. The annual temperature and precipitation at 2008 in Langtang Valley are 3.6°C and 690 mm.

Sample collection

Snow samples were collected on the glacial surface at two sites at altitudes of 5300 m (28.23°N, 85.62°E) and 5504 m (28.23°N, 85.63°E). One sample was collected at each site. Snow at no more than 5 cm depth was taken using a sterile spoon, and 3 L of snow was placed in sterile Nalgene bottles. Two water samples were collected using similar bottles from the moraine lake located at the foot of the glacier (5291 m, 28.23°N, 85.61°E) and in a glacial stream (5237 m, 28.23°N, 85.61°E). The lake is small with area about 0.4 km^2. The lake is alimented by snowmelt from the glacier. The stream is jointly fed by lakes in its upper catchment and glacial melting. And the discharge is about 0.2 cm^3/s. Extreme care was taken at all times to ensure minimal contamination. Spoons and bottles for collecting samples were sterilized in the laboratory and used only once for one sample. All samples were placed in a cooler with frozen phase change materials during the 3 days of transport. After reaching the laboratory, melted snow and water from the lake and stream for determination of cell abundance were immediately fixed with 1.5% formaldehyde (final concentration) and frozen at −80°C until analysis. About 1 L of snow melt water and of lake/stream water were immediately filtered through 0.22 μm filters (Millipore) and the filters frozen at −80°C until further analysis. Water for measuring ion concentration was stored at −20°C until further analysis.

Laboratory analysis

Cell abundance was analyzed using flow cytometry (Beckman Coulter, Epics Altra II), with SYBR Green I applied as the nucleic acid stain (Marie et al. 1997). The flow cytometer was equipped with a 100-mW 488-nm water-cooled argon-ion laser (Cohenrent Inc) and a

standard filter set-up. An external sample injector (Harvard Apparatus PHD 2000) was employed for accurate quantification (Jiao et al. 2005). Fluorescent beads (1 μm in diameter, Polyscience Inc) were added to the sample for internal reference (supplement). The major chemical species were measured using a Dionex Ion Chromatograph model 2010 (Buck et al. 1992). Dissolved organic carbon (DOC) was analyzed using a TOC-Vcph (Shimadzu Corp., Japan). Each sample was measured three times with a standard deviation lower than 10%.

Community DNA extraction, PCR amplification, and 16S rRNA gene clone library construction

The microbial community DNA was extracted using the NucleoSpin Soil DNA Isolation Kit following the manufacturer's instructions. The bacterial 16S rRNA genes of the extracted community DNA were amplified with the primer sets 27F (AGAGTTTGATCATGGCTCAG) and 1392R (ACG GGC GGT GTG TRC) (Brosius et al. 1978). The reaction mixture (30 μL) consisted of 1 U of LA Taq (TaKaRa Co., Dalian, China), 0.2 mM each 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 30 cycles (94°C for 1 min, 56°C for 1 min and 72°C for 1.5 min), and then by a final extension at 72°C for 8 min.

The PCR products were purified using an agarose gel DNA purification kit (TaKaRa Co., Dalian, China), ligated into pGEM-T vector (TaKaRa Co., Dalian, China), and then transformed into *E. coli* DH5α. The presence of inserts was checked using ampicillin resistance selection and colony PCR. Eighty to 100 clones from each library were randomly selected for sequencing.

Phylogenetic analysis

All sequences obtained were examined for chimeric artifacts using Pintail tool online (http://www.bioinformatics-toolkit.org), and clones identified as potential chimeras were discarded. Sequences of each library were calculated with DOTUR (Schloss and Handelsman 2005) and those with similarity greater than 97% were grouped into one operational taxonomic unit (OTU). Sequences were assigned to the genus level grouping with 80% confidence using the "Classifier" program of RDP (Cole et al. 2005). The closest neighbors were retrieved from the NCBI (http://www.ncbi.nih.gov/BLAST) through blasting. A phylogenetic tree including the OTUs obtained and their closest relatives was constructed using MEGA software (Kumar et al. 2008). Neighbor-joining phylogenies were constructed from dissimilatory distances and pair-wise comparisons with the Jukes–Cantor distance model. Bootstrap analysis of 1000 replicates was performed.

Statistical analyses

Coverages of clone libraries were calculated using the equation: Coverage = 1 − (*N*/Individuals), where *N* is the number of clones that occurred only once (Kemp and Aller 2004). The diversity index (Rarefaction richness, Shannon, Simpson, Evenness) was calculated using the statistical program PAST (http://folk.uio.no/ohammer/past). The Chao1 richness index was calculated using DOTUR (Schloss and Handelsman 2005).

Nucleotide sequence accession numbers

The unique bacterial 16S rRNA gene clone sequences obtained in this study have been deposited in the GenBank database under accession numbers: HQ333271–HQ333343, HQ333405–HQ333473.

Results

Concentrations of major ions and DOC

The nutrient conditions of the snow and lake/stream water in the Yala Glacier were different (Table 1). Concentrations of ions except NH_4^+ in the water were much higher than those in snow. Two snow samples in different altitude had similar ion contents. Lake and stream water exhibited comparable chemical features, but stream water contained relatively more NO_3^- and NH_4^+, while lake water had more DOC.

Cell abundance

Cell abundance of snow and water in the Yala Glacier ranged from 1.1×10^4 to 25×10^4 cells mL^{-1} (Table 1), with the highest abundance in the moraine lake and the lowest abundance in surface snow at 5504 m a.s.l. Microbes were more abundant in water than in snow, but they varied to a different degree in snow and water. Cell abundance in the moraine lake was twice that in the glacier stream, while abundance in the snow at 5300 m was nearly nine times that at 5504 m.

Clone library analysis

Four 16S rRNA gene clone libraries (YAS1, YAS3, YA-L and YA-S) for the snow at two altitudes and the water in the moraine lake and the stream from the Yala Glacier were constructed (Table 1). A total of 360 clones were

Table 1 Location, cell abundance, diversity of bacterial 16S rRNA gene libraries, ion concentration, and dissolved organic carbon (DOC) in snow and water at the Yala Glaciers

Samples	Habitat	Altitude (m)	Cell abundance (10^4 cells mL^{-1})	Diversity indices of gene libraries						Concentration of major ions and dissolved organic carbon (μg L^{-1})								
				OTUs	Individuals	Chao1 (95% CI)	Shannon	Simpson	Evenness	Coverage (%)	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NH_4^+	NO_3^-	SO_4^{2-}	DOC
YAS1	Surface snow	5504	1.1	36	90	163.0 (78.5–419.4)	2.7	0.9	0.4	68.9	49	92	63	970	105	582	606	651	
YAS3	Surface snow	5300	9.0	37	84	253.0 (124.0–601.2)	3.0	0.9	0.5	70.2	52	136	113	3346	148	481	617	230	
YA-L	Moraine lake	5291	24.7	32	87	95.5 (58.1–199.0)	2.8	0.9	0.5	75.9	398	351	404	6709	315	139	957	3302	521
YA-S	Glacier stream	5237	11.3	37	84	79.1 (56.5–139.6)	3.4	1.0	0.7	78.6	398	453	435	8095	198	–	1431	4414	422

NH_4^+ in YA-S is under detected limited, representing with –. DOC values for snow lack due to insufficient number of samples for measure

Individuals number of clones sequenced, *OTUs* number of operational taxonomic units observed, *Chao1* Chao1 nonparametric richness estimate with 95% confidence interval in parentheses

subjected to sequence analysis, and 345 sequences were obtained and identified as normal 16S rRNA gene sequences. All 345 sequences were grouped into 142 OTUs (supplement). Coverages of the four libraries were from 68.9 to 78.6%. The richness and diversity index of four libraries were various (Table 1) and in the range of other snow and glacier meltwater libraries in polar and high mount glaciers (Larose et al. 2010; Liu et al. 2006a).

Sequence analysis

The sequences in the Yala Glacier were classified into Alpha, Beta, Gamma, Delta-Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, TM7 candidate phylum, OD1 and unclassified. Sixty-three percent of the sequences were classified into 41 genera. Twenty-seven genera only occurred in one library: eight in YAS-1, nine in YAS-3, six in YA-L and four in YA-S. The two snow libraries had seven common genera and the water libraries six common genera, but only one genus (*Methylobacillus*) existed in snow and water libraries (Table 2). There were no common genera among the four libraries.

The two snow libraries were dominated by clones related to the Cyanobacteria, which accounted for about half of the total clones (48% in YAS1 and 56% in YAS3), followed by clones affiliated to the Alphaproteobacteria, Firmicutes and Actinobacteria (Fig. 2). The Betaproteobacteria and Bacteroidetes represented only a small percentage (<5%) of the libraries. In the YAS1 library, 35% of the sequences were closely related to sequences from alpine or polar environments, and 27% of the sequences were related to soil bacteria. In the YAS3 library, sequences similar to those from soil, aquatic and cold environments accounted for 36, 14 and 14% of the total sequences (Table 3).

The YA-L library sequences contained seven bacterial classes, dominated by the Bacteroidetes (46%) and Betaproteobacteria (34%), followed by the Actinobacteria (12%). Seventeen sequences, accounting for 53% of the total, were closely related to aquatic bacteria, and 11 sequences (34% of the total) were related to sequences from snow or ice in glaciers. Sequences in the Betaproteobacteria, Bacteroidetes and Actinobacteria also dominated in the YA-S library, accounting for 43, 32 and 13% of the total sequences. In this library, 38% of the sequences were similar to those recovered from lakes and rivers, and 11% were similar to those in snow and glaciers.

Six OTUs were common in snow libraries and they represented 44 and 49% of the total clones in YAS1 and YAS3. Thirteen common OTUs occurred in water libraries, which represented 76 and 70% of the total clones in moraine lake and stream. Only one OTU, representing two clones, existed both in snow and water libraries.

Table 2 Common genera and their percentages in libraries among snow, moraine lake, and glacier stream on Himalayan glaciers

Phylum	Genus	YAS1 (%)	YAS3 (%)	YA-L (%)	YA-S (%)	ESS[a] (%)	L1[b] (%)	L2[b] (%)	Other cryohabitats
Alphaproteobacteria	*Brevundimonas*	1.1	1.2			2.4	16.7		E
	Sandarakinorhabdus			1.1	2.4			1.6	E
Betaproteobacteria	*Methylobacillus*	1.1		1.1					E
	Polaromonas			21.8	17.9	16.7	7.1		E
	Rhodoferax			2.3	7.1				E
Actinobacteria	*Ilumatobacter*			4.6	4.8			9.8	
	Modestobacter	1.1	1.2						
Firmicutes	*Bacillus*	5.6	9.5						E
	Tumebacillus	1.1	1.2						E
Bacteroidetes	*Arcicella*			27.6	3.6			1.6	E
	Flavobacterium			13.8	28.6	2.4	42.9	19.7	E
	Hymenobacter	1.1	2.4						E
Cyanobacteria	Gp I	1.1	4.8						E
	Streptophyta	36.7	46.4						E
Total percentage		48.9	66.7	72.4	64.3	21.4	66.7	32.8	

E representing that this genus also existed in other cryohabitats

[a] Data from Liu et al. (2007), surface snow on East Rongbuk Glacier

[b] Data from Liu et al. (2006a, b), L1 and L2 were moraine lakes at 5140 and 5152 m at the base of the East Rongbuk Glacier

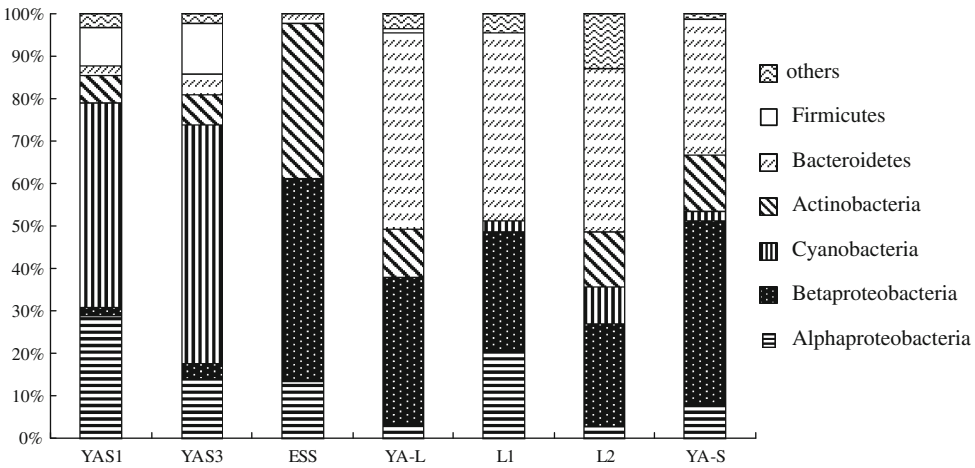

Fig. 2 Major group affiliations of the 16S rRNA gene clone libraries from Himalayan glaciers. YAS-1 and YAS-3 represent surface snow at 5504 and 5300 m on the Yala Glacier; YA-L and YA-S represent a moraine lake and a glacial stream at the Yala Glacier; ESS represents surface snow at 6300 m on the East Rongbok Glacier (Liu et al. 2007); and L-1 and L-2 represent moraine lakes at the East Rongbok Glacier (Liu et al. 2006a)

Discussion

Cell abundance varied at snow, moraine lake and glacial stream at the Yala Glacier

Several factors play important roles in the cell abundance in the snow. Earlier studies showed that the amount of mineral material present and the growth of the microbes were the major factors which influence the microbial abundance in the snow (Segawa et al. 2005). Amato et al. (2007a) found concentrations of microorganisms were clearly linked to the chemical composition of cloud water and ocean represented a major source for cloud water. Christner et al. (2008) studied rain and snow from different regions and indicated that terrestrial vegetation and soils were an important source of the biological ice nuclei in the atmosphere. And the concentration of these particles was related to the season and precipitation chemistry. Meanwhile, snow chemistry studies in the Himalayas regions showed that both terrestrial and marine source material were deposited on the glaciers (Kang et al. 2004). In our study, cell abundance in snow at 5504 m decreased sharply comparing with that at 5300 m, accompanied by a drop in K^+, Mg^{2+}, and Ca^{2+} concentrations which are an index of the amount of dust particles transported to glaciers (Kang et al. 2002; Yao et al. 2004). The results from chemical composition of fresh snow on the Nepal Himalayas (Marinoni et al. 2001) showed that Na^+/Cl^- ratio during

Table 3 Nearest neighbors of 16S rRNA gene sequences in snow, moraine lake and stream in the Yala Glacier

Category	Clone	Nearest neighbor			RDP classifier
		Sequence	Identity (%)	Isolated environment	Genus
Alphaproteobacteria	YAS1-1-46	FJ540928	99	Soil	*Bradyrhizobium*
Alphaproteobacteria	YAS-3-48	FJ774973	99	Plant	*Brevundimonas*
Alphaproteobacteria	YAS1-1-69	HM446258	100	Tomato ecosystem	*Brevundimonas*
Alphaproteobacteria	YAS1-1-63	EU803702	100	Lake water	*Methylobacterium*
Alphaproteobacteria	YA-S-15	EU641717	99	Lake	*Phenylobacterium*
Alphaproteobacteria	YAS-3-94	AM231587	100	Drinking water	*Roseomonas*
Alphaproteobacteria	YAS-3-68	EU869608	98	Antarctic stream sediment	*Rubellimicrobium*
Alphaproteobacteria	YA-L-81	AY902680	99	Lake	*Sandarakinorhabdus*
Alphaproteobacteria	YA-S-25	AY902680	99	Lake	*Sandarakinorhabdus*
Alphaproteobacteria	YA-S-13	AJ620198	98	Lake	*Sphingopyxis*
Alphaproteobacteria	YAS-3-19	AF058665	98	Plant	Unknown genus
Alphaproteobacteria	YAS-3-2	EF075650	99	Forest	Unknown genus
Alphaproteobacteria	YAS-3-44	GU906603	97	Soil	Unknown genus
Alphaproteobacteria	YAS-3-70	EU218989	97	Oligotrophic lake	Unknown genus
Alphaproteobacteria	YAS-3-71	DQ129276	96	Urban aerosol	Unknown genus
Alphaproteobacteria	YAS-3-75	DQ648974	94	Soil	Unknown genus
Alphaproteobacteria	YAS1-1-12	AM940520	99	Arctic soil	Unknown genus
Alphaproteobacteria	YAS1-1-28	GU219533	97	Volcanic rocks	Unknown genus
Alphaproteobacteria	YAS1-1-49	GU219654	98	Volcanic rocks	Unknown genus
Alphaproteobacteria	YAS1-1-54	GU219654	98	Volcanic rocks	Unknown genus
Alphaproteobacteria	YAS1-1-5	AB464939	96	Glacier	Unknown genus
Alphaproteobacteria	YAS1-1-84	EF220105	98	Antarctic soil	Unknown genus
Alphaproteobacteria	YAS1-1-87	EU751320	97	Sandstone formations	Unknown genus
Alphaproteobacteria	YA-L-69	FR667277	99	High mountain lake, Spain	Unknown genus
Alphaproteobacteria	YA-L-95	FN668059	99	Switzerland lake	Unknown genus
Alphaproteobacteria	YA-S-11	HM156124	100	Soil from glacier forefield	Unknown genus
Betaproteobacteria	YAS-3-8	GU473026	100	Soil	*Burkholderia*
Betaproteobacteria	YA-L-16	HM355747	100	Plant	*Delftia*
Betaproteobacteria	YAS1-1	GQ396966	98	Soil	*Herbaspirillum*
Betaproteobacteria	YAS-3-76	DQ177478	99	Qinghai-Tibet permafrost	*Massilia*
Betaproteobacteria	YAS-3-89	AF443565	99	Semiarid soil	*Massilia*
Betaproteobacteria	YAS1-1-94	FJ826053	99	Plant	*Methylobacillus*
Betaproteobacteria	YA-L-60	GU937478	100	Plant	*Methylobacillus*
Betaproteobacteria	YA-L-12	DQ521549	99	Lake Vida ice cover, Antarctica	*Polaromonas*
Betaproteobacteria	YA-S-17	FN296773	99	High mountain lake, Spain	*Polaromonas*
Betaproteobacteria	YA-S-21	EU263783	99	Kuytun 51 Glacier	*Polaromonas*
Betaproteobacteria	YA-S-29	EF423330	98	Tianshan glacier	*Polaromonas*
Betaproteobacteria	YA-L-47	DQ628931	99	Arctic glacier	*Rhodoferax*
Betaproteobacteria	YA-L-77	FJ547045	99	Switzerland lake	*Rhodoferax*
Betaproteobacteria	YA-S-2	FN297003	99	High mountain lake, Spain	*Rhodoferax*
Betaproteobacteria	YA-S-38	EU636023	96	Collins glacier, Antarctica	*Rhodoferax*
Betaproteobacteria	YA-L-13	GU246818	99	Qiangyong glacier	Unknown genus
Betaproteobacteria	YA-L-63	FN668017	99	Switzerland lake	Unknown genus
Betaproteobacteria	YA-L-71	FJ612163	99	Lake	Unknown genus
Betaproteobacteria	YA-L-87	FJ694630	99	River	Unknown genus
Betaproteobacteria	YA-S-26	FN668017	99	Switzerland lake	Unknown genus
Betaproteobacteria	YA-S-39	FJ694403	99	River	Unknown genus

Table 3 continued

Category	Clone	Nearest neighbor			RDP classifier
		Sequence	Identity (%)	Isolated environment	Genus
Betaproteobacteria	YA-S-49	EU636040	98	Collins glacier, Antarctica	Unknown genus
Betaproteobacteria	YA-S-58	GU305830	96	Oligotrophic lake	Unknown genus
Betaproteobacteria	YA-S-59	EF018476	98	Soil	Unknown genus
Betaproteobacteria	YA-S-63	EF018476	96	Soil	Unknown genus
Betaproteobacteria	YA-S-80	HM565485	100	Mittivakkat glacial sediment	Unknown genus
Betaproteobacteria	YA-S-84	FJ694545	99	River	Unknown genus
Betaproteobacteria	YA-S-92	AY989283	96	Soil	Unknown genus
Betaproteobacteria	YA-S-98	EU442037	98	Nam Co Lake, Tibet	Unknown genus
Betaproteobacteria	YA-S-99	GU321360	98	Nam Co Lake, Tibet	Unknown genus
Gammaproteobacteria	YA-L-1	HM163509	100	Plant	*Acinetobacter*
Deltproteobacteria	YAS1-1-10	FJ152793	97	Alkaline saline soil	*Kofleria*
Actinobacteria	YAS1-1-86	NR-028884	99	Antarctic lake	*Friedmanniella*
Actinobacteria	YA-L-28	DQ675460	99	Moraine lake at Mt. Everest	*Ilumatobacter*
Actinobacteria	YA-L-32	HM129836	99	Nam Co Lake, Tibet	*Ilumatobacter*
Actinobacteria	YA-L-61	FN668348	99	Switzerland lake	*Ilumatobacter*
Actinobacteria	YA-S-33	FN296962	99	High mountain lake, Spain	*Ilumatobacter*
Actinobacteria	YA-S-93	HM129625	99	Nam Co Lake, Tibet	*Ilumatobacter*
Actinobacteria	YAS-3-95	EU297027	99	Soil	*Microlunatus*
Actinobacteria	YAS-3-93	EU181492	100	Marine sediment	*Modestobacter*
Actinobacteria	YAS1-1-81	AF409007	99	Soil	*Modestobacter*
Actinobacteria	YAS1-1-89	AM932258	100	Mushroom	*Nonomuraea*
Actinobacteria	YAS-3-60	AY831385	98	Aerobic granules	*Quadrisphaera*
Actinobacteria	YAS-3-84	FR667171	99	Soil	Unknown genus
Actinobacteria	YAS-3-99	X92358	98	Soil	Unknown genus
Actinobacteria	YAS1-1-26	HM222666	99	Deep-sea sediment	Unknown genus
Actinobacteria	YAS1-1-70	EU636020	99	Collins glacier, Antarctica	Unknown genus
Actinobacteria	YA-L-4	HM129891	99	Nam Co Lake, Tibet	Unknown genus
Actinobacteria	YA-L-40	EF520360	98	Lake	Unknown genus
Actinobacteria	YA-L-64	GU784868	99	Glacier	Unknown genus
Actinobacteria	YA-L-92	HM129891	99	Nam Co Lake, Tibet	Unknown genus
Actinobacteria	YA-S-14	EU375416	98	Puma Yumco Lake, Tibet	
Actinobacteria	YA-S-60	FJ694584	99	River	Unknown genus
Firmicutes	YAS-3-1	GU369561	98	Lake water	*Tumebacillus*
Firmicutes	YAS-3-16	FN429099	98	Soil	*Bacillus*
Firmicutes	YAS-3-3	HM113638	98	Alaskan soil	*Bacillus*
Firmicutes	YAS-3-37	AB487679	94	Soil	*Acetivibrio*
Firmicutes	YAS-3-92	EF074872	99	Soil	*Bacillus*
Firmicutes	YAS1-1-3	EF074504	99	Pasture	*Bacillus*
Firmicutes	YAS1-1-50	GU256501	99	Soil	*Bacillus*
Firmicutes	YAS1-1-90	GQ183258	98	Wetland	*Bacillus*
Firmicutes	YAS1-1-42	AY170379	99	Lagoon	*Clostridium*
Firmicutes	YAS1-1-75	DQ129361	99	Urban aerosol	*Tumebacillus*
Firmicutes	YAS1-1-40	AY660701	99	Siberan permafrost	Unknown genus
Firmicutes	YA-L-29	AM882998	99	Waste compost	*Weissella*
Bacteroidetes	YAS-3-32	AM988899	98	Lake water	*Chryseobacterium*
Bacteroidetes	YAS-3-39	GQ128143	98	Soil in Mila mount, Tibet	*Hymenobacter*
Bacteroidetes	YAS-3-79	DQ365993	95	Antarctic soil	*Segetibacter*

Table 3 continued

Category	Clone	Nearest neighbor			RDP classifier
		Sequence	Identity (%)	Isolated environment	Genus
Bacteroidetes	YAS1-1-71	EU382214	96	Qinghai-Tibet plateau soil	*Hymenobacter*
Bacteroidetes	YAS1-1-8	EF221070	97	Antarctic soil	Unknown genus
Bacteroidetes	YA-L-11	FN297373	99	High mountain lake, Spain	*Arcicella*
Bacteroidetes	YA-L-23	EU642274	100	Lake	*Arcicella*
Bacteroidetes	YA-L-52	GU246876	100	Muztag Ata Glacier	*Arcicella*
Bacteroidetes	YA-L-100	FN984857	98	High mountain lake, Spain	*Ferruginibacter*
Bacteroidetes	YA-L-10	EU267872	98	Kuytun 51 Glacier	*Flavobacterium*
Bacteroidetes	YA-L-7	EU267872	99	Kuytun 51 Glacier	*Flavobacterium*
Bacteroidetes	YA-L-14	AM934668	97	Water	*Flavobacterium*
Bacteroidetes	YA-L-43	DQ628952	99	Glacier	*Flavobacterium*
Bacteroidetes	YA-L-74	EF190151	100	Snow at 6350 m Mt. Everest	*Flavobacterium*
Bacteroidetes	YA-L-5	FN668091	99	Switzerland lake	*Sediminibacterium*
Bacteroidetes	YA-S-28	DQ675462	99	Moraine lake at Mt. Everest	*Arcicella*
Bacteroidetes	YA-S-10	EU442926	98	Nam Co Lake, Tibet	*Flavobacterium*
Bacteroidetes	YA-S-12	EF190151	98	Snow at 6350 m Mt. Everest	*Flavobacterium*
Bacteroidetes	YA-S-24	HQ000018	97	Pond	*Flavobacterium*
Bacteroidetes	YA-S-34	EF190151	99	Snow at 6350 m Mt. Everest	*Flavobacterium*
Bacteroidetes	YA-S-41	EF377928	96	Soil	*Flavobacterium*
Bacteroidetes	YA-S-56	EU860082	99	Freshwater	*Flavobacterium*
Bacteroidetes	YA-S-70	EU267872	98	Kuytun 51 Glacier	*Flavobacterium*
Bacteroidetes	YA-S-79	DQ675472	99	Moraine lake at Mt. Everest	*Flavobacterium*
Cyanobacteria	YAS-3-10	Z81323	99	Plastid:chloroplast	Unknown genus
Cyanobacteria	YAS-3-100	EU161552	99	Plastid:chloroplast	Unknown genus
Cyanobacteria	YAS1-1-100	AP005672	99	Plastid:chloroplast	Unknown genus
Cyanobacteria	YAS-3-18	GQ998717	100	Plastid:chloroplast	Unknown genus
Cyanobacteria	YAS1-1-47	GQ998717	100	Plastid:chloroplast	Unknown genus
Cyanobacteria	YAS-3-24	AM940794	99	Arctic soil	Unknown genus
Cyanobacteria	YAS-3-53	AY425770	98	Volcanic deposit	*GpI*
Cyanobacteria	YAS-3-82	DQ471915	99	Monuments	*GpI*
Cyanobacteria	YAS-3-87	AJ544082	97	Sphagnum bog	*GpI*
Cyanobacteria	YAS1-1-92	EU753646	96	Dry stromatolite	*GpI*
Cyanobacteria	YA-S-1	HM129797	100	Nam Co Lake, Tibet	*GpIIa*
Cyanobacteria	YAS-3-55	HM119276	99	Volcano subglacial hyaloclastite	Unknown genus
Cyanobacteria	YAS-3-46	EU160009	98	Rhizosphere soil	Unknown genus
Cyanobacteria	YAS-3-90	EF522323	99	Rock	Unknown genus
Cyanobacteria	YAS1-1-45	EU705095	94	Aerosol	Unknown genus
Cyanobacteria	YAS1-1-73	FJ790635	96	Quartz in a Tibet desert	Unknown genus
Cyanobacteria	YAS1-1-13	FJ790635	96	Quartz in a Tibet desert	Unknown genus
Cyanobacteria	YAS1-1-32	FJ849316	99	Arctic stream epilithon	Unknown genus
Acidobacteria	YAS-3-14	FJ694458	98	River	*Gp3*
Acidobacteria	YAS1-1-34	FJ790570	97	Tuff in a Tibet desert	*Gp4*
Acidobacteria	YAS1-1-36	EF219808	98	Antarctic soil	*Gp1*
Chloroflexi	YAS-3-62	EF651681	97	Soil	Unknown genus
OD1	YA-L-54	GQ340335	90	Reservoir	Unknown genus
TM7	YA-S-95	AY274161	89	Gold mine tailings	Unknown genus

the monsoon period was very near the sea–salt ratio (0.86) indicating the strong marine contribution related to the monsoon circulation. However, Na^+/Cl^- ratios in our two snow samples were 0.47 and 0.35, which indicated the weak marine source. It is consisted with that these snow samples were collected on the pre-monsoon season when marine contributed less aerosol to the glacier. The relationship between the ion concentration and cell abundance indicated that snow microbe were sourced from terrestrial environment. And snow at 5300 m possibly received relatively more microbes attached to particles. Not only cell abundance but also the biomass of snow algae was observed to clearly decrease with altitude following the change in environmental conditions in this glacier (Yoshimura et al. 1997). The microbe could grow well as the algae due to the relatively high temperature and nutrients at lower altitude. More input and growth of microbe made the cell more abundant at the 5300 m than at 5504 m in the Yala Glacier. But this changed trend was distinctly different from that in the East Rongbuk Glacier, where bacterial abundance in surface snow increases with altitude (Liu et al. 2007). This may be due to the relatively high altitude (from 6300 to 8800 m) and harsh survival conditions in the East Rongbuk Glacier resulting in snow bacteria only being transported to the glacier, but without any growth.

Cell abundances in aquatic niches were higher than that in snow. Bacterial abundance in the moraine lake and stream of the Yala Glacier was much higher than those in the glacial runoff and stream of a Svalbard glacier (ranged from 1.4×10^4 to 4.8×10^4 cells mL^{-1}) in the Arctic (Mindl et al. 2007) and in the melt water streams of an Antarctic glacier (ranged from 0.3×10^4 to 1.1×10^4 cells mL^{-1}) in the McMurdo Dry Valley (Mikucki et al. 2004). They were also higher than in moraine lakes (ranged from 0.3×10^4 to 8.4×10^4 cells mL^{-1}) in the East Rongbuk Glacier (Liu et al. 2006a). However, they were lower than in the Bench Glacier (ranged from 6.6×10^4 to 37×10^4 cells mL^{-1}), Alaska (Skidmore et al. 2005). The DOC concentrations in the water and the annual temperature in the Yala Glacier were higher than in the East Rongbuk, Svalbard and Antarctic glaciers. It seems that relatively high nutrient and temperature resulted in the microbes being more abundant in water from the Yala Glacier.

Cell abundance in snow and aquatic niches showed strong linear negative relationship with NH_4^+ concentrations while other ion concentrations were found to increase from high-altitude snow to stream. The lower altitude snow should contain less ions than that in higher site due to their being near the ion source (Kang et al. 2007). Ion concentrations in the lake and stream were definitely higher than that in the snow as the chemical and physical weathering added more chemical into water. All the ions changed consistently except the NH_4^+. With the decrease of the NH_4^+ concentration, the NO_3^- concentration increased. It implied that more nitrifying bacteria in the low altitude oxidized NH_4^+ into NO_3^-.

Different microbial communities in snow, moraine lake and glacier stream at the Yala Glacier

Microbial communities in snow at 5300 and 5504 m were similar; both were dominated by the Cyanobacteria and owned same groups. While the number of sequences with nearest neighbors from polar/alpine environments increased, the number of sequences with their nearest neighbors (and with DNA) belonging to the Cyanobacteria decreased as the altitude increased.

On the Yala Glacier, water and snow were highly diverse systems. The microbial communities in the moraine lake and glacier stream were totally different from those in snow. The Bacteroidetes and Betaproteobacteria, which are the major groups in most freshwater environments around the world (Zwart et al. 2002), were dominant in the moraine lake and stream at the Yala Glacier. However, most of them were closely related to sequences from alpine/polar environments. In the moraine lake 34% of sequences and in the stream 11% of sequences had their nearest neighbor from snow and glaciers, while very few genera were detected in common in the snow and lake/stream (Table 2). Significant differences between snow and meltwater communities are detected in Arctic and Himalayan glaciers (Larose et al. 2010; Liu et al. 2007). This indicated the diversity of microbes in glacial snow and aquatic habitats. However, within the aquatic habitats, the moraine lake and the stream had similar microbial features, and common genera accounted for the major part (72 and 64%, respectively) of the bacterial communities. Microbes in different moraine lakes on the East Ronbuk Glacier (Liu et al. 2006a), meltwater and lake at Arctic glaciers also had similar community structure and common genera (Larose et al. 2010; Mindl et al. 2007).

Microbial communities were similar in lake/stream and different in snow at the Yala and East Rongbuk glaciers

Microbe in the snow in the Yala Glaciers was different from that in another Himalaya glacier, East Rongbuk Glaciers. The Cyanobacteria dominated in snow on the Yala Glaciers but were much fewer on the East Rongbuk Glaciers, whereas the Gammaproteobacteria dominated on the East Rongbuk Glaciers but were much fewer on the Yala Glaciers. In case this variation in the microbial communities was due to the change from the surface snow

to the deep snow in the snowpit, we compared the microbes only in surface snow in the two glaciers on the Himalayas. The surface snow microbial communities of the two glaciers were different (Fig. 2). Only one common genus (*Brevundimonas* in the Alphaproteobacteria) existed in the snow on both glaciers, and the genus accounted for a small percentage in the libraries (Table 2). Microbes in the surface snow directly reflected the input of microorganisms from outside the glacier and the instant selection effect within the glacier. The significant difference between the microbes in the snow on the two glaciers on the Himalayas was attributed to different atmospheric, climatic and environmental conditions. In the spring the two glaciers were under the influence of different atmosphere cycles. On the East Rongbuk Glacier, westerly and local atmospheric cycles carried microbes from the Tibetan Plateau, while on the Yala Glacier the monsoon was the major carrier of microbes. The height of the Himalayas can block some microbial sources and the two glaciers had different microbial sources. Meanwhile, the harsh climatic and environmental conditions on the northern slope (such as relatively low temperature, strong winds, less oxygen and high altitude) mean that some microbes are unable to survive.

The diversities of the moraine lake and stream libraries in the Yala Glacier were higher than moraine lakes in the East Rongbuk Glacier but only slightly lower than the meltwater and meltwater river in the Arctic glacier (Larose et al. 2010). The aquatic microbial communities on the northern and southern slopes were not significantly different and were both dominated by the Bacteroidetes and Betaproteobacteria groups with four common genera (Table 2). The moraine lake and streams on the two glaciers located at similar altitudes (around 5200 m). The abundance of nutrients and possibly more allochthonous input sources to the Yala Glacier, which is near the Latang National Park, were most likely to be responsible for the relatively high bacterial diversity in the lake and stream.

Conclusions

The microbial abundance in surface snow at different altitudes, the moraine lake and the glacial stream in the Yala Glacier increased with altitude and were consistent with variations in the ion and DOC concentrations. Microbes in snow at different altitudes resembled one another. The moraine lake had similar bacteria to that of the glacier stream. However, microbial communities in the snow were different from those in the moraine lake and stream. Microbial abundance and diversity in the snow in the Yala Glacier varied distinctly from that in the East Rongbuk Glacier on the northern slope of the Himalayas due to different physiochemical conditions. Aquatic bacteria had similar community features in the Yala and East Rongbuk Glacier. This indicated that the snow habitat in different glaciers was easily affected by climatic and environmental factors, while the aquatic habitat was comparatively more stable.

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