

Bacterial biodiversity from Roopkund Glacier, Himalayan mountain ranges, India

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Abstract The bacterial diversity of two soil samples collected from the periphery of the Roopkund glacial lake and one soil sample from the surface of the Roopkund Glacier in the Himalayan ranges was determined by constructing three 16S rRNA gene clone libraries. The three clone libraries yielded a total of 798 clones belonging to 25 classes. *Actinobacteria* was the most predominant class (>10% of the clones) in the three libraries. In the library from the glacial soil, class *Betaproteobacteria* (24.2%) was the most predominant. The rarefaction analysis indicated coverage of 43.4 and 41.2% in the samples collected from the periphery of the lake thus indicating a limited bacterial diversity covered; at the same time, the coverage of 98.4% in the glacier sample indicated most of the diversity was covered. Further, the bacterial diversity in the Roopkund glacier soil was low, but was comparable with the bacterial diversity of a few other glaciers. The results of principal component analysis based on the 16S rRNA gene clone library data, percentages of OTUs and biogeochemical data revealed that the lake soil samples were different from the glacier soil sample and the biogeochemical properties affected the diversity of microbial communities in the soil samples.

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Introduction

Studies on bacterial diversity, based on the culture-independent 16S rRNA approach of water, soil, ice etc., of cold habitats such as Arctic (Bottos et al. 2008; Perreault et al. 2007; Kirchman et al. 2003), Antarctica (Yergeau et al. 2007; Niederberger et al. 2008; Stackebrandt et al. 2004; Tindall 2004; Bowman and McCuaig 2003; Neelson 1999; López-García et al. 2001; Brambilla et al. 2001; Bowman et al. 2000; Shivaji et al. 2004; Karr et al. 2003, 2006; Smith et al. 2006; Li et al. 2006; Prabakaran et al. 2007; Mikucki and Priscu 2007; Sjöling and Cowan 2003) and Himalayan glaciers (Xiang et al. 2005; Liu et al. 2006, 2009; Zhang et al. 2006, 2008; Gangwar et al. 2009) have established that the culture-independent approach reveals a greater degree of microbial diversity compared to the culture-dependent approach. The 16S rRNA gene clone libraries of cold regions like tundra soils, sub-glacial samples and permafrost samples revealed many sequences that were related to the *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Cytophagae–Flavobacteriae–Bacteroides* (CFB group), *Planctomyces* and *Gemmatimonadetes* (Cheng and Foght 2007; Neufeld et al. 2004; Steven et al. 2007; Carpenter et al. 2000; Amato et al. 2007; Gangwar et al. 2009). The Himalayas also has several distinct habitats such as glacial ice, permafrost, tundra wetlands, tundra soil, sub-glacial soil, peri-glacial soil and peri-glacial lakes. Earlier studies have reported bacterial diversity from sub-glacial samples and surface soil samples from the Himalayas (Liu et al. 2006, 2009), but the bacterial diversity of lakes formed due to glacial retreat water at high altitudes is lacking. In the current study,

culture-independent approach was used to establish the bacterial diversity of soil samples collected from the Roopkund glacier and periphery of Roopkund glacial lake located in the Himalayan mountain ranges, India.

Materials and methods

Sampling site and sample collection

Roopkund glacier is located near the base of two Himalayan peaks namely Trisul (7120 m) and Nandghungti (6310 m), respectively, at an altitude of 5029 m. The glacial melt water pours into a shallow lake, the ‘Roopkund lake’ or the ‘Skeleton lake’, which is about 2 m deep. About 5 g of ice-free soil samples (RKS1 and RKS6) were collected in the month of September 2005 from about 1 m from the periphery of the Roopkund glacial lake (Fig. 1). In addition, another soil sample, RKS7, was also collected at the same time from the Roopkund glacier. Prior to collection, 1 cm of the surface soil was removed with a sterile spatula and using another sterile spatula the soil was collected, transferred into sterile polythene bags, transported to the laboratory under sterile conditions and stored at -20°C until use. The air temperature was 2°C at the time of sample collection.

Soil analysis

The physical and chemical characteristics of RKS1, RKS6 and RKS7 were analysed. Soil samples were thawed, dried, ground and allowed to pass through a 2-mm sieve. Soil pH (Rhoades 1982), water content (Blakemore et al. 1987) and the chemical characteristics (Jackson 1967) were determined



Fig. 1 The Roopkund Glacier Lake, Himalayas, India, showing the sites of collection of soil samples RKS1 and RKS6 (filled circle). The Roopkund glacier is located about 100 m to the North-east of the lake

using standard methods. Organic matter was determined in terms of organic carbon according to the method of Walkley and Black (1934). Further, the available/mineralizable Nitrogen (N) was determined by the method of Subbiah and Asija (1956), and the total element content was determined in soil after acid digestion. Total Phosphorous (P) was estimated spectrophotometrically by the ammonium vanadomolabdate method of Bray and Kurtz (1945). Macronutrients (Na, K, Ca and Mg) and micronutrients (Zn, Cu, Fe and Mn) were determined using an atomic absorption spectrophotometer (Perkin Elmer Analyst 100 model). Each sample was analysed in duplicate. Sulphate in the soil was determined by extracting soil with conc. H_2SO_4 and conc. HNO_3 followed by analysis on ICP OR AAS (APHA 1995).

Bacterial count

Total bacterial count in the sediment samples was determined by epifluorescence using *BacLight*TM Bacterial Viability Kit (Invitrogen, Oregon, USA) as per the instructions given in the kit. Bacteria were counted in a Petroff-Hausser Counter using a fluorescent microscope (Axioplan 2, Zeiss, Germany).

Extraction of total DNA from soil and PCR amplification of the 16S rRNA gene

Total DNA was isolated from the soil samples essentially according to the methods described earlier (Shivaji et al. 2004; Tsai and Olson 1991, 1992). Primers 16S3 (5'-TCC TAC GGG AGG CAG CAG-3') and 16S4 (5'-GGC GGT GTG TAC AAG GCC C-3') corresponding to positions 339–356 and 1402–1384, respectively, of the *Escherichia coli* 16S rRNA gene (Lane, 1991) were used to amplify about 1.0 kb fragment of the total 1.5 kb 16S rRNA gene. Amplification was done as described earlier (Reddy et al. 2000; Shivaji et al. 2004). The PCR amplicon was electrophoresed on a 1.0% agarose gel and visualized following staining with ethidium bromide (1 $\mu\text{g}/\text{ml}$). The PCR product was purified with the Quiaquick PCR purification kit (Qiagen Inc, Chatsworth, USA) according to the instructions provided.

Cloning and library construction of soil 16S rRNA gene sequences

The purified PCR product obtained earlier was cloned into pMOS Blue Blunt End vector system (Amersham Biosciences, New Jersey, USA) following the instructions of the manual. Transformants were selected on a LB agar plate containing 20 $\mu\text{g}/\text{ml}$ X-gal and 60- $\mu\text{g}/\text{ml}$ ampicillin and incubated at 37°C overnight. Clones were maintained on LB agar plates containing X-gal and ampicillin.

PCR amplification of the 16S rRNA gene, sequencing and phylogenetic analysis

The 16S rRNA gene was amplified from the white colonies by colony PCR using the vector-targeted M13 forward (5'-GTAAAACGACGGCCAGT-3') and M13 reverse (5'-GGAAACAGCTATGACCATG-3') primers, respectively (Amersham Biosciences, New Jersey, USA). The amplified product was checked on 1% agarose gel for quality and then subjected to treatment with 1 µl of ExoSAP-IT (USB Corporation, USA) at 37°C for 15 min followed by incubation at 80°C. The ExoSAP-IT treated amplicon was sequenced using the primers M13 forward, M13 reverse, pD (5'-CAGCAGCCGCGTAATAC-3') and pF* (5'-ACGAGCTGACGACAGCCATG-3') (Reddy et al. 2000). The sequences obtained were trimmed for vector and chimeric sequences using Gene Tool version 2 (<http://www.biotoools.com>) and then aligned using the Autoassembler software (Macintosh software package from PE-Applied Bio-systems, Foster City, CA, USA). Approximately 1050-bp-long sequence obtained for all the clones was subjected to BLAST sequence similarity search (Altschul et al. 1990) and EzTaxon (Chun et al. 2007) to identify the nearest taxa. All the sequences were aligned with sequences, belonging to the nearest taxa, downloaded from the database (<http://www.ncbi.nlm.nih.gov>) using CLUSTAL W (Thompson et al. 1997). Pair-wise evolutionary distances were computed using the DNADIST programme with the Kimura 2-parameter model as developed by Kimura (1980). Phylogenetic and molecular evolutionary analyses were conducted using Neighbour-Joining option of MEGA version 4 software package (Tamura et al. 2007). Bootstrap analysis was performed employing 1000 replicate data sets in order to assess stability among the clades recovered in the phylogenetic tree.

Statistical analyses

In order to compare the bacterial diversity within the three samples, 16S rRNA gene sequences showing $\geq 97\%$ sequence similarity were grouped into the same OTU (phylotype). Shannon–Wiener Diversity Index (<http://www.changbioscience.com/genetics/shannon.html>) was used to calculate Shannon index (H'), Evenness (J') (Lefauconnier et al. 1994) and the Simpson's index (D) (Magurran 1996). Rarefaction analysis was done using the site Online Calculation (http://biome.sdsu.edu/fastgroup/cal_tools.htm). Coverage of 16S rRNA gene clone libraries was calculated as described previously (Good 1954). Rarefaction curves were generated to compare the relative diversity and coverage of each.

The three 16S rRNA gene clone libraries (RUGL1, RUGL6 and RUGL7) were compared using a JAVA

based software Comm Cluster (Hur and Chun 2004) using three different cut-off values (97, 90 and 80%) and an ordination analysis was performed using principal component analysis (PCA). PCA was performed using the SPSS statistical computing package (version 16.0; SPSS, Inc., Chicago, IL, USA) and was employed to group or separate samples based on the biogeochemical parameters [altitude, total bacterial count, water content, pH value, organic carbon, organic matter, mineralisable Nitrogen (N), NO_3^- , SO_4^{2-} , total Phosphorous (P), Mg, Zn, Mn, Fe and Ca] and the percentages of OTUs in each sample. PCA analysis was also performed to compare the present 16S rRNA gene clone libraries with the earlier published data of clone libraries from different glaciers (refer to Table 4).

Nucleotide sequence accession numbers

All the sequences of the 16S rRNA gene clone library were deposited in GenBank with accession numbers GQ420851–GQ421153, GQ366384–GQ366653, GQ366655–GQ366699, EU852102–EU852155, EU852157–EU852260, EU852262–EU852266, EU852268–EU852274 and EU852276–EU852289.

Results

Viable bacterial count and the physico-chemical characteristics of the soil samples from Roopkund lake and Roopkund glacier

The total bacterial count in the soil samples (RKS1, RKS6 and RKS7) from the Roopkund lake and the glacier varied from 0.9 to 4.2×10^7 bacteria g^{-1} of soil. The pH and water content of the three soil samples were similar (Table 1). But, the organic carbon levels varied with very low content in the glacial soil sample RKS7 (0.29%) and high in the RKS1 (1.74%) and RKS6 (1.26%). The other chemical characteristics like mineralisable N, NO_3^- , SO_4^{2-} , total P, Mg, Zn, Mn and Fe content in the glacial lake samples (RKS1 and RKS6) were similar, but differed from RKS7, the glacial soil sample (Table 1).

Bacterial diversity from Roopkund lake and Roopkund glacier

In the present study, two soil samples collected from the vicinity of the Roopkund Glacial Lake (RKS1 and RKS6) and one from the glacier (RKS7) were analysed for bacterial diversity. The soil samples yielded about 30–80 µg DNA g^{-1} of soil. About 200 ng of the DNA was used for constructing a 16S rRNA gene library. The

Table 1 Physico-chemical characteristics and the total bacterial count of two soil samples (RKS1 and RKS6) collected in the vicinity of Roopkund glacier lake and a soil sample (RKS7), collected from the Roopkund glacier, Himalayas, India

Soil parameters	RKS1	RKS6	RKS7
Soil texture	Fine, moist, blackish, silty with small stones and devoid of vegetation	Fine, moist, blackish, silty with small stones and devoid of vegetation	Coarse (sandy), moist, yellowish-brown, with small stones and devoid of vegetation
Altitude (m)	4771	4771	4871
Total bacterial count/g sediment (10 ⁷)	4.2	3.5	0.9
Water content (%)	30	32	28
pH	7.4	7.3	7.15
Organic carbon (%)	1.74	1.26	0.29
Organic matter (%)	3.9	2.82	0.64
Mineralisable N (ppm)	228.6	215.7	128.3
NO ³⁻ (ppm)	45	50	40
SO ₄ ²⁻ (ppm)	446.4	681.6	801.6
Total P (ppm)	523	522	217
Mg (ppm)	16930	17680	4805
Zn (ppm)	84	82	76
Mn (ppm)	427	430	227
Fe (ppm)	30212	30337	14087
Ca (ppm)	4710	15660	1735
16S rRNA gene clone libraries	RUGL1	RUGL6	RUPGL7

libraries constructed from RKS1, RKS6 and RKS7 were designated RUGL1, RUGL6 and RUPGL7, respectively, and consisted of 302, 311 and 185 clones, respectively, with an insert size of approximately 1 kb. BLAST sequence similarity analysis of the clone libraries RUGL1 and RUGL6 indicated that clones belonging to the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospira*, *Proteobacteria*, *Verrucomicrobia* and TM7_s TM7a, respectively, were present in both the libraries (Table 2, Supplementary Table 1). In contrast to these two libraries, the 186 clones in RUPGL7 could be assigned to only two higher taxa namely *Actinobacteria* and *Firmicutes* (Table 2, Supplementary Table 1).

Frequency of distribution of the clones indicated that in the two libraries (RUGL1 and RUGL6) derived from the Roopkund glacial lake soil, clones belonging to phyla *Acidobacteria* (17.2 and 13.0%), *Actinobacteria* (10.6 and 32.8%), CFB group (10.6 and 6.4%), *Chloroflexi* (8.6 and 12.2%) and *Proteobacteria* (42.7 and 23.2%) were the most predominant (representing >10%) of the total clones. In contrast, in RUPGL7 clones assigned to *Actinobacteria* (86.5%) and *Firmicutes* (13.5%) were predominant (Tables 2, 4, Supplementary Table 1). The affiliation of each and every clone to the nearest phylogenetic neighbour based on 16S rRNA gene sequence is also presented in Supplementary Table 1.

Proteobacteria

In the 16S rRNA gene clone libraries of Roopkund glacial lake soils (RUGL1 and RUGL6) (Table 2, Supplementary Table 1) clones affiliated to all the five classes of the phylum *Proteobacteria* namely *Alpha*-, *Beta*-, *Gamma*-, *Delta*- and *Epsilonproteobacteria* (*Epsilonproteobacteria* was absent in RUGL6 library) were present (Table 2, Supplementary Table 1). Phylogenetic analysis also confirmed the affiliation of the clone sequences to the five classes of phylum *Proteobacteria* (Figs. 2a, 3a). All the clones clustered with their related sequences (Figs. 2a, 3a) except RUGL1-52, RUGL1-298, RUGL1-415 and RUGL1-612 clones showed >90% pairwise sequence similarity based on BLAST with *Geobacter lovleyi* SZ^T (*Deltaproteobacteria*), *Desulfuromonas thiophila* DSM 8987^T (*Deltaproteobacteria*), *Thiobacillus* sp. DDR2W1u61 (*Betaproteobacteria*) and *Geoalkalibacter ferrihydriticus* Z-0531^T (*Deltaproteobacteria*) but did not cluster with their corresponding phylogenetic neighbours (Fig. 2a). Clones affiliated to *Proteobacteria* were absent in RUPGL7 library (Fig. 4).

Actinobacteria

Clones affiliated to *Actinobacteria* were present in libraries RUGL1, RUGL6 and RUPGL7 (32, 102 and 160 clones),

Table 2 Comparison of the three 16S rRNA gene libraries (RUGL1, RUGL6 and RUPGL7) constructed using two soil samples (RKS1 and RKS6) collected in the vicinity of Roopkund glacier lake and a soil sample (RKS7) collected from the Roopkund glacier, Himalayas, India, based on BLAST analysis of the 16S rRNA gene sequences

Phylum/class	RUGL1		RUGL6		RUPGL7	
	Number of clones	Library (%)	Number of clones	Library (%)	Number of clones	Library (%)
Phylum- <i>Acidobacteria</i>	52	17.2	40	13	0	0
Class- <i>Acidobacteria</i>	43	14.2	38	12.2	0	0
Class- <i>Holophagae</i>	2	0.7	0	0	0	0
Class- <i>Soilbacteres</i>	7	2.3	2	0.6	0	0
Phylum- <i>Actinobacteria</i>	32	10.6	102	32.8	160	86.5
Class- <i>Actinobacteria</i>	32	10.6	102	32.8	160	86.5
Phylum- <i>Bacteroidetes</i>	32	10.6	20	6.4	0	0
Class- <i>Bacteroidia</i>	5	1.7	6	1.9	0	0
Class- <i>Flavobacteria</i>	8	2.7	5	1.6	0	0
Class- <i>Sphingobacteria</i>	19	6.2	9	2.9	0	0
Phylum- <i>Chlorobi</i>	5	1.7	1	0.3	0	0
Class- <i>Chlorobea</i>	5	1.7	1	0.3	0	0
Phylum- <i>Chloroflexi</i>	26	8.6	38	12.2	0	0
Class- <i>Anaerolineae</i>	5	1.7	8	2.6	0	0
Class- <i>Caldilineae</i>	2	0.7	2	0.6	0	0
Class- <i>Chloroflexi</i>	15	4.9	28	9	0	0
Class- <i>Dehalococcoidetes</i>	2	0.7	0	0	0	0
Class- <i>Thermomicrobia</i>	2	0.7	0	0	0	0
Phylum- <i>Firmicutes</i>	5	1.7	4	1.3	25	13.5
Class- <i>Bacilli</i>	1	0.3	0	0	25	13.5
Class- <i>Clostridia</i>	4	1.3	4	1.3	0	0
Phylum- <i>Elusimicrobia</i>	1	0.3	0	0	0	0
Class- <i>Elusimicrobia</i>	1	0.3	0	0	0	0
Phylum- <i>Gemmatimonadetes</i>	8	2.7	5	1.6	0	0
Class- <i>Gemmatimonadetes</i>	8	2.7	5	1.6	0	0
Phylum- <i>Nitrospira</i>	6	2	8	2.6	0	0
Class- <i>Nitrospira</i>	6	2	8	2.6	0	0
Phylum- <i>Planctomycetes</i>	0	0	2	0.6	0	0
Class- <i>Planctomycetacia</i>	0	0	2	0.6	0	0
Phylum- <i>Proteobacteria</i>	129	42.7	72	23.2	0	0
Class- <i>Alphaproteobacteria</i>	9	3	10	3.2	0	0
Class- <i>Betaproteobacteria</i>	73	24.2	38	12.2	0	0
Class- <i>Gammaproteobacteria</i>	16	5.3	8	2.6	0	0
Class- <i>Deltaproteobacteria</i>	28	9.3	16	5.1	0	0
Class- <i>Epsilonproteobacteria</i>	3	1	0	0	0	0
Phylum- <i>Verrucomicrobia</i>	4	1.3	3	1	0	0
Class- <i>Verrucomicrobiae</i>	4	1.3	3	1	0	0
Phylum-TM7_s TM7a	2	0.7	16	5.1	0	0
Total	302	100	311	100	185	100

and they shared a 16S rRNA gene sequence similarity of 83–100% with the nearest phylogenetic neighbour (Table 2, Supplementary Table 1). In RUGL1 library, all the clones related to *Actinobacteria* formed two major clades (Fig. 2b) along with the other reported strains of

Actinobacteria. In RUGL6 library also all the clone sequences related to *Actinobacteria* clustered with the Actinobacterial clade except one sequence RUGL6-301 which clustered with *Solobacteres* (Fig. 3b). In RUPGL7 library all the clones phylogenetically related to

Cryobacterium roopkundense RuGL₇^T (which was earlier isolated from the same habitat (Reddy et al. 2009) clustered with *Cryobacterium roopkundense* (In Fig. 4 only 7 sequences out of 160 clone sequences were taken for the tree construction) (Fig. 4).

Acidobacteria

In RUGL1 phylum *Acidobacteria* was represented by 17.2% of the total clones in the library and were affiliated to four genera *Acidobacterium*, *Edaphobacter*, *Geothrix* and *Solibacter*. These clones related to the class *Acidobacteria* formed three branches and clustered with the branch of the class *Halopahagae* (Fig. 2b). Further, the clones related to *Solibacter* formed two branches along with *Solibacter usitatus* Ellin6076 and clustered with the branch of *Clostridia* (Fig. 2b). In RUGL6, 13% of the clones belonged to phylum *Acidobacteria*, and they formed two clades (10 and 11 clones) and branched from *Verrucomicrobiae* and *Planctomycetacia*, respectively (Fig. 3b). It is interesting that two clones related to *Solibacteres* clustered with uncultured *Solibacter* sp. AMIB8 (Fig. 3b). Clones related to *Acidobacteria* were not present in the RUPGL7 library.

Bacteroidetes

Clones similar to the phylum *Bacteroidetes* constituted 10.6 and 6.4% of the total clones in RUGL1 and RUGL6 libraries, respectively (Table 2, Supplementary Table 1) and shared a 16S rRNA gene sequence similarity of 85–99 and 83–99%, respectively, with the nearest phylogenetic neighbour (Supplementary Table 1). All clone sequences related to *Bacteroidetes* clustered within the *Bacteroidetes* clade which appeared to be polyphyletic (Figs. 2b, 3b). Clones related to *Bacteroidetes* were not present in the RUPGL7 library.

Chloroflexi

Phylum *Chloroflexi* was represented by 9 and 12.1% of the total clones in RUGL1 and RUGL6 libraries (Table 2, Supplementary Table 1), respectively, and formed a single clade in both RUGL1 and RUGL6 libraries (Figs. 2b, 3b). Clones related to *Chloroflexi* were not present in the RUPGL7 library.

Firmicutes and Elusimicrobia

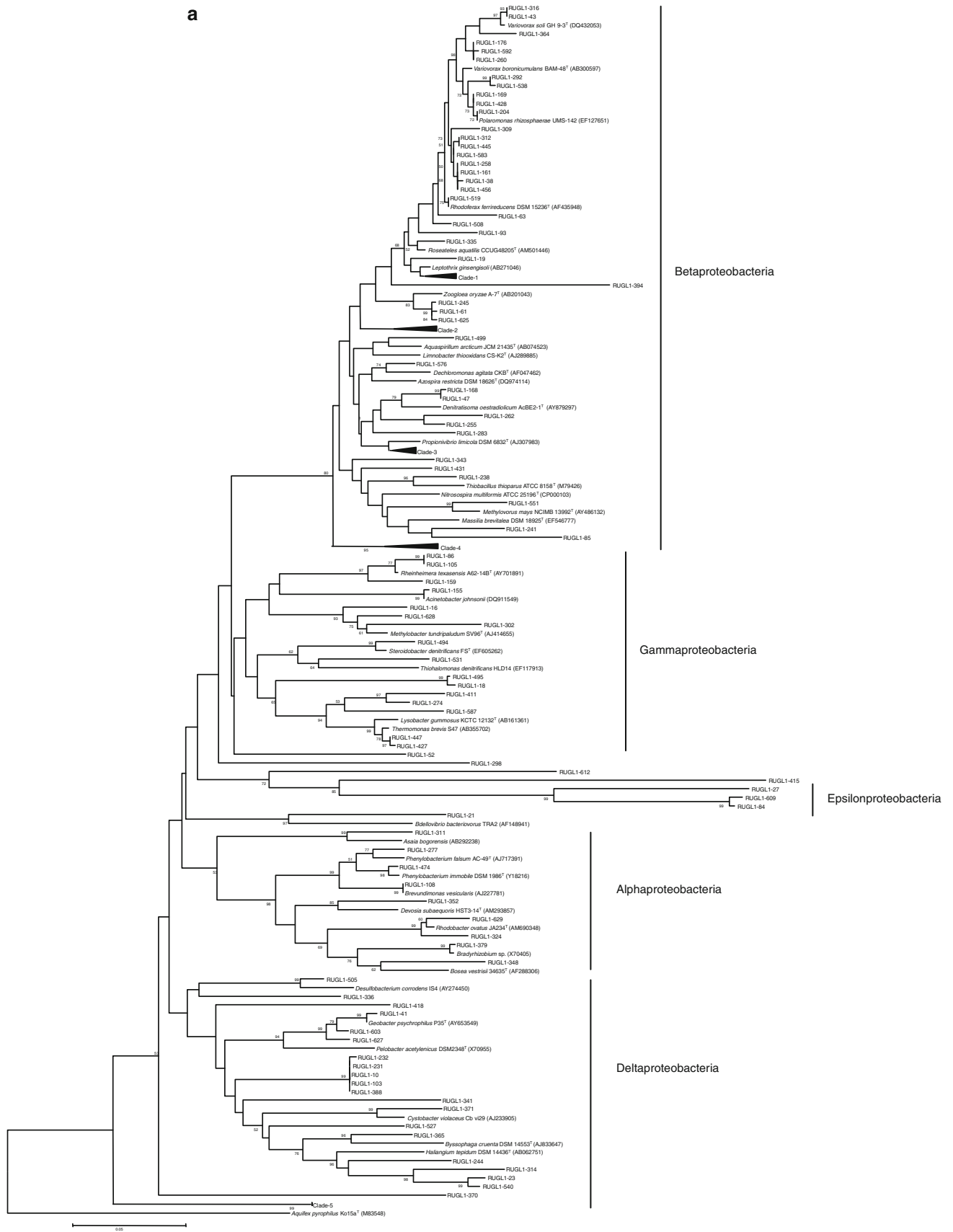
Clones affiliated to phylum *Firmicutes* constituted only 1.7 and 1.3%, respectively, in RUGL1 and RUGL6 (Tables 2, 4, Supplementary Table 1). All the *Firmicutes* sequences were related to *Clostridia* and in the phylogenetic trees

Fig. 2 Neighbour Joining phylogenetic tree of 16S rRNA gene clones from RUGL1, a library constructed using RKS1, a soil sample from the vicinity of Roopkund glacier lake, showing the phylogenetic relationship of clones affiliated to *Proteobacteria* (a) and clones affiliated to *Acidobacteria*, *Actinobacteria*, *Bacilli*, *CFB*, *Chlorobea*, *Chloroflexi*, *Clostridia*, *Elusimicrobia*, *Gemmatimonadetes*, *Holophagae*, *Nitrospira*, *Solibacteres*, TM7_s TM7a and *Verrucomicrobiae* (b). In a the following clones have been compressed: 9, 56, 300, 321, 425, 559 and 567 as Clade-1; 3, 170, 333, 467, 471, 518 and 580 as Clade-2; 303, 334, 442, 507, 530, 537, 569, 577 and 605 as Clade-3; 57, 174, 178, 247, 296, 305 and 591 as Clade-4 and 253, 271, 275 and 297 as Clade-5. In b the following clones have been compressed 1, 29, 83, 95, 164, 276, 403, 416, 434, 461, 464 and 514 as Clade-1; 37, 251, 318, 344, 440, 468, 557 and 626 as Clade-2; 12, 114, 223, 339, 350, 382, 391, 410, 449, 493, 511 and 589 as Clade-3; 6, 59, 67, 167, 197, 201, 209, 385, 398, 413, 554 and 595 as Clade-4; 11, 28, 81, 397, 470 and 604 as Clade-5; 230, 363, 366, 380 and 572 as Clade-6; and 71, 196, 248, 483, 617 as Clade-7. Phylogenetic trees were constructed by Neighbour-Joining method. Numbers at the nodes are bootstrap values. The bar represents 0.05 and 0.02 substitutions per alignment position in a and b, respectively

they formed two and three branches, respectively, in RUGL1 and RUGL6 libraries (Figs. 2b, 3b). One clone RUGL1-448 showing 83% pairwise sequence similarity with *Thermacetogenium phaeum* PB^T belonging to the class *Bacilli* clustered with RUGL1-632 which is related to *Elusimicrobium minutum* Pei191 (Fig. 2b). In RUPGL7 13.5% of all clones (Tables 2, 4, Supplementary Table 1) are phylogenetically close to *Bacillus firmus* AU9 (Fig. 4). (In Fig. 4 only five sequences out of 24 clone sequences were taken for the tree construction). One clone sequence clustered with *Bacillus foraminis* CV53^T which is its phylogenetic relative (Fig. 4).

Chlorobi, *Gemmatimonadetes*, *Nitrospira*, *Planctomycetes* and *Verrucomicrobia*

BLAST analysis indicated that clones affiliated to phylum *Chlorobi* (5 and 1 clones in RUGL1 and RUGL6 libraries), *Gemmatimonadetes* (8 and 5 clones in RUGL1 and 6 libraries), *Nitrospira* (6 and 8 clones in RUGL1 and 6 libraries), *Planctomycetes* (only two clones in the RUGL6 library), *Verrucomicrobia* (4 and 3 clones in RUGL1 and 6 libraries) (Table 2, Supplementary Table 1) shared a 16S rRNA gene sequence similarity with the nearest phylogenetic neighbours (Supplementary Table 1). Further, all the clone sequences clustered with the respective phylogenetic neighbour except RUGL1-266 which branched from the *Solibacteres*, RUGL1-400 which clustered with *Chloroflexi* and RUGL1-404, which clustered with RUGL1-448 (related to *Bacilli*) (Figs. 2b, 3b). Clone sequences related to *Chlorobi* branched from the CFB group in both libraries and clone sequences related to the phyla *Gemmatimonadetes* and *Nitrospira* formed separate branches and clustered



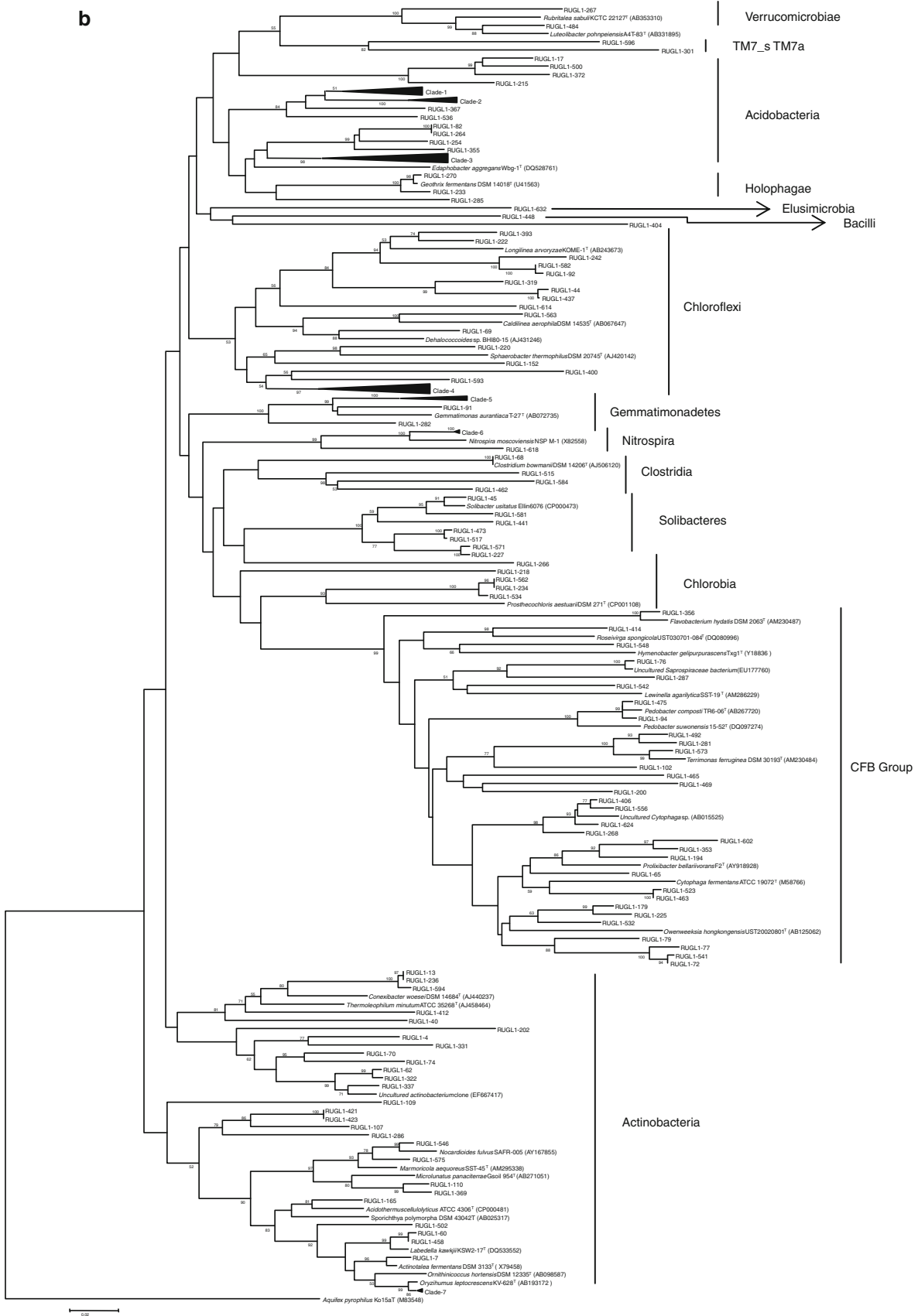


Fig. 2 continued

together (Figs. 2b, 3b). Clones related to above five phyla were not present in the RUPGL7 library.

TM7_s TM7a

Few of the clones were related to the candidate phylum TM7_s TM7a (2 and 16 clones in RUGL1 and 6 libraries, respectively) (Table 2, Supplementary Table 1) and they shared a 16S rRNA gene sequence similarity of 80–93% and clustered together (Figs. 2b, 3b). Clones related to phylum TM7_s TM7a were not present in the RUPGL7 library.

In the present study, unculturable bacteria were also identified in various phyla in RUGL1 and RUGL6 libraries (Supplementary Table 1), and the maximum number was seen in phylum *Acidobacteria* (9.9 and 12% in RUGL1 and 6 libraries, respectively).

Statistical analysis

The three libraries from the three soil samples (RKS1, RKS6 and RKS7) are represented by 302, 311 and 185 clones, respectively, of 16S rRNA gene. These clones based on 16S rRNA gene sequence similarity criteria of >97% could be categorised into 171, 183 and 3 phylotypes (Fig. 5; Table 3, Supplementary Table 1). The most common phylotype was *Cryobacterium psychrophilum*, which was shared by all the three libraries (Supplementary Table 1). Species richness varied from 3 to 183 and the diversity coverage ranged from 41.2 to 98.4% (Table 3). The rarefaction curves indicated that the bacterial populations in both the samples from Roopkund lake did not plateau whereas the soil sample from Roopkund glacier, with diversity coverage of 98.4% plateaued (Fig. 5). The rarefaction curve analysis implied that these are likely to be minimal estimates of diversity. These observations are supported by bacterial diversity parameters, such as Shannon index, Simpson's index, Coverage, Chao1 and Evenness (Table 3).

The sampling sites RKS1 and RKS6 which were close to each other and located on the vicinity of the Roopkund glacial lake were found to be closely associated in the ordination diagram based on the first two principal components (Fig. 6). It was also evident from the clone library sequences that samples from the vicinity of the Roopkund lake RKS1 and RKS6 clustered together at the cut-off values of (97, 90 and 80%) (Fig. 6a, b, c), whereas the sample collected from the Roopkund glacier (RKS7) differed from the lake samples.

The result of PCA based on percentage of a specific OTU (Phyla) in the three libraries (RUGL1, RUGL6 and RUPGL7) is shown in Fig. 7a, and it appears that the principal component factors 1 and 2 (PC1-74.084% and

PC2-25.216%) explain 99.3% of the total variances. From the PCA plot considerable differences in the distribution of phyla in the three soil samples has been observed. The phyla *Actinobacteria* and *Firmicutes* which are dominant in RUPGL7 library grouped closely and were separated from the other phyla present only in RUGL1 and RUGL6 libraries. The result of PCA based on the biogeochemical properties is shown in Fig. 7b and the principal component factors 1 and 2 (PC1-87.107% and PC2-12.893%) explained 100% of the total variances. The PCA plot indicates that altitude and sulphate content which are higher in the glacier sample (RKS7) grouped closely. Further, when all the other parameters were checked it was observed that except for water content, nitrate and calcium the remaining parameters appeared to be associated. Thus, it may imply that altitude, sulphate, nitrate, calcium and water content are the key parameters that influence the observed differences in the percentage of specific OTUs in the three 16S rRNA gene clone libraries.

Principal component analysis based on percentage of specific OTUs (Phyla) in three samples from the present study and eight samples from the published literature (RKS1; RKS6; RKS7; Tibetan Plateau Glaciers, moraine lakes, Mount Everest; glacial meltwater, Mount Everest; John Evans glacier, Canada; Bench Glacier, Alaska; Schirmacher Oasis, Antarctica; Siberian tundra; Samoylov Island, Siberia) are shown in Fig. 7c. The principal component factors 1 and 2 (PC1-72.962% and PC2-16.544%) explain 89.506% of the total variances. The PCA plot indicated that RKS7 from the Roopkund glacier clustered separately from all the other samples; RKS6 and Samoylov Island sample clustered together and all the other samples including RKS1 grouped closely. This analysis also suggested that the bacterial diversity in the Roopkund glacier is different from all the other samples studied.

Discussion

In the present study, two soil samples collected from the vicinity of the Roopkund Glacial Lake (RKS1 and RKS6) and one from the glacier (RKS7) were analysed for bacterial diversity. The results indicated a low bacterial count and decreased species richness in the glacial soil (RKS7) compared to the glacial lake soils (RKS1 and RKS6) and this may be due to the decreased organic carbon %, organic matter %, mineralisable N and total P in glacial soil samples compared to the lake soil samples (Table 1). This observation is in accordance with earlier studies which had demonstrated that factors such as organic carbon content (LaMontagne et al. 2003; Zhou et al. 2002; Fierer et al. 2007) and nutrient availability (Fierer et al. 2003), influence microbial community composition and diversity. The

high carbon in the lake soils might also be due to the decomposition of human carcasses indicated by the presence of skeletons present near the lake and also the availability of water for the microbial metabolic activities (Bill 1994).

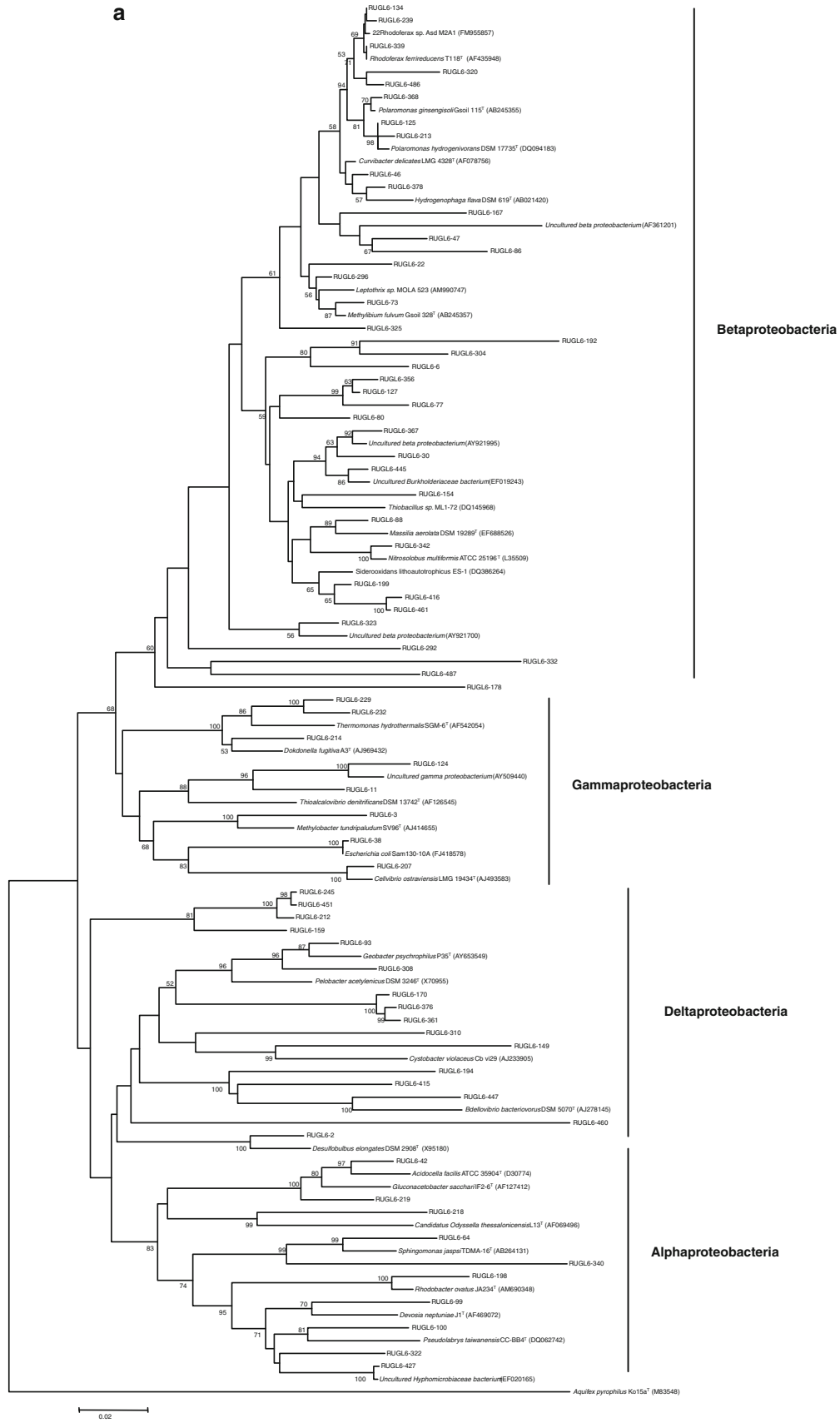
The results obtained by PCA based on the 16S rRNA gene clone library data, percentages of OTUs and biogeochemical properties indicated that the three samples (RKS1, RKS6 and RKS7) and their microbial communities were different, but the samples from the lake (RKS1 and RKS6) are similar compared to the sample from glacier (RKS7). The results of PCA also suggested that biogeochemical properties such as altitude, sulphate, nitrate, calcium and water content may influence the heterogeneity observed in the three samples with respect to the specific OTUs. High altitude, sulphate content and soil texture might influence the decreased diversity observed in the glacial soil sample. However, in an earlier study Zhang et al. (2008) demonstrated that the bacterial diversity was not related to Ca^{2+} concentration but factors such as pH (Eichorst et al. 2007), and water content (Treves et al. 2003) influence microbial community composition and diversity. In the present study also water content appears to be a key parameter that influences the observed differences in the percentage of specific OTUs in the glacial soil compared to the lake soil 16S rRNA gene clone libraries. Earlier studies have indicated that coarse sediments are less favourable than fine sediment as a microbial substrate (Certini et al. 2004; Kastovská et al. 2005), and this indeed may be the reason for the decreased diversity observed in the glacial soil compared to the lake soil since the texture of the soil from the glacier was coarse (sandy), moist, yellowish-brown and had small stones whereas the soil from the lake was fine, moist, blackish, silty, but also had small stones. An overall comparison indicated that the physical and chemical properties of the glacial soil sample and lake soil samples determined in this study are related to other glacial habitats (Liu et al. 2006; Mindl et al. 2007; Foght et al. 2004; Zhang et al. 2008). For instance, the pH, the water content (Foght et al. 2004), organic carbon content (Liu et al. 2006; Mindl et al. 2007; Foght et al. 2004), nitrogen content (Foght et al. 2004), phosphorous (Mindl et al. 2007) and bacterial count (Mindl et al. 2007) were comparable with the earlier studies from cold habitats.

BLAST sequence similarity analysis of the clone libraries RUGL1 and RUGL6 constructed using the soil samples from the lakes indicated that clones belonging to the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospira*, *Proteobacteria*, *Verrucomicrobia* and *TM7_s TM7a* were present in both the libraries (Table 2, Supplementary Table 1) except clones belonging to phylum

Fig. 3 Neighbour-Joining phylogenetic tree of 16S rRNA gene clones from RUGL6, a library constructed using RKS6, a soil sample from the vicinity of Roopkund glacier lake, showing the phylogenetic relationship of clones affiliated to *Proteobacteria* (a) and clones affiliated to *Acidobacteria*, *Actinobacteria*, *CFB*, *Chloroflexi*, *Clostridia*, *Gemmatimonadetes*, *Nitrospira*, *Planctomycetes*, *Solibacteres*, *TM7_s TM7a* and *Verrucomicrobiae* (b). In b the following clones have been compressed 28, 54, 62, 72, 87, 117, 128, 182, 203, 247, 278, 372, 395, 399, 411, 412 and 472 as Clade-1; 34, 126, 222, 306, 373, 463, 466, 474 and 485 as Clade-2; 36, 68, 113, 120, 131, 141, 150, 152, 168, 175, 197, 265, 327, 360, 382, 394, 410, 413, 468, 470 and AM292622 as Clade-3; 97, 111, 112, 135, 195 and 489 as Clade-4; 1, 20, 92, 101, 102, 107, 109, 115, 205, 261, 264, 316, 351, 404, 414, 432, and 441 as Clade-5; 91, 291, 329, 348, 364 and 467 as Clade-6; 17, 29, 183, 225, 235, 244, 289, 303, 420 and AY571791 as Clade-7; 5, 8, 24, 40, 138, 153, 158, 174, 177, 184, 186, 188, 193, 215, 228, 241, 277, 319, 328, 330, 343, 384, 421, 436, 442, 444, 455, 457, 471 and EU192969 as Clade-8; 23, 25, 82, 83, 123, 196, 242, 255, 294, 314, 324 and 422 as Clade-9; 56, 78, 118, 132, 172, 226, 266, 283, 288, 302, 333, 337, 374, 428, 439, 453 and AM902609 as Clade-10; 98, 151, 176, 237, 243, 271, 313, 354, 371, 379, 401, 407 and 426 as Clade-11; 66, 116, 169, 202, 240, 254, 257, 349, 391, 469, 475 and *Prolixibacter* as Clade-12. Numbers at nodes are bootstrap values. The bar represents 0.02 substitutions per alignment position

Planctomycetes and *Elusimicrobia* which were present only in one of the libraries and constituted a very small fraction of the total library (<1%), indicating that the variation may due to variation in recovery of the specific DNA in the two samples. It is not surprising that the two libraries are very similar with respect to the various taxa present since both the soil samples are from the vicinity of the same lake and the physico-chemical characteristics are also very similar (Table 1). In contrast to the aforementioned two libraries, library RUPGL7 (consisting of 185 clones) from the glacial soil, which is a harsh habitat compared to the soil samples collected from the periphery of the glacial lake could be assigned to only two higher taxa, namely *Actinobacteria* and *Firmicutes* (Table 2, Supplementary Table 1). Xiang et al. (2004) reported only two phyla *Proteobacteria* and *Cytophaga-Flavobacterium-Bacteroides* from Malan ice core drilled from the Tibetan Plateau, which is a cold desert like the present site.

Frequency of distribution of the clones indicated that in the two libraries (RUGL1 and RUGL6) derived from the Roopkund glacial lake soil, clones belonging to phyla *Acidobacteria* (17.2 and 13.0%), *Actinobacteria* (10.6 and 32.8%), *CFB* group (10.6 and 6.4%), *Chloroflexi* (8.6 and 12.2%) and *Proteobacteria* (42.7 and 23.2%) were the most predominant (representing >10%) of the total clones (Supplementary Table 1). All these phyla have been reported earlier in different cold habitats of John Evans glacier, Canada (Cheng and Foght 2007); Tibetan plateau glacier (Liu et al. 2009); Samoylov island, Siberia (Wagner et al. 2009) and Schirmacher oasis soil, Antarctica (Shivaji et al. 2004). However, in the Bench glacier, Alaska, only clones belonging to phyla *Acidobacteria* (1.1%), *CFB*



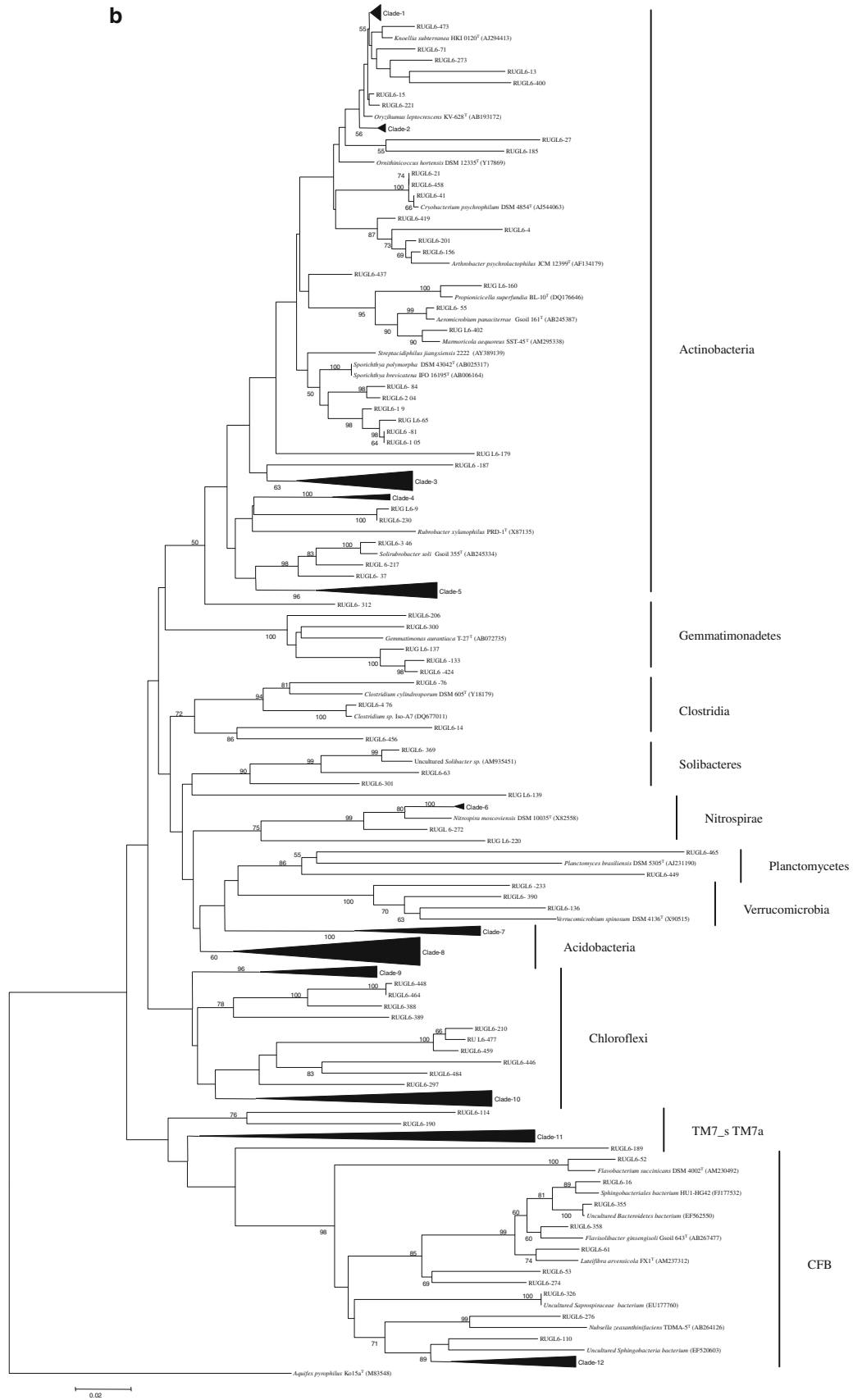


Fig. 3 continued

Fig. 4 Neighbour Joining phylogenetic tree of 16S rRNA gene clones from RUPGL7, a library constructed using RKS7, a soil sample from the Roopkund glacier showing the phylogenetic relationship of clones with their nearest phylogenetic relatives. In this library consisting of 185 clones a majority of the clones (160) were phylogenetically close to *Cryobacterium roopkundense* (93–100%), and the remaining (25 clones) were close to *Bacillus firmus* (95–99%) and *Bacillus foraminis* (96.9%). The tree was constructed using 15, 16, 19, 88, 135, 219, 351, 24, 208, 216, 266, 381 and 327 clones of *Cryobacterium roopkundense*, *Bacillus firmus* and *Bacillus foraminis*, respectively. Numbers at nodes are bootstrap values. The bar represents 0.02 substitutions per alignment position

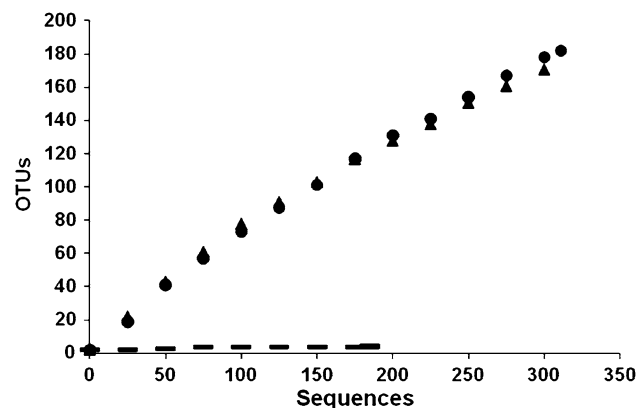
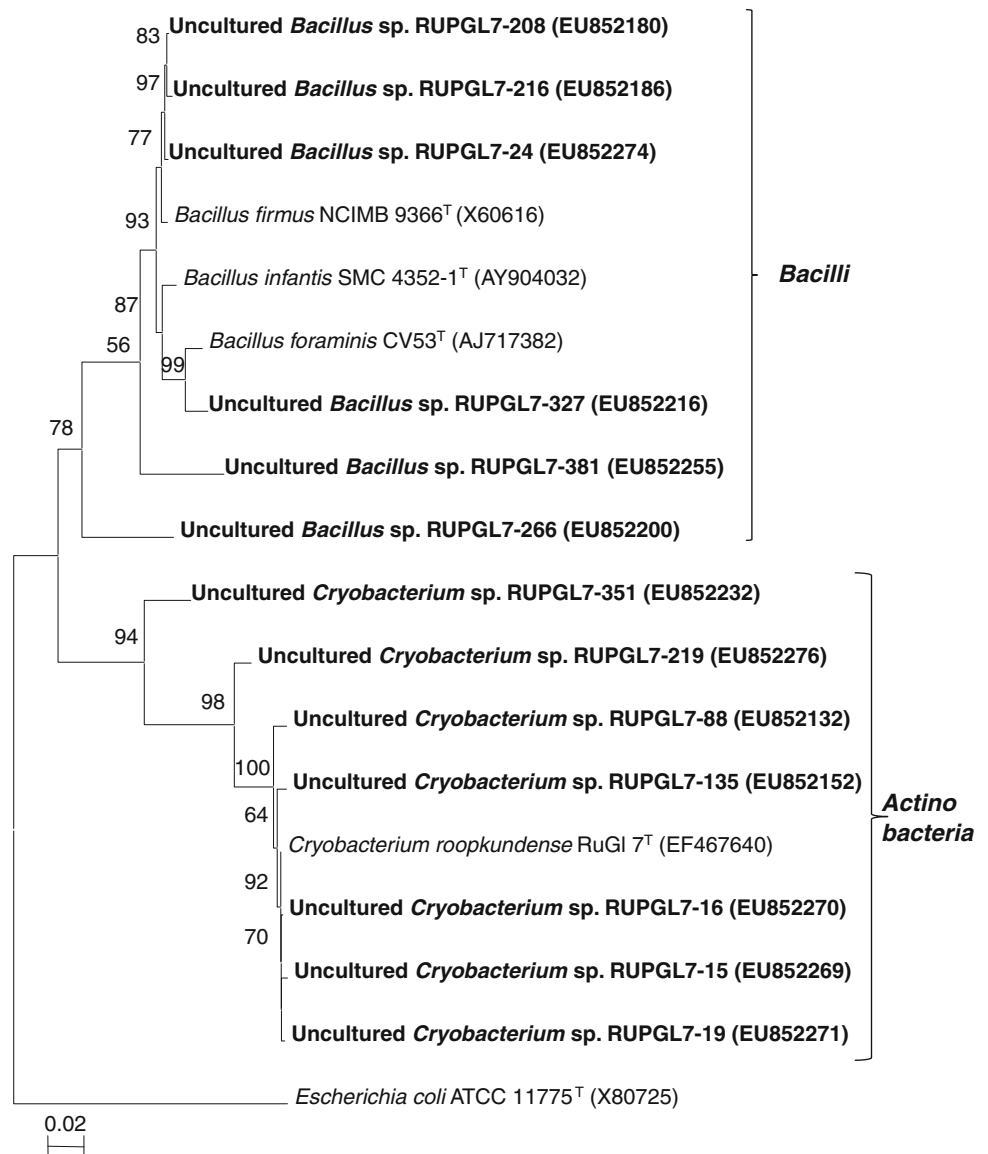
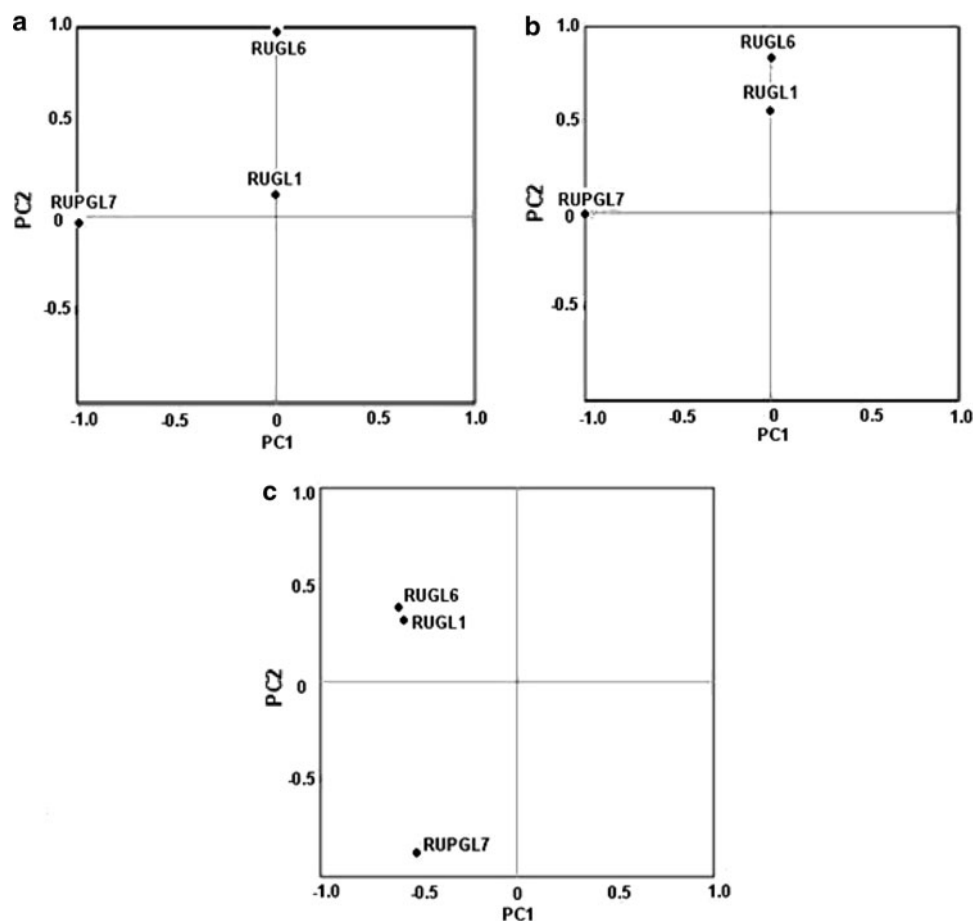


Fig. 5 Rarefaction curves of observed OTUs in the two soil samples collected in the vicinity of Roopkund glacier (RKS1 and RKS6) and a soil sample collected from the Roopkund glacier (RKS7). The symbols represent the rarefaction curves generated for the clone libraries from the three samples RKS1 (filled circle), RKS6 (filled triangle) and RKS7 (minus) respectively

Table 3 Estimated numbers of phylotypes in the three 16S rRNA gene libraries (RUGL1, RUGL6 and RUPGL7) constructed using two soil samples (RKS1 and RKS6) collected in the vicinity of Roopkund glacier lake and a soil sample (RKS7) collected from the Roopkund glacier, Himalayas, India, using different diversity indices

	RUGL1	RUGL6	RUPGL7
Number of clones	302	311	185
Species richness (S)	171	183	3
Coverage (%)	43.4	41.2	98.4
Evenness (J')	0.95	0.91	0.38
Shannon (H')	4.89	4.78	0.42
Simpson's (D)	0.0097	0.0182	0.76
Chao1	355.4	673	-1.0

Fig. 6 Ordination diagrams based on principal component analysis using frequency tables obtained from the three 16S rDNA clone libraries (RUGL1, RUGL6 and RUPGL7) defined at different cutoff similarity values of 97% (a), 90% (b) and 80% (c) using Comm Cluster Software. *PC 1* principal component analysis factor 1, *PC 2* principal component analysis factor 2



group (1.1%), *Proteobacteria* (96.8%) and *Spirochaetes* (1.1%) were present (Skidmore et al. 2005) (Table 4). In contrast, in RUPGL7 clones assigned to *Actinobacteria* (86.5%) and *Firmicutes* (13.5%) were predominant (Tables 2, 4, Supplementary Table 1), thus indicating a decrease in bacterial diversity in the glacial soil.

Phylum *Proteobacteria* is the most dominating community in the RUGL1 and RUGL6 libraries (129 and 72 clones) constructed using Roopkund glacial lake soil (Supplementary Table 1). Earlier studies on the bacterial diversity of glaciers have also indicated predominance of *Proteobacteria* under cold conditions (Cheng and Foght 2007; Liu et al. 2006, 2009; Skidmore et al. 2005; Shivaji et al. 2004; Wagner et al. 2009; Zhou et al. 1997). As compared to Phylum *Proteobacteria* which was represented only in libraries RUGL1 and RUGL6 phylum *Actinobacteria* appears to be a dominating community in RUGL1, RUGL6 and RUPGL7. Majority of the *Actinobacteria* clones of RUGL1 and RUGL6 libraries (26 and 78) are related to different known strains which belong to 36 different genera (Supplementary Table 1). But, in RUPGL7 library, 160 clones are related to *Cryobacterium*

psychrophilum (Suzuki et al. 1997) of the class *Actinobacteria*. Earlier studies on the bacterial diversity of glaciers have also indicated predominance of *Actinobacteria* (Burkert et al. 2003; Glöckner et al. 2000; Cheng and Foght 2007; Mosier et al. 2007; Liu et al. 2006, 2009; Wagner et al. 2009; Zhou et al. 1997). Clones with similarity to Phylum *Acidobacteria* were present in libraries of RUGL1 and RUGL6. In fact, in this phylum vast majority of the clones were similar to uncultured *Acidobacterium* (12%). Earlier studies on the bacterial diversity of glaciers have also indicated predominance of *Acidobacteria* (Cheng and Foght 2007; Skidmore et al. 2005; Shivaji et al. 2004; Liu et al. 2009; Wagner et al. 2009; Zhou et al. 1997).

In the phylum *Bacteroidetes*, the class *Spingobacteria* represents highest number of clones (19 and 9) in RUGL1 and RUGL6 libraries (Supplementary Table 1), respectively, and the remaining clones belong to classes *Bacteroidia* and *Flavobacteria*, thus confirming earlier studies (Cheng and Foght 2007; Skidmore et al. 2005; Shivaji et al. 2004; Liu et al. 2006, 2009; Wagner et al. 2009; Zhou et al. 1997).

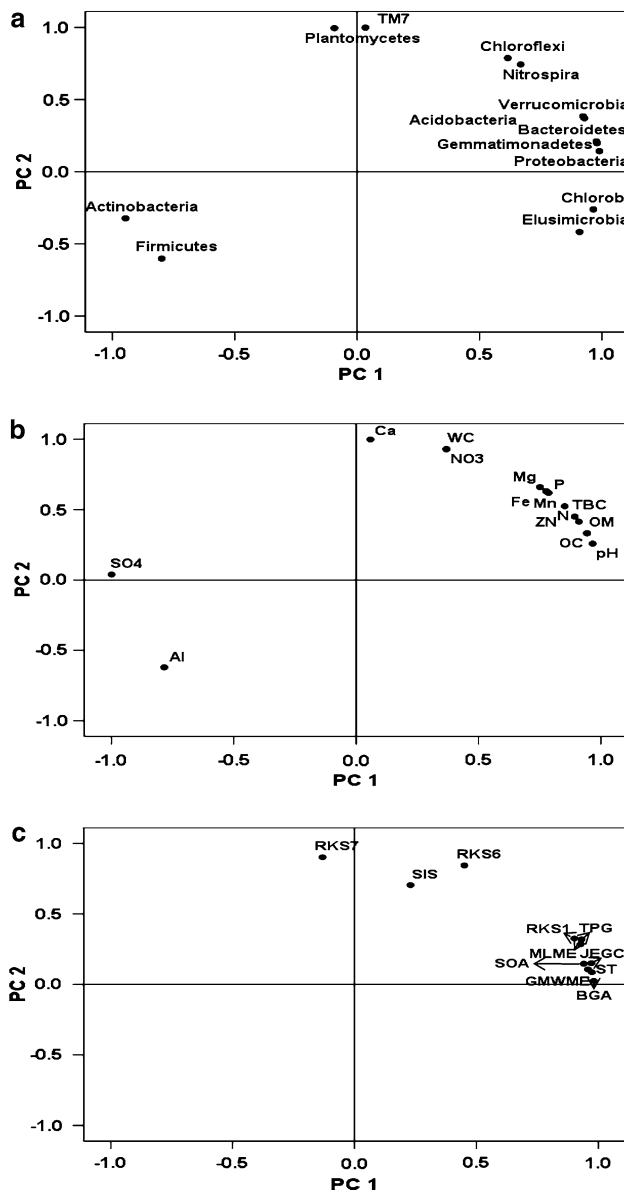


Fig. 7 **a** Principal component analysis based on percentage of specific OTUs from three 16S rRNA gene clone libraries (PC1-74.084% and PC2-25.216%). **b** PCA plot based on biogeochemical properties (altitude, total bacterial count, water content, pH value, organic carbon, organic matter, N, NO_3^- , SO_4^{2-} , total P, Mg, Zn, Mn, Fe and Ca) of three soil samples (PC1-87.107% and PC2-12.893%). **c** PCA plot based on percentage of specific OTUs from 11 16S rRNA gene clone libraries (PC1-72.962% and PC2-16.544%). The data for eight of the libraries were taken from published literature (See footnote to Table 4). *PC 1* principal component analysis factor 1; *PC 2* principal component analysis factor 2; *TPG* Tibetan Plateau Glaciers, *MLME* Moraine lakes, Mount Everest; *GMWME* Glacial meltwater, Mount Everest; *JEGC* John Evans glacier, Canada; *BGA* Bench Glacier, Alaska; *SOA* Schirmacher Oasis, Antarctica; *ST* Siberian tundra; *SIS* Samoylov Island, Siberia

An interesting feature is that most of the clones in the phylum *Chloroflexi* identified with uncultured bacteria. This is a clear indication that in these cold habitats clones

belonging to the phylum *Chloroflexi* are diverse and attempts are needed to culture these organisms. The presence of *Chloroflexi* in other cold habitats (Costello and Schmidt 2006; Li et al. 2008; Lysnes et al. 2004; Reed et al. 2006; Shivaji et al. 2004; Liu et al. 2006; 2009; Wagner et al. 2009) is also documented.

A phylogenetic analysis of the clones affiliated to phylum *Firmicutes* indicated a distinct difference between the clones in the soil from the glacial lake soil (RKS1 and RKS6) compared to glacial soil (RKS7). In the lake soil, though the frequency of clones belonging to *Firmicutes* was low the diversity was significantly higher with the clones showing similarity to genera *Caldalkalibacillus*, *Clostridium*, *Desulfosporosinus* and *Syntrophobotulus*, whereas all the clones in the glacial soil were affiliated only to the genus *Bacillus* (Supplementary Table 1). As of now, only one new species of *Bacillus* has been described from a Himalayan glacier (Reddy et al. 2008). The presence of *Firmicutes* in other cold habitats (Liu et al. 2009; Wagner et al. 2009; Zhou et al. 1997) is also documented.

Clones belonging to many other phyla such as *Chlorobi*, *Verrucomicrobia*, *Planctomycetes*, *Nitrospira* and *Gemmatimonadetes* were present in at least two of the three libraries, but the frequency of distribution was low (<3%). This observation is in agreement with earlier studies which had also indicated that clones affiliated to *Chlorobi* (Zhou et al. 1997), *Verrucomicrobia* (Cheng and Foght 2007; Liu et al. 2006, 2009; Wagner et al. 2009; Zhou et al. 1997), *Planctomycetes* (Cheng and Foght 2007; Liu et al. 2006, 2009; Zhou et al. 1997), *Nitrospira* (Shivaji et al. 2004) and *Gemmatimonadetes* (Shivaji et al. 2004; Steven et al. 2007) are present in other cold habitats (Table 4) at a low frequency. Phylum *Elusimicrobia* is represented only by a single clone in the RUGL1 library, and reports on the presence of these bacteria in cold habitats is lacking. Apart from all the above-known phyla, few clones were related to the candidate phylum TM7_s TM7a (Marcy et al. 2007). The presence of the candidate phylum TM7 was reported from Tibetan plateau glacier sample but at a very low frequency (0.1%). The presence of these clones in Roopkund lake soil may be justified by the presence of human skeletons at the edge of the skeleton lake (Bill 1994) since these were first reported from the human oral cavity (Marcy et al. 2007).

This study demonstrates that the diversity of bacteria in two soil samples obtained from the vicinity of the Roopkund glacial lake and a sample from the Roopkund glacier is comparable with that reported from other cold habitats such as glacial soil, oasis soil and tundra soil samples (Table 4). For example, members of *Actinobacteria*, *Gammaproteobacteria*, *Betaproteobacteria* and

Table 4 Comparison of the bacterial diversity in different cold habitats as determined by 16S rRNA gene clone libraries

Phylogenetic group	1 RKS1	1 RKS6	2 RKS7	3	4	5	6	7	8	9	10
<i>Acidobacteria</i>	17.2	13	0	0.4	0	0	1.5	1.1	2.5	16.3	5.4
<i>Actinobacteria</i>	10.6	32.8	86.5	17.5	13.5	0	5.3	0	9	4.7	16.2
<i>Chlamydiae</i>	0	0	0	0	0	0	0	0	12	0	0
<i>CFB group</i>	10.6	6.4	0	12.8	24.3	28.6	24.8	1.1	4	2.3	24.3
<i>Chlorobi</i>	1.7	0.3	0	0	0	0	0	0	0	2.3	0
<i>Chloroflexi</i>	8.6	12.2	0	0.1	0	0	0	0	4	0	22.9
<i>Cyanobacteria</i>	0	0	0	4	0	0	0	0	0	0	0
<i>Deinococcus-Thermus</i>	0	0	0	0.2	0	0	0	0	0	0	0
<i>Fibrobacteres</i>	0	0	0	0	2.7	0	0	0	0	0	0
<i>Firmicutes</i>	1.7	1.3	13.5	5.5	0	0	0	0	0	4.7	14.9
<i>Elusimicrobia</i>	0.3	0	0	0	0	0	0	0	0	0	0
<i>Gemmatimonadetes</i>	2.7	1.6	0	0	0	0	0	0	4	0	0
<i>Nitrospira</i>	2	2.6	0	0	0	0	0	0	1	0	0
<i>Plantomycetes</i>	0	0.6	0	0.1	2.7	0	1.5	0	0	2.3	0
<i>Proteobacteria</i>	42.7	23.2	0	58.4	54	71.4	64.6	96.8	54	62.8	10.8
<i>Spirochaetes</i>	0	0	0	0	0	0	0	1.1	0	2.3	0
<i>Verrucomicrobia</i>	1.3	1	0	0.4	2.7	0	2.3	0	0	2.3	2.7
Unclassified	0	0	0	0.5	0	0	0	0	9	0	0
OP11	0	0	0	0	0	0	0	0	0	0	2.7
TM7	0.7	5.1	0	0.1	0	0	0	0	0	0	0

Values represent the percentage of clones

CFB, *Cytophaga–Flavobacterium–Bacteroides*

Cold habitats: 1, Roopkund glacial lake soil, Himalayas, India^a; 2, Roopkund glacier soil, Himalayas, India^a; 3, Tibetan Plateau Glaciers^b; 4, Moraine lakes, Mount Everest^c; 5, Glacial meltwater, Mount Everest^c; 6, John Evans glacier, Canada^d; 7, Bench Glacier, Alaska^e; 8, Schirmacher Oasis soil sample, Antarctica^f; 9, Siberian tundra soil sample^g; 10, Samoylov Island, Siberia^h

Data from the present study^a are compared with that of Liu et al. (2009)^b; Liu et al. (2006)^c; Cheng and Foght (2007)^d; Skidmore et al. (2005)^e; Shivaji et al. (2004)^f; Zhou et al. (1997)^g; Wagner et al. (2009)^h

Acidobacteria have been reported from Tibetan plateau glacier, China; Mount Everest, Nepal; John Evans glacier, Canada; Bench Glacier, Alaska; Schirmacher Oasis soil, Antarctica and Siberian tundra soil samples (Table 4). The presence of *Bacillus* is reported for the first time from a glacier, though an isolate was also reported from the Pindari glacier of Himalayas earlier (Reddy et al. 2008). *Bacillus* and *Cryobacterium* were also found in Guliya and Vostok (Antarctica) ice core samples (Christner et al. 2001, 2003). The occurrence of related phylotypes in geographically diverse cold environments is indicative of the ability of microorganisms to adapt similar strategies to survive freezing and remain active at low temperatures (Abyzov et al. 1998; Priscu and Christner 2003). Noticeably, neither enteric nor pathogenic bacteria were detected in Roopkund glacier though enteric bacteria have been isolated from glacial ice on Ellesmere Island (Dancer et al. 1997) and surface

snow of Tateyama Mountains in Japan (Segawa et al. 2005).

Conclusions

Bacterial diversity of Roopkund glacier and the glacial lake based on 16S rRNA gene sequence analysis led to the identification of *Actinobacteria*, *Bacilli*, *Acidobacteria* and *Proteobacteria* as the major groups in two soil samples collected near Roopkund glacier. But, molecular sequences representing *Proteobacteria* could not be detected in the glacial soil. PCA indicated that biogeochemical properties such as altitude, sulphate, nitrate, calcium and water content might influence the heterogeneity observed in the three samples with respect to the specific OTUs.

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