Extremophiles (2011) 15:1–22
DOI 10.1007/s00792-010-0333-4

ORIGINAL PAPER

Bacterial diversity of soil in the vicinity of Pindari glacier, Himalayan mountain ranges, India, using culturable bacteria and soil 16S rRNA gene clones

S. Shivaji · M. S. Pratibha · B. Sailaja · K. Hara Kishore · Ashish K. Singh · Z. Begum · Uttam Anarasi · S. R. Prabagaran · G. S. N. Reddy · T. N. R. Srinivas

Received: 12 August 2010 / Accepted: 22 October 2010 / Published online: 9 November 2010

Abstract Three 16S rRNA gene clone libraries (P1L, P4L and P8L) were constructed using three soil samples (P1S, P4S and P8S) collected near Pindari glacier, Himalayas. The three libraries yielded a total of 703 clones. *Actinobacteria*, *Firmicutes* and *Proteobacteria* were common to the three libraries. In addition to the above P1L and P8L shared the phyla *Acidobacteria*, *Bacteroidetes*, *Gemmatimonadetes* and *Planctomycetes*. Phyla *Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Dictyoglomi*, *Fibrobacteres*, *Nitrospirae*, *Verrucomicrobia*, candidate division SPAM and candidate TM7s TM7a phylum were present only in P1L. Rarefaction analysis indicated that the bacterial diversity in P4S and P8S soil samples was representative of the sample. Principal component analysis (PCA) revealed that P1S and P8S were different from P4S soil sample. PCA also indicated that arsenic content, pH, Cr and altitude influence the observed differences in the percentage of specific OTUs in the three 16S rRNA gene clone libraries. The observed bacterial diversity was similar to that observed for other Himalayan and non-polar cold habitats. A total of 40 strains of bacteria were isolated from the above three soil samples and based on the morphology 20 bacterial strains were selected for further characterization. The 20 bacteria belonged to 12 different genera. All the isolates were psychro-, halo- and alkalitolerant. Amylase and urease activities were detected in majority of the strains but lipase and protease activities were not detected. Long chain, saturated, unsaturated and branched fatty acids were predominant in the psychrotolerant bacteria.

Keywords Himalayan glacier · Pindari glacier · 16S rRNA gene clone library · Psychrophiles · Psychrophilic enzymes · Cellular fatty acids

Communicated by T. Matsunaga.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-010-0333-4) contains supplementary material, which is available to authorized users.

S. Shivaji (✉) · M. S. Pratibha · B. Sailaja · K. Hara Kishore · A. K. Singh · Z. Begum · U. Anarasi · S. R. Prabagaran · G. S. N. Reddy · T. N. R. Srinivas
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India
e-mail: shivas@ccmb.res.in

Introduction

Seventy percent of the world's freshwater is frozen in glaciers, and the Himalayas have the largest concentration of glaciers outside the Polar Regions, covering about 33,000 km^2. The region has been referred to as the 'Water Tower of Asia', as it provides around 8.6×10^6 m^3 of water annually (Dyurgerov and Meier 1997). Almost 70% of the glaciers in the Himalayas are retreating at a startling rate due to climate change (Ageta and Kadota 1992; Yamada 1991; Yamada et al. 1992; Fushimi et al. 1985). The change in climate will also affect the diversity of prokaryotic organisms. Due to the threat posed to the Himalayan glaciers from global warming and human intervention, there is an immediate need to explore and preserve their biodiversity. Microorganisms that exist in these cold environments play a key function in food chains, biogeochemical cycles, and mineralization of pollutants (Brinkmeyer et al. 2003; Dong et al. 2006; Feller and Gerday 2003).

Recent studies on bacterial diversity using snow, ice, water soil and sediments samples of the alpine cold habitats have revealed that psychrotolerant microbes are predominant in these habitats (Liu et al. 2006, 2009; Skidmore et al.

2005; Xiang et al. 2005, 2009; Zhang et al. 2006, 2008; Nemergut et al. 2007; Gangwar et al. 2009). As compared to the alpine glaciers comparatively little is known about the bacterial diversity of the Himalayan glaciers. In our previous studies, several novel bacterial species such as *Planococcus stackebrandtii* (Mayilraj et al. 2005), *Paenibacillus glacialis* (Kishore et al. 2010), *Dyadobacter hamtensis* (Chaturvedi et al. 2005), *Bacillus cecembensis* (Reddy et al. 2008b), *Exiguobacterium indicum* (Chaturvedi and Shivaji 2006), *Leifsonia kafniensis* (Pavan Kumar et al. 2009), *Cryobacterium roopkundense* (Reddy et al. 2010) and *Leifsonia pindariensis* (Reddy et al. 2008a) were isolated from various habitats including snow, ice, water, and soil samples in the vicinity of glaciers. In this study, both culture-independent and culture-dependent approaches were used to establish the bacterial diversity of soil samples collected near Pindari glacier located in the Himalayan mountain ranges, India. In addition, we screened the culturable bacteria for cold-active enzymes and fatty acid profiles. The primary objectives of the study were to establish the bacterial diversity of a Himalayan glacier, to compare the diversity with other alpine and polar glaciers and other cold habitats and to bioprospect for cold-active enzymes.

Materials and methods

Sampling site and sample collection

The Pindari glacier (30°16′4″N, 80°00′36″E) is located in the upper reaches of the Kumaon Himalayas in India to the southeast of Nanda Devi. Three soil samples were collected enroute to the Pindari glacier. Soil samples P1S and P4S were collected near two huge ice blocks lying 3 and 4 km away from the Pindari glacier. The P8S soil sample was collected from the Pindari stream about 8 km from the glacier (Fig. 1). The altitudes at which P1S, P4S and P8S were collected were 3,490, 3,430 and 3,200 m, respectively. About 5 g of ice-free soil samples (P1S, P4S and P8S) were collected in the month of September 2005. 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.

Soil analysis

The physical and chemical characteristics of P1S, P4S and P8S 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 using standard methods. The total element content

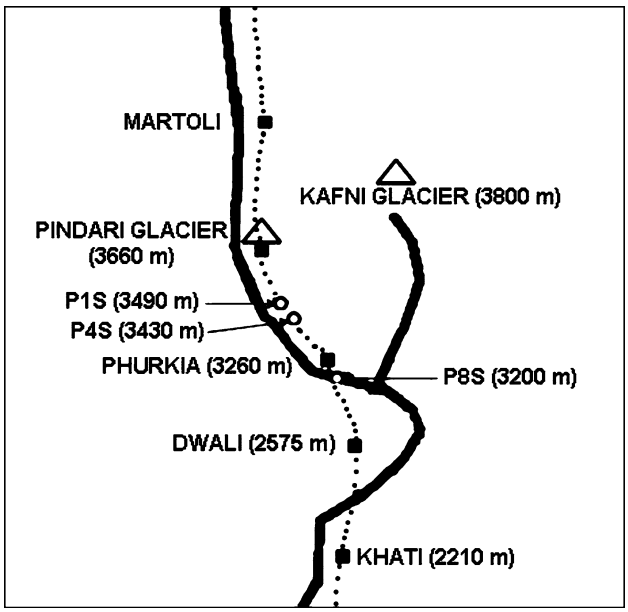


Fig. 1 The trekking route to the Pindari glacier (*broken line*), Himalayas, India, showing the sites of collection of soil samples P1S, P4S and P8S (*solid circle*)

was determined in soil after acid digestion. Micronutrients/elements (Li, Be, Al, V Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, In, Cs, Pb, Bi and U) were determined using an atomic absorption spectrophotometer (Perkin Elmer Analyst 100 model). Each sample was analysed in triplicate and the mean values were calculated.

Bacterial count

Total bacterial count (TBC) in the sediment samples was determined by epifluorescence using *Bac*Light™ 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).

Isolation and characterisation of bacterial strains

Approximately 1 g of the soil sample was suspended in 0.9% NaCl solution and shaken for 2 h at 15°C. For isolation of the bacteria, 100 µl of the water sample was plated on Luria–Bertani (LB) agar plates [tryptone (1.0% w/v), yeast extract (0.5% w/v), NaCl (1.0% w/v) and agar (2.0% w/v)] and incubated at 4°C for 15 days (Reddy et al. 2008a). Colony count was recorded and different morphotypes were purified and maintained on LB agar medium (Shivaji et al. 1989).

The growth at different temperatures (4, 18, 30, 37°C), pH (2, 5, 6, 7, 9, 11) and tolerance to various salt concentrations (2, 5, 8, 10% w/v) was checked using ABM

[peptone (3% w/v), yeast extract (2% w/v) and agar (2% w/v)] agar plates. Salt tolerance was monitored at 18°C and pH 7, temperature optimum was done at pH 7 in the absence of added salt and optimum pH was determined at 18°C and in the absence of any added salt. In all cases ABM agar plates were used. Extracellular enzymatic activities like amylase (Priest 1977), lipase (Booth 1978), protease (Priest 1977) and urease were checked by streaking the cultures on ABM agar plates supplemented with either 0.2% soluble starch, 1% Tween-60 along with 0.01% $CaCl_2$, 0.3% casein and 2% urea (filter sterilized), respectively, and incubating the plates at 4 and 18°C for 5–10 days. Fatty acid methyl esters were prepared and analysed as previously described (Shivaji et al. 2007) using cells grown at 18°C. DNA was isolated from all the cultures and the 16S rRNA gene was amplified, sequenced and phylogenetic analysis were carried out as described earlier (Reddy et al. 2000; Srinivas et al. 2009; Vardhan Reddy et al. 2009).

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 μg/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 above was cloned into pMOS Blue Blunt End vector system (Amersham Biosciences, New Jersey and USA) following the instructions of the manual. Transformants were selected on a LB agar plate containing 20 μg/ml X-gal and 60-μg/ml ampicillin and incubated at 37°C overnight. Clones were maintained on LB agar plates containing X-gal and ampicillin.

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

The 16S rRNA gene was amplified from the transformants by colony PCR using the vector-targeted M13 forward (5′-GTA AAA CGA CGG CCA GT-3′) and M13 reverse (5′-GGA AAC AGC TAT GAC CAT G-3′) primers, respectively and sequenced using the primers M13 forward, M13 reverse, pD (5′-CAG CAG CCG CGG TAA TAC-3′) and pF* (5′-ACG AGC TGA CGA CAG CCA TG-3′) primers (Pradhan et al. 2010). Vector-based sequences and chimeric sequences were eliminated using Gene Tool version 2 (http://www.biotools.com). Sequences were then subjected to BLAST to identify the nearest taxa and aligned with sequences belonging to the nearest taxa, (http://www.ncbi.nlm.nih.gov) using CLUSTAL X. Phylogenetic trees were constructed using Neighbour Joining

Table 1 Physicochemical characteristics and the total bacterial count of three soil samples (P1S, P4S and P8S) collected near the Pindari glacier, Himalayas, India

Soil parameters	P1S	P4S	P8S
Altitude (m)	3,490	3,430	3,200
Soil texture	Fine, black with small stones and grass	Course, yellowish-brown, sandy	Fine, dark brown with small stones
pH	7.2	7.9	7.1
Water content (%)	35	18	28
Ni (ppm)	22.7	18.0	21.0
Cu (ppm)	27.4	15.6	22.1
Rb (ppm)	61.3	52.2	57.5
In (ppb)	17.0	8.5	11.7
Cd (ppb)	457.8	108.4	205.5
Pb (ppm)	26.9	7.6	8.8
Zn (ppm)	138.5	76.9	80.5
Cr (ppm)	35.0	36.5	30.5
As (ppm)	5.8	10.7	5.3
Fe (ppm)	8,616.9	12,493.0	10,753.0
Be (ppm)	0.5	0.7	0.6
Al (ppm)	13,450	13,970	13,940
Li (ppm)	10.7	16.0	15.6
Cs (ppm)	3,390.9	4,685.9	5,150.9
Mn (ppm)	435.9	441.3	449.9
Bi (ppb)	198.9	266.3	424.9
U (ppb)	926.4	995.5	1,498.5
V (ppm)	28.6	29.2	32.7
Se (ppb)	1,036.0	912.9	1,481.0
Sr (ppm)	32.7	11.1	34.2
Ga (ppm)	9.5	5.7	9.6
Total bacterial count/g of soil (10^8)	8.7	2.2	3.9
16S rRNA gene clone libraries	P1L	P4L	P8L

method (Pradhan et al. 2010). Bootstrap analysis, based on 1,000 replicate datasets, was performed to assess stability among the clades.

Statistical analyses of the cloned libraries

In order to compare the bacterial diversity within the three samples, 16S rRNA gene sequences of the isolates showing ≥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 (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 (P1L, P4L and P8L) 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 principle 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, and micronutrients/elements like Li, Be, Al, V Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, In, Cs, Pb, Bi and U) and the percentages of OTUs in each sample (higher taxa ≥1% in the libraries were considered).

Nucleotide sequence accession numbers

All the sequences of the 16S rRNA gene clone libraries were deposited in GenBank with accession numbers GQ287410, GQ287411, GQ287413–GQ287461, GQ287463–GQ287472, GQ287475–GQ287618, GQ329113–GQ329187, GQ329189–GQ329300 and GQ329303–GQ329607.

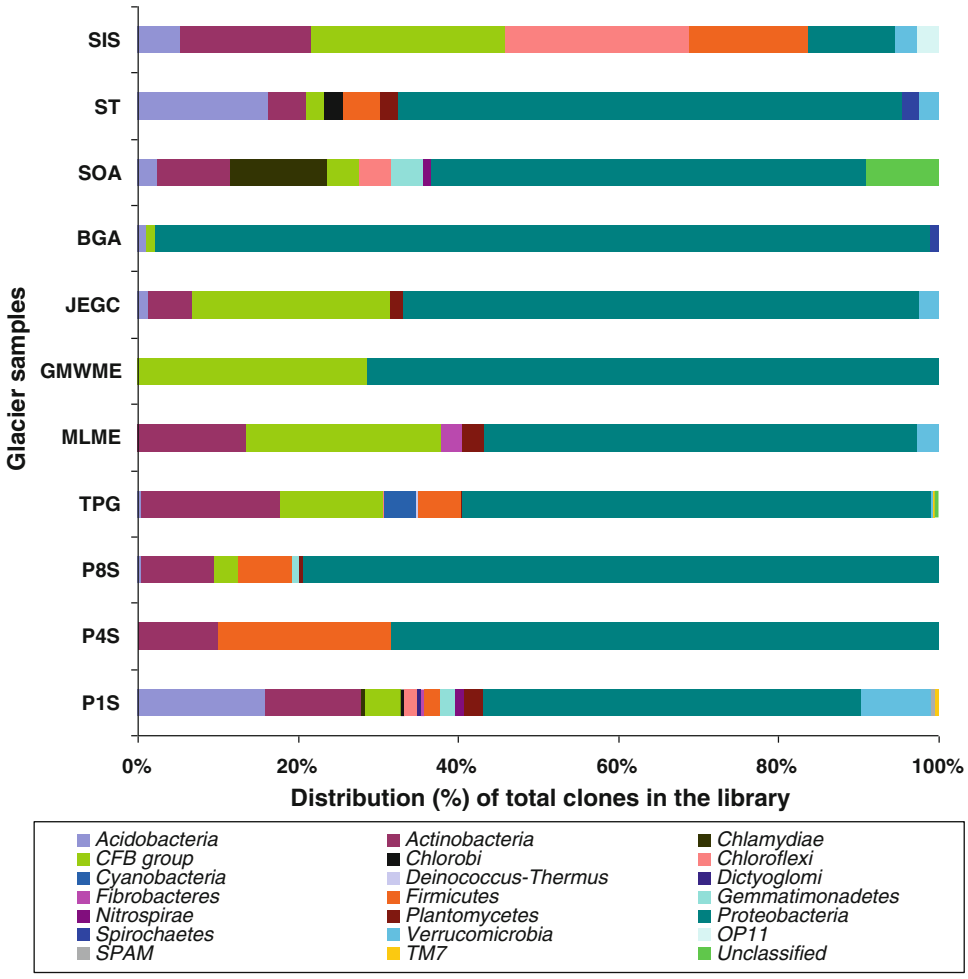


Fig. 2 Comparison of the bacterial diversity in different cold habitats as determined by 16S rRNA gene clone libraries. P1S, soil sample, Pindari glacier, India (present study); P4S, soil sample, Pindari glacier, India (present study); P8S, soil sample, Pindari glacier, India (present study); *TPG* Tibetan Plateau glaciers (Liu et al. 2009), *MLME* Moraine lakes, Mount Everest (Liu et al. 2006); *GMWME* glacial meltwater, Mount Everest (Liu et al. 2006); *JEGC* John Evans glacier, Canada (Cheng and Foght 2007); *BGA* Bench glacier, Alaska (Skidmore et al. 2005); *SOA* soil sample, Schirmacher Oasis, Antarctica; *ST* soil sample, Siberian tundra; *SIS* Samoylov Island, Siberia (Wagner et al. 2009). *CFB Cytophaga–Flavobacterium–Bacteroides.* Values represent the percentage of clones. Data from the present study are compared with that of Liu et al. (2006), (2009), Cheng and Foght (2007), Skidmore et al. (2005), Shivaji et al. (2004), Zhou et al. (1997), Wagner et al. (2009)

Results

Bacterial abundance and the physicochemical characteristics of the soil samples collected near Pindari glacier

Three soil samples (P1S, P4S and P8S) were collected from three different sites near the Pindari glacier at an altitude of 3,200, 3,430 and 3,490 m, respectively. The pH ranged from 7.1 to 7.9 and the water content varied from 18 to 35% (Table 1). The soil texture and chemical characteristics (elemental) like Ni, Cu, Rb, In, Cd, Pb, Zn, Cr, As, Fe, Be, Al, Li, Cs, Mn, Bi, U, V, Se, Sr and Ga were similar in P1S and P8S but differed considerably with P4S (Table 1). The total bacterial count in the soil samples (P1S, P4S and P8S) varied from 2.2 to 8.7 $\times$ 10^8 bacteria g^{-1} of soil.

Table 2 Comparison of the three 16S rRNA gene libraries (P1L, P4L and P8L) constructed using three soil samples (P1S, P4S and P8) collected near the Pindari glacier, Himalayas, India, based on BLAST analysis of the 16S rRNA gene sequences

Phylum/class	P1L		P4L		P8L	
	Number of clones	Library (%)	Number of clones	Library (%)	Number of clones	Library (%)
Phylum-*Proteobacteria*	**98**	**47.2**	**204**	**68.4**	**157**	**79.3**
Class-*Alphaproteobacteria*	32	15.4	1	0.3	10	5.1
Class-*Betaproteobacteria*	31	14.9	195	65.4	18	9.1
Class-*Gammaproteobacteria*	24	11.6	8	2.7	128	64.6
Class-*Deltaproteobacteria*	11	5.3	0	0	1	0.5
Phylum-*Acidobacteria*	**33**	**15.9**	**0**	**0**	**1**	**0.5**
Class-*Acidobacteria*	25	11.1	0	0	1	0.5
Class-*Holophagae*	1	0.5	**0**	**0**	0	0
Class-*Soilbacteres*	7	3.9	0	0	**0**	**0**
Phylum-*Actinobacteria*	**25**	**12.1**	**30**	**10.1**	**18**	**9.1**
Class-*Actinobacteria*	25	12.1	30	10.1	18	9.1
Phylum-*Firmicutes*	**4**	**1.9**	**64**	**21.5**	**13**	**6.6**
Class-*Bacilli*	3	1.4	64	21.5	9	4.6
Class-*Clostridia*	1	0.5	0	0	4	2.0
Phylum-*Bacteroidetes*	**9**	**4.4**	**0**	**0**	**6**	**3.0**
Class-*Flavobacteria*	6	2.9	0	0	0	0
Class-*Sphingobacteria*	3	1.5	**0**	**0**	6	3.0
Phylum-*Chlamydiae*	**1**	**0.5**	0	0	0	0
Class-*Chlamydiae*	1	0.5	**0**	**0**	**0**	**0**
Phylum-*Chlorobi*	**1**	**0.5**	0	0	0	0
Class-*Ignavibacteria*	1	0.5	**0**	**0**	**0**	**0**
Phylum-*Chloroflexi*	**3**	**1.5**	0	0	0	0
Class-*Caldilineae*	3	1.5	**0**	**0**	**0**	**0**
Phylum-*Dictyoglomi*	**1**	**0.5**	0	0	0	0
Class-*Dictyoglomia*	1	0.5	**0**	**0**	**0**	**0**
Phylum-*Fibrobacteres*	**1**	**0.5**	0	0	0	0
Class-*Fibrobacteres*	1	0.5	**0**	**0**	**0**	**0**
Phylum-*Gemmatimonadetes*	**4**	**1.9**	0	0	**2**	**1.0**
Class-*Gemmatimonadetes*	4	1.9	**0**	**0**	2	1.0
Phylum-*Nitrospirae*	**2**	**1.0**	0	0	0	0
Class-*Nitrospira*	2	1.0	**0**	**0**	**0**	**0**
Phylum-*Planctomycetes*	**5**	**2.4**	0	0	**1**	**0.5**
Class-*Planctomycetacia*	5	2.4	**0**	**0**	1	0.5
Phylum-*Verrucomicrobia*	**18**	**8.7**	0	0	0	0
Class-*Verrucomicrobiae*	18	8.7	**0**	**0**	**0**	**0**
Candidate division SPAM	**1**	**0.5**	**0**	**0**	**0**	**0**
Candidate phylum TM7	**1**	**0.5**	0	0	0	0
Total	**207**	**100**	**298**	**100**	**198**	**100**

The bold values represent the number of clones in each phylum and the number of clones in each library

Culture-independent bacterial diversity from soil samples collected near Pindari glacier

16S rRNA gene clone libraries

The soil samples (P1S, P4S and P8S) yielded about 40–70 μg DNA g^{-1} of soil. About 200 ng of the DNA was used for the construction of a 16S rRNA gene library. The libraries constructed using P1S, P4S and P8S were designated P1L, P4L and P8L, respectively and consisted of 207, 298 and 198 clones, respectively with an insert size of approximately 1 kb, respectively. BLAST sequence similarity analysis of the three clone libraries indicated that clones in P1L, P4L and P8L were affiliated to 16 (14 phyla plus candidate division spam and candidate phylum TM7), 3 and 7 phyla, respectively. Clones belonging to three phyla *Actinobacteria*, *Firmicutes* and *Proteobacteria* were common to all the three libraries. Phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes, Gemmatimonadetes*, *Planctomycetes* and *Proteobacteria* were present both in P1L and P8L and the phyla *Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Dictyoglomi*, *Fibrobacteres*, *Nitrospirae*, *Verrucomicrobia*, candidate division SPAM and candidate TM7s TM7a candidate phylum were present only in P1L (Fig. 2; Table 2; Supplementary Table 1).

Frequency of distribution of the clones indicated that in the three libraries (P1L, P4L and P8L) clones belonging to phyla *Acidobacteria* (15.9, 0 and 0.5%), *Actinobacteria* (12.1, 10.1 and 9.1%), *Firmicutes* (1.9, 21.5 and 6.6%) and *Proteobacteria* (47.2, 68.4 and 79.3) were the most predominant (representing >10%) of the total clones (Table 2; Supplementary Table 1). The affiliation of each and every clone to the nearest phylogenetic neighbour based on the BLAST analyses of the 16S rRNA gene sequence is also presented in Supplementary Table 1.

Proteobacteria

In the 16S rRNA gene clone libraries (P1L, P4L and P8L) (Table 2; Supplementary Table 1), clones affiliated to the three classes of the phylum *Proteobacteria*, namely *Alpha-*, *Beta-* and *Gamaproteobacteria* were present (Table 2; Supplementary Table 1). *Deltaproteobaceria* was absent in P4L. In the P1L library 58 out of the 98 clones which belonged to the phylum *Proteobacteria* clustered with their nearest phylogenetic neighbour (Fig. 3a) and 36 clones clustered within the same class. The remaining four clone sequences P1s-297, P1s-53, P1s-302 and P1s-136 formed a cluster within the phylum *Proteobacteria* but in a different class. For instance P1s-297 and P1s-53, showed 88 and 86% pairwise sequence similarity with *Rhodovibrio salinarum* and *Roseomonas vinacea* (*Alphaproteobacteria*) but clustered with the deltaproteobacterial sequences; P1s-302 showed 88% with *Polaromonas aquatica* (*Betaproteobacteria*) but clustered as an out group to the gammaproteobacterial clade and P1s-136 showed 91% with *Dyella marensis* (*Gammaproteobacteria*) but clustered with the betaproteobacterial sequences (Fig. 3a). In the P4L and P8L libraries all the clones affiliated to phylum *Proteobacteria* clustered with their related sequences. But four clone sequences P4s-2, P4s-144, P4s-238 and P4s-321 in P4L and three clone sequences P8s-131, P8s-319 and P8s-108 in P8L clustered separately from their nearest phylogenetic neighbours (Figs. 4a, 5a). In Fig. 5a only 32 sequences out of the 157 clone sequences were considered (based on 97% cut off) for phylogenetic analysis.

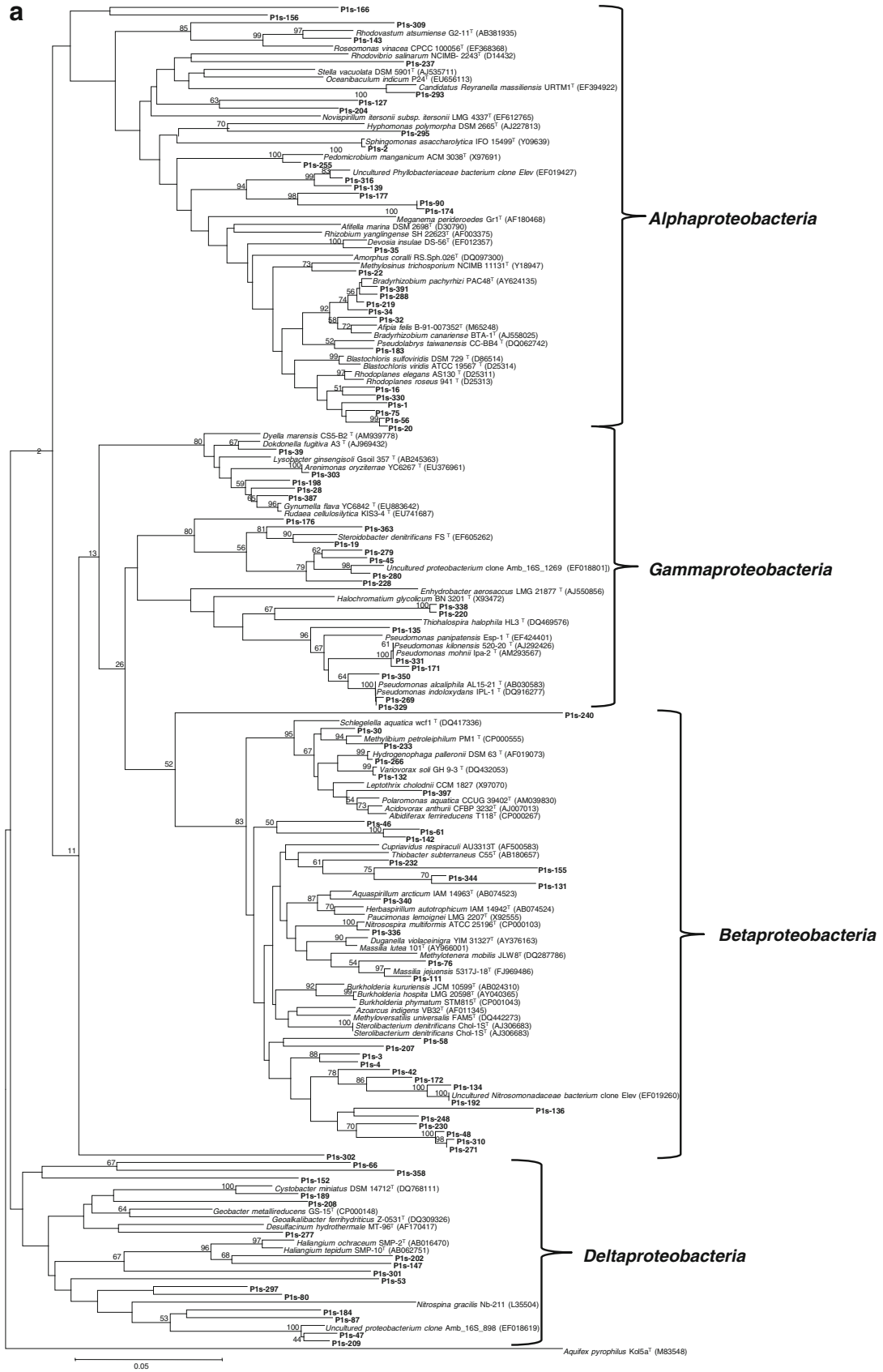
Fig. 3 Neighbour Joining phylogenetic tree of 16S rRNA gene clones from P1L library, showing the phylogenetic relationship of clones affiliated to *Proteobacteria* (**a**) and clones affiliated to phyla *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Chlamydiae*, *Chlorobia*, *Chloroflexi*, *Dictyoglomi*, *Fibrobacteres*, *Gemmatimondetes*, *Nitrospirae, Planctomycetes*, SPAM (candidate division), TM7_s TM7a (candidate phylum) and *Verrucomicrobia* (**b**). In **b** the following clones have been compressed P1s-262, P1s-146, P1s-165, P1s-191, P1s-211, P1s-133, P1s-299, P1s-178, P1s-145, P1s-247, P1s-94, P1s-43, P1s-157, P1s-359 and P1s-33 as Clade-1; P1s-67, P1s-216 and 335 as Clade-2; and P1s-37, P1s-158, P1s-185 and P1s-339 as Clade-3. 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

Actinobacteria

Clones affiliated to *Actinobacteria* were present in libraries P1L, P4L and P8L (25, 30 and 18 clones) and they shared a 16S rRNA gene sequence similarity of 84–100% with the nearest phylogenetic neighbour (Table 2; Supplementary Table 1). All the clone sequences of the three libraries clustered with the related sequences except P4s-243, which appeared as an out group to the *Actinobacteria* clade (Fig. 4b). Further P8s-99, P8s-235 and P8s-341 formed a separate cluster and branched from the *Acidothermus*, *Frankia* and *Pseudonocardia* cluster; whereas P8s-227 and P8s-129 clustered together and branched from the *Clostridia* clade (Fig. 5b). Two clone sequences P4s-79 and P4s-102 appeared to be phylogenetically affiliated to *Cryobacterium roopkundense* RuGl_7^{T} (which was earlier isolated by us from the same habitat (Vardhan Reddy et al. 2009) (Fig. 4b).

Firmicutes

Representatives of clones affiliated to phylum *Firmicutes* were common to all the three libraries (Table 2; Supplementary Table 1). All clone sequences related to the class *Bacilli* clustered with their nearest phylogenetic neighbour except P1s-242, P4s-227 and P4s-199 (Figs. 3b, 4b, 5b).


a
Alphaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Deltaproteobacteria
0.05

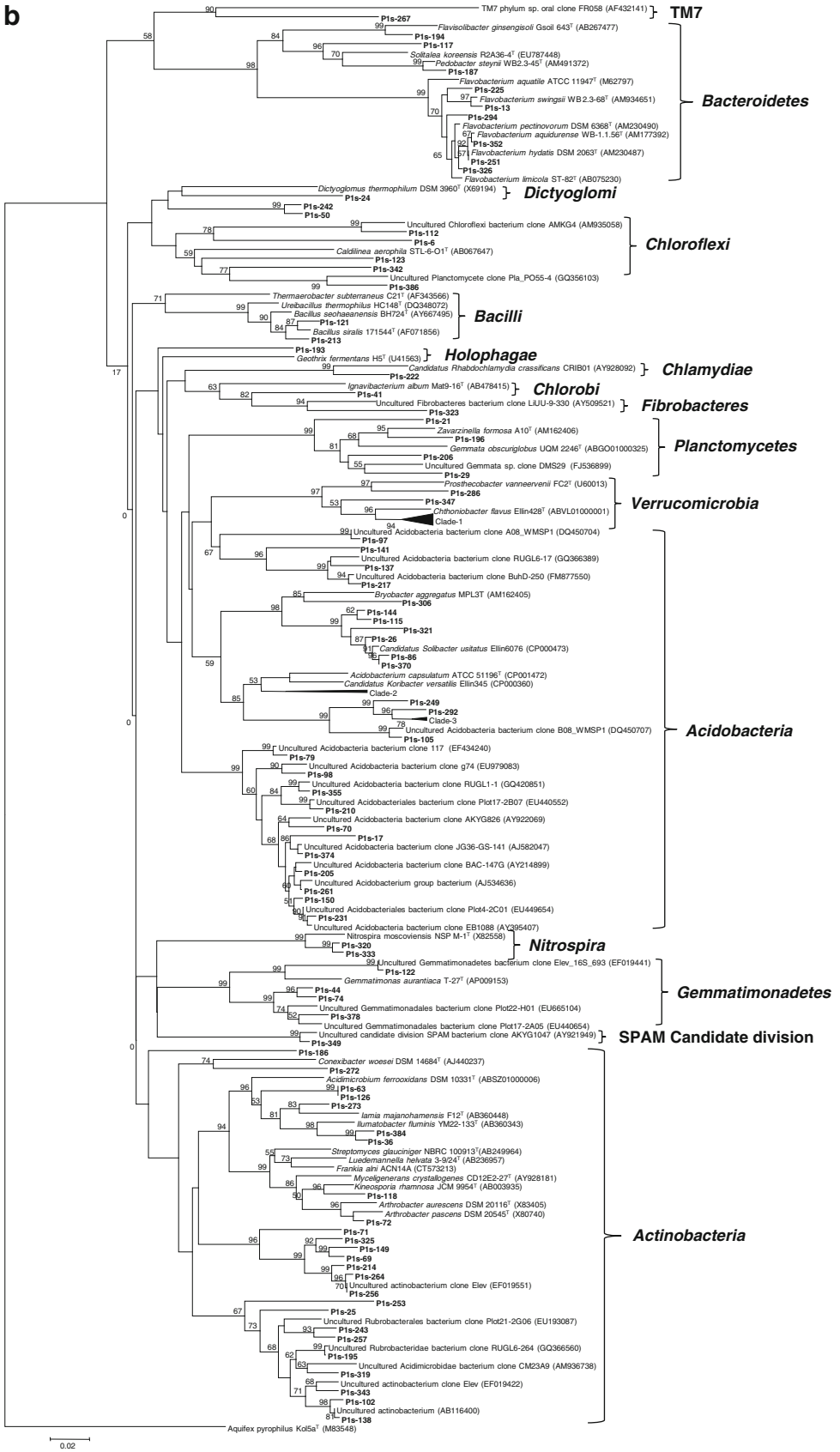


Fig. 3 continued

But the clones related to *Clostridia* clustered with the related sequences (Figs. 3b, 5b). Clones related to the class *Clostridia* were absent in P4L library.

Acidobacteria

In P1L phylum *Acidobacteria* was represented by 15.9% of the total clones in the library and the clones were affiliated to five genera *Acidobacterium*, *Bryobacterium*, *Geothrix*, *Koribacter* and *Solibacter* and the remaining 15 clones were related to unculturable acidobacterial clones (Supplementary Table 1). Majority of the clone sequences clustered with related sequences in the phylogenetic tree except P1s-249 and P1s-292 which clustered with clade-3 of *Koribacter versatilis* and P1s-141 which appeared to be affiliated to unculturable acidobacterial sequences (Fig. 3b). The clones related to the class *Acidobacteria* of the P1L library formed four branches (Fig. 3b). The single clone which represents the phylum *Acidobacteria* in P8L library showed 97% pairwise sequence similarity with *Edaphobacter modestus* and clustered with the same (Fig. 5b).

Bacteroidetes, Gemmatimonadetes and Planctomycetes

Clones similar to the phylum *Bacteroidetes* constituted 4.4 and 3.0% of the total clones in P1L and P8L libraries, respectively (Table 2; Supplementary Table 1) and all the clone sequences clustered in the *Bacteroidetes* clade which appeared to be polyphyletic (Figs. 3b, 5b). Clones affiliated to *Gemmatimonadetes* (4 and 2 clones in P1L and P8L libraries, respectively) and *Planctomycetes* (5 and 1 clones in P1L and P8L libraries) formed a cluster with the nearest phylogenetic neighbours (Figs. 3b, 5b).

Chloroflexi, Chlorobi, Chlamydiae, Dictyoglomi, Fibrobacteres, Gemmatimonadetes, Verrucomicrobia, Nitrospirae and Planctomycetes, Candidate division SPAM and candidate phylum TM7_s TM7a

Clones affiliated to the above phyla were specific only to P1L and appeared at a very low frequency. (Table 2; Supplementary Table 1). Phylogenetic analysis indicated that clones affiliated to the above phyla clustered with their respective phylogenetic neighbours (Fig. 3b) except P1s-6 which clustered within the phylum *Chloroflexi* (Fig. 3b).

Statistical analysis

The 16S rRNA gene clones from the three soil samples (P1S, P4S and P8S) based on 16S rRNA gene sequence similarity criteria of >97% could be categorised into 158, 44 and 57 phylotypes (Fig. 6; Supplementary Table 1). Interestingly, none of the phylotypes were common to all the three soil samples (Supplementary Table 1). The common phylotypes which are present in P1L and P8L libraries are *Frankia alni*, *Gemmatimonas aurantiaca*, *Haliangium tepidum*, *Kineosporia rhamnosa*, *Methylibium petroleiphilum*, *Pseudomonas alcaliphila*, *Pseudomonas indoloxydans* and *Steroidobacter denitrificans* (Supplementary Table 1). Species richness varied from 44 to 158 and the diversity coverage ranged from 23.7 to 85.2% (Table 3). The rarefaction curves indicated that the bacterial populations in P4S and P8S soil samples plateaued, whereas in P1S soil sample, it did not (Fig. 6). 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 P1S and P8S were closely associated in the ordination diagram based on the first two principal components (Fig. 7). It was also evident from the clone library sequences that samples P1S and P8S sites clustered together at all three cut off values of (97, 90 and 80%) (Fig. 7a–c), whereas the P4S soil sample differed from the other two samples.

The result of PCA based on percentage of a specific OTU (Phyla with ≥1% clones were considered) in the three libraries (P1L, P4L and P8L) is shown in Fig. 8a, and it appears that the principal component factors 1 and 2 (PC1, 82.457%; PC2, 17.543%) explain 100% of the total variances. From the PCA plot considerable differences in the distribution of phyla in the three soil samples has been observed. The phylum *Firmicutes* which is dominant in P4L library and the phylum *Proteobacteria* which is dominant in both P4L and P8L libraries were grouped differently and were separated from the other phyla which are present either in P1L and P8L libraries but dominant in P1L library. The result of PCA based on the biogeochemical properties is shown in Fig. 8b and the principal component factors 1 and 2 (PC1, 83.318%; PC2, 11.682%) explained 95% of the total variances.

The PCA plot indicates that arsenic content and pH which are higher in the P4S soil sample grouped closely (Fig. 8b; Table 1). Further, when all the other parameters were checked it was observed that Cr and altitude were separated from the remaining parameters (Fig. 8b). Thus, it may imply that altitude, pH, As, and Cr are the key parameters that influence the observed differences in the percentage of specific OTUs in the three 16S rRNA gene clone libraries. The factors which are numerically high in P1S like Pb, Zn, Cd, TBC, In, Cu, Rb, Ni, Ga, Sr and

water content (Table 1) grouped closely on the right side of the PCA plot. P1S also has the highest bacterial content and this may be influenced by water as indicated by the PCA grouping. Further, factors which were high in P8S like Be, Fe, Li, Co, Al, Cs, Mn, Bi, U, V and Se (Table 1) grouped closely on the top left side of the PCA plot (Fig. 8b). Thus, PCA does discriminate the three soil samples.

Culture-dependent bacterial diversity from soil samples collected near Pindari glacier

Characterisation of the bacterial strains isolated from the soil samples collected near Pindari glacier

Only a total of 40 bacterial strains were isolated from the 3 soil samples which were subsequently grouped into 20 morphotypes based on their colony morphology (Table 4). All the 20 isolates were psychrotolerant and could grow between 4 and 30°C or 4–37°C (Table 4). All the strains could grow without NaCl in the medium. The tolerance to NaCl varied from 0.5 to 2.0 M (Table 4). All strains could grow either in the pH range of 6–11 or 7–11. Thus, all the isolates are psychro-, halo- and alkalitolerant. Amylase and urease activities were detected in the majority of the strains (19 and 18, respectively) at 4 and 20°C (Table 4) but lipase and protease activities were not detected at 4 and 20°C. Out of 20 strains 13 and 11 strains showed both amylase and urease activities at 4°C.

Fatty acid profiles of the isolated strains from soil samples collected near Pindari glacier

In the present study, long chain fatty acids (C_{13}–C_{20} fatty acids) were observed in all strains compared to short chain fatty acids (C_6–C_{12} fatty acids) (Supplementary Table 2). The results also clearly indicated that in the psychrotolerant bacteria iso-, anteiso-, methylated-, cyclo-, hydroxyl- and unsaturated fatty acids together constituted a significant proportion of the total fatty acid composition varying from a minimum of 0.5% to a maximum of 86.8%. The composition of fatty acids in all the strains varied considerably with iso-fatty acids ranging from 3.9 to 63.1%, antesio-fatty acids from 1.2 to 51.8%, hydroxyl-fatty acids from 0.7 to 30.1%, saturated fatty acids from 40.4 to 86.8% and unsaturated fatty acids from 11.9 to 59.6%. The methyl- and cyclo-fatty acids were present only in 6 and 9 strains ranging from 0.5 to 11.6 and 0.9–21.5%, respectively. Out of 20 strains 6 strains contained 3 different polyunsaturated fatty acids (C18:3 ω6, 9, 12c; C20:2 ω6, 9c; C20:4 ω6, 9, 12, 15c) with composition varying from 1.9 to 11.1, 1.7–17.1 and 0.1–10.3%, respectively.

Fig. 4 Neighbour Joining phylogenetic tree of 16S rRNA gene clones from P4L library, showing the phylogenetic relationship of clones affiliated to *Proteobacteria* (**a**) and clones affiliated to *Actinobacteria* and *Firmicutes* (**b**). In **a** the following clones have been compressed P4s-221, P4s-229, P4s-175, P4s-1, P4s-223, P4s-379, P4s-135, P4s-258, P4s-66, P4s-247, P4s-349, P4s-286, P4s-21, P4s-192, P4s-129, P4s-355, P4s-307, P4s-353, P4s-340, P4s-184, P4s-5, P4s-138, P4s-293, P4s-282, P4s-3, P4s-357, P4s-173, P4s-91, P4s-120, P4s-202, P4s-157, P4s-151, P4s-305, P4s-22, P4s-59, P4s-105, P4s-78, P4s-123, P4s-76, P4s-44, P4s-133, P4s-96, P4s-304, P4s-94, P4s-30, P4s-87, P4s-106, P4s-167, P4s-347, P4s-56, P4s-245, P4s-80, P4s-228, P4s-334, P4s-148, P4s-24, P4s-203, P4s-48, P4s-141, P4s-18, P4s-136, P4s-325, P4s-109, P4s-108, P4s-295, P4s-236, P4s-371, P4s-55, P4s-170, P4s-140, P4s-147, P4s-52, AM747630, P4s-95, P4s-97, P4s-177, P4s-185, P4s-336, P4s-381, P4s-257, P4s-279, P4s-125, P4s-367, P4s-169, P4s-122, P4s-71, P4s-9, P4s-217, P4s-292, P4s-239, P4s-276, P4s-183, P4s-174, P4s-230, P4s-41, P4s-240, P4s-50, P4s-7, P4s-92, P4s-142, P4s-100, P4s-196, P4s-73, P4s-287, P4s-235, P4s-372, P4s-255, P4s-46, P4s-248, P4s-256, P4s-67, P4s-139, P4s-274, P4s-353, P4s-265, P4s-273, P4s-266, P4s-219, P4s-209, P4s-374, P4s-291, P4s-315, P4s-341, P4s-354, P4s-222, P4s-333, P4s-205, P4s-53, P4s-262, P4s-193, P4s-160, P4s-40, P4s-290, P4s-131, P4s-300, P4s-358, P4s-288, P4s-153, P4s-74, P4s-107, P4s-316, P4s-303, P4s-158, P4s-12, P4s-110, P4s-198, P4s-285, P4s-42, P4s-337, P4s-156, P4s-43, P4s-85, P4s-377, P4s-47, P4s-132, P4s-249, P4s-241, P4s-280, P4s-180, P4s-179, P4s-137, P4s-329, P4s-332, P4s-64, P4s-143, P4s-23, P4s-218, P4s-19, P4s-188, P4s-10, P4s-189, AM747632, P4s-39, P4s-93, P4s-277, P4s-86, P4s-261, AM747631, P4s-317, P4s-124, P4s-208, P4s-260, P4s-270, P4s-45, P4s-8, P4s-6, P4s-206, P4s-346, P4s-284, P4s-35, P4s-31 and P4s-298 as Clade-1. In **b** the following clones have been compressed P4s-145, P4s-103, P4s-152, P4s-234 and P4s-384 as Clade-1; P4s-281, P4s-299, P4s-126, P4s-373, P4s-343, P4s-88 and P4s-224 as Clade-2; P4s-350, P4s-331, P4s-155, P4s-313, P4s-319, P4s-226, P4s-62, P4s-162, P4s-231, P4s-150, P4s-212, P4s-82, P4s-70, P4s-176, EU815300, P4s-348 and P4s-164 as Clade-3; P4s-89, P4s-242, P4s-117, P4s-104, P4s-309, P4s-200, L37605 and P4s-15 as Clade-4; P4s-163, P4s-339, P4s-264, P4s-271, P4s-16, P4s-161, P4s-172, P4s-335, AF061009, P4s-149, P4s-376, P4s-324, P4s-232, P4s-330, P4s-178, P4s-181, P4s-58, P4s-28, P4s-342 and P4s-302 as Clade-5. *Numbers at nodes* are bootstrap values. The *bar* represents 0.05 substitutions per alignment position

Identification of bacterial strains isolated from soil samples collected near Pindari glacier

Taxonomic analysis of all the 20 strains of different morphotype, isolated from soil samples near Pindari glacier indicated that 3 strains were Gram-negative and 17 were Gram-positive. BLAST sequence similarity search based on 16S rRNA gene sequence of the strains indicated that four strains belonged to the genus *Arthrobacter*, three strains each belonged to the genera *Bacillus* and *Sporosarcina*, two strains belonged to the genus *Rhodococcus* and one strain each belonged to the genera, *Advenella*, *Brachybacterium*, *Flavobacterium*, *Lysinibacillus*, *Microterricola*, *Paenisporosarcina*, *Pseudomonas* and *Viridibacillus*, respectively (Table 5). Phylogenetic analysis based on 16S rRNA gene sequences confirmed the affiliation of 19 out of the 20 isolates with their nearest phylogenetic neighbour (Fig. 9;

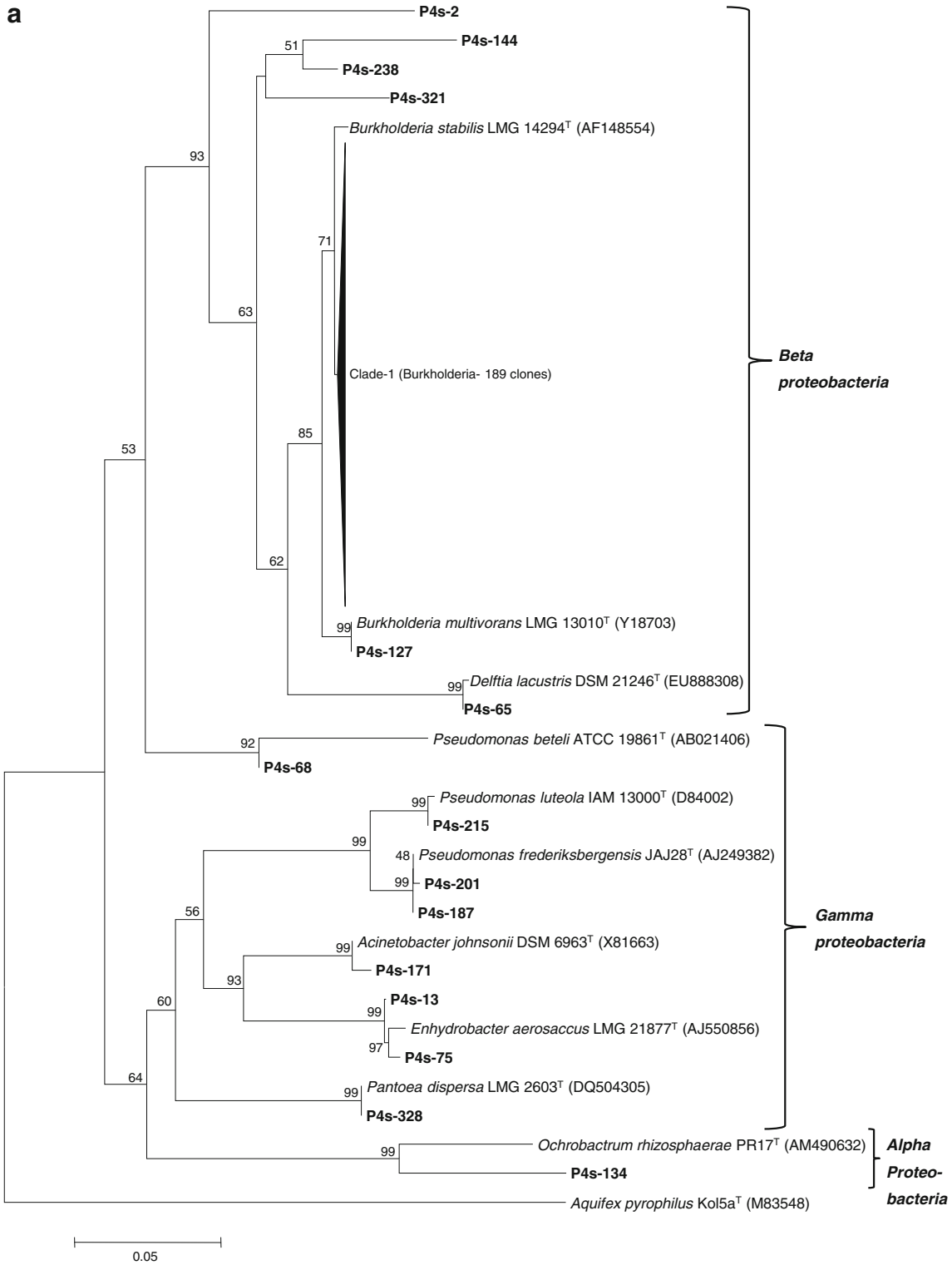


Table 5). The only strain that did not cluster with the nearest phylogenetic neighbour was strain PA-8 which formed a clade with *Lysinibacillus xylanilyticus* instead of *Lysinibacillus fusiformis* (Fig. 9; Table 5). Many of the strains were closely related to one another and exhibited more than 99% sequence similarity at the 16S rRNA gene

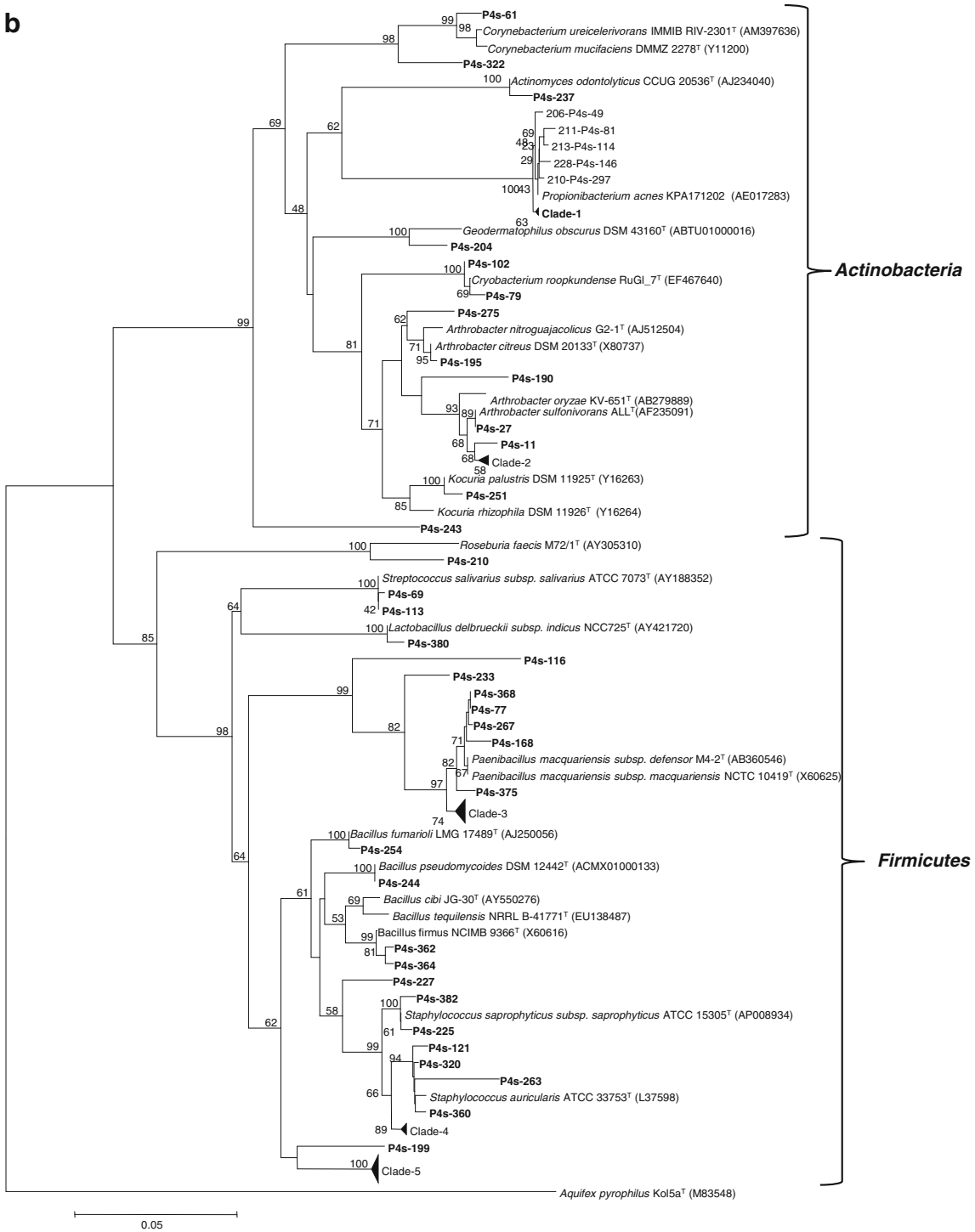


Fig. 4 continued

sequence level like PN-8 and PN-11 which are closely related to one another (100%) and are affiliated to *Rhodococcus qingshengii* djl-6^T (99.9 and 100%, respectively) and PA-3 and PON-4 which are closely related to one another (100%) and are affiliated to *Sporosarcina aquimarina* SW28^T (99.2 and 99%, respectively) (Fig. 9).

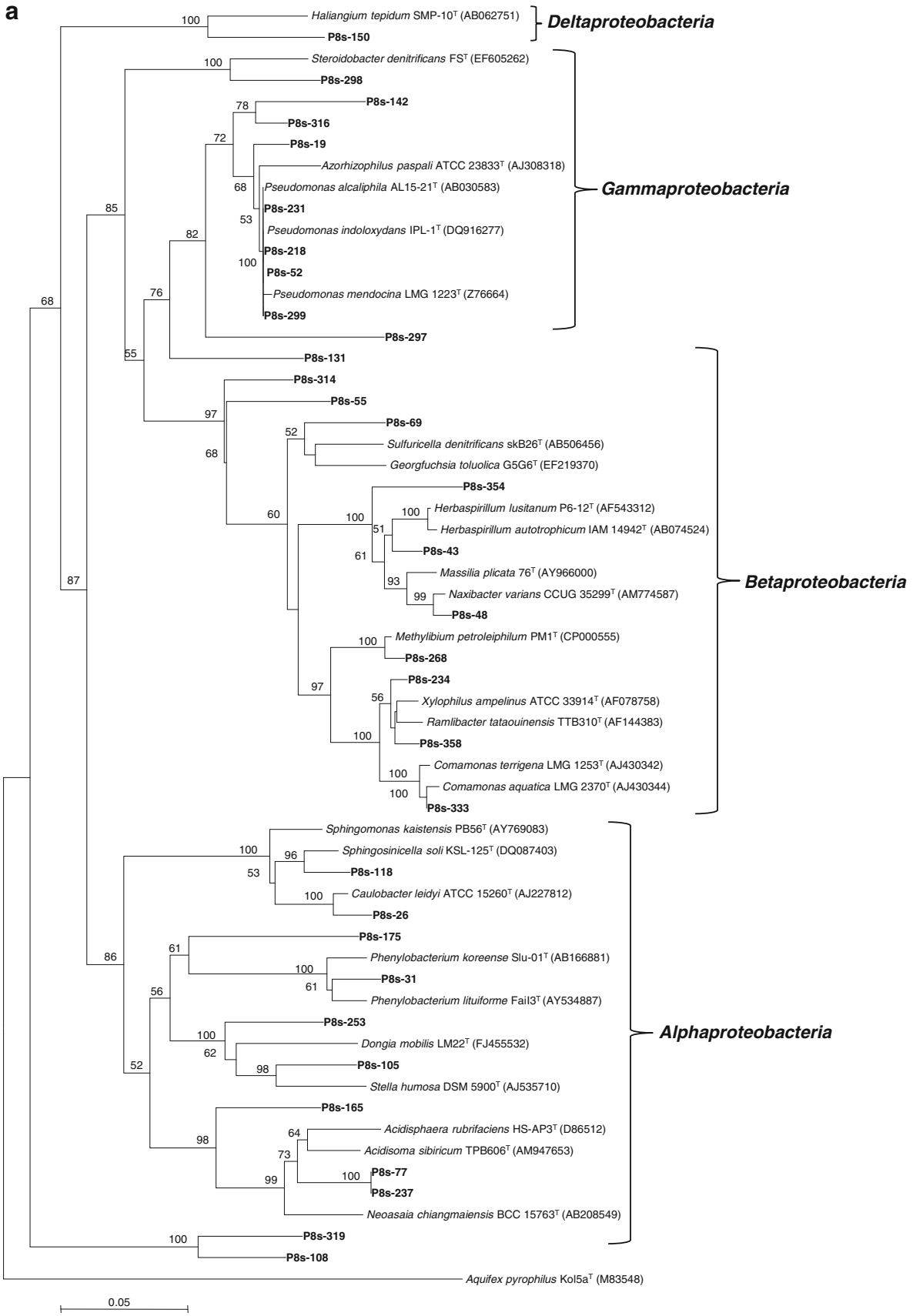
Discussion

In the present study, bacterial diversity was studied using two soil samples P1S and P4S collected from the edge of two huge ice blocks located 3 and 4 km away from the Pindari glacier. These ice blocks may have found their way

downhill from the glacier due to an avalanche which is not very uncommon in the Himalayas. In fact our trek to the Pindari glacier was abandoned since by the time we reached the site where we collected P1S we experienced heavy rain fall followed by huge boulders of stone and large blocks of ice coming down from the Pindari glacier warning us about the likelihood of an avalanche. The diversity of these two samples was compared with P8S, a soil sample that was collected from an ice-free region 8 km from the glacier near the Pindari stream. The results indicated a low bacterial count and decreased species richness in the P4S soil sample compared to the other soils (P1S, P8S).

Comparison of the bacterial diversity observed in the present study with that of other Himalayan cold habitats like the moraine lakes and glacial meltwaters of Mount Everest (Liu et al. 2006), snow over Tibetan plateau glacier (Liu et al. 2009), Malan ice core from the Tibetan plateau (Xiang et al. 2004), ice core from the Muztag Ata glacier (Xiang et al. 2005), solid ice, firn, and snow, at the terminus, middle, and top of the Kuytun 51 glacier (Xiang et al. 2009), soil samples from the western Himalayas (Gangwar et al. 2009), Puruogangri ice (Zhang et al. 2006, 2008) and soil from Roopkund lake and glacier (Pradhan et al. 2010) indicated that the diversity of bacteria in the three soil samples near Pindari glacier are comparable with that reported from the above cold habitats (Fig. 2) with respect to the presence of *Actinobacteria, Proteobacteria* and *Acidobacteria*. Further clones belonging to these three phyla have also been reported from Tibetan plateau glacier, China; John Evans glacier, Canada; Bench glacier, Alaska; Schirmacher Oasis soil, Antarctica and Siberian tundra soil samples (Fig. 2). The occurrence of identical phyla in geographically diverse cold environments is indicative of the ability of microorganisms to adapt to similar strategies to survive freezing and remain active at low temperatures (Abyzov et al. 1998; Priscu and Christner 2003).

In the present study clones belonging to phyla *Proteobacteria*, *Actinobacteria* and *Firmicutes* were common to P1L, P4L and P8L (Fig. 2; Table 2; Supplementary Table 1). Phylogenetic analysis of the clones affiliated to phylum *Firmicutes* indicated a distinct difference between the clones in the P1L and P8L libraries compared to P4L and the only genus common to the three libraries was *Bacillus* (Supplementary Table 1). As of now, only one new species of *Bacillus* has been described from a Himalayan glacier (Reddy et al. 2008b). *Bacillus* strains were also reported earlier from Guliya and Vostok (Antarctica) ice core samples (Christner et al. 2001, 2003). In addition to phyla *Proteobacteria*, *Actinobacteria* and *Firmicutes*, clones belonging to many other phyla such as *Verrucomicrobia, Chlamydiae*, *Chlorobi*, *Chloroflexi*, *Dictyoglomi*, *Fibrobacteres*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, candidate division SPAM and candidate phylum TM7s TM7a were present in one or two libraries but the frequency of distribution was low (0.5–2.4%; Table 2). These observations are in agreement with earlier studies which had also indicated that clones affiliated to *Verrucomicrobia* (Cheng and Foght 2007; Liu et al. 2006, 2009; Pradhan et al. 2010; Wagner et al. 2009; Zhou et al. 1997), *Chlamydiae* (Shivaji et al. 2004), *Chlorobi* (Zhou et al. 1997; Pradhan et al. 2010), *Chloroflexi* (Costello and Schmidt 2006; Li et al. 2008; Liu et al. 2006, 2009; Lysnes et al. 2004; Pradhan et al. 2010; Reed et al. 2006; Shivaji et al. 2004; Wagner et al. 2009), *Fibrobacteres* (Liu et al. 2006), *Gemmatimonadetes* (Pradhan et al. 2010; Shivaji et al. 2004; Steven et al. 2007), *Nitrospirae* (Pradhan et al. 2010; Shivaji et al. 2004), *Planctomycetes* (Pradhan et al. 2010; Cheng and Foght 2007; Liu et al. 2006, 2009; Zhou et al. 1997) and candidate division SPAM (Lipson and Schmidt 2004) are present in other cold habitats (Fig. 2) at a low frequency. Phylum *Dictyoglomi* is represented only by a single clone in the P1L library and reports on the presence of this bacterium in cold habitats is lacking. Apart from all the above known phyla, a single clone in the P1L library was related to the candidate phylum TM7 (Marcy et al. 2007). This candidate phylum TM7 was reported earlier from a Tibetan plateau glacier (Liu et al. 2009) and Roopkund lake samples (Pradhan et al. 2010) but at a very low frequency (0.1–5.1%). Among the three libraries, bacterial biodiversity was very scarce in P4L and was represented by only *Proteobacteria*, *Actinobacteria* and *Firmicutes* (Fig. 2; Table 2; Supplementary Table 1). Limited diversity observed in P4L is in accordance with earlier studies by Xiang et al. (2004) and Liu et al. (2006) who had reported only *Proteobacteria* and *Cytophaga–Flavobacterium–Bacteroides* from Malan ice core from the Tibetan Plateau and glacial melt water from the Mount Everest, which are cold deserts like the present site, respectively. Earlier observations on Roopkund glacial soil also indicated that the predominant phyla were *Actinobacteria* and *Firmicutes* (Pradhan et al. 2010). Similarly, Skidmore et al. (2005) reported only four phyla *Acidobacteria*, *Cytophaga–Flavobacterium–Bacteroides*, *Proteobacteria* and *Spirochaetes* from the Bench glacier, Alaska. The observed difference in the bacterial biodiversity in P1L, P4L and P8L may also reflect the intrinsic changes observed in the physicochemical characteristics of the three samples. In fact the PCA analysis indicated that altitude, pH, As, and Cr are the key parameters that influence the observed diversity (Fig. 8). Therefore, the limited diversity in P4L compared to P1L and P8L could be attributed to the high pH, high As and low water content in P4L compared to the other two samples (Table 1). Factors such as pH (Eichorst et al. 2007) and water content (Treves et al. 2003) are known to influence microbial community composition and diversity (Zhang et al. 2008). Earlier


a
Haliangium tepidum SMP-10T (AB062751)
P8s-150
Deltaproteobacteria
Steroidobacter denitrificans FST (EF605262)
P8s-298
P8s-142
P8s-316
P8s-19
Azorhizophilus paspali ATCC 23833T (AJ308318)
Pseudomonas alcaliphila AL15-21T (AB030583)
P8s-231
Pseudomonas indoloxydans IPL-1T (DQ916277)
P8s-218
P8s-52
Pseudomonas mendocina LMG 1223T (Z76664)
P8s-299
Gammaproteobacteria
P8s-297
P8s-131
P8s-314
P8s-55
P8s-69
Sulfuricella denitrificans skB26T (AB506456)
Georgfuchsia toluolica G5G6T (EF219370)
P8s-354
Herbaspirillum lusitanum P6-12T (AF543312)
Herbaspirillum autotrophicum IAM 14942T (AB074524)
P8s-43
Massilia plicata 76T (AY966000)
Naxibacter varians CCUG 35299T (AM774587)
P8s-48
Betaproteobacteria
Methylibium petroleiphilum PM1T (CP000555)
P8s-268
P8s-234
Xylophilus ampelinus ATCC 33914T (AF078758)
Ramlibacter tataouinensis TTB310T (AF144383)
P8s-358
Comamonas terrigena LMG 1253T (AJ430342)
Comamonas aquatica LMG 2370T (AJ430344)
P8s-333
Sphingomonas kaistensis PB56T (AY769083)
Sphingosinicella soli KSL-125T (DQ087403)
P8s-118
Caulobacter leidyi ATCC 15260T (AJ227812)
P8s-26
P8s-175
Phenylobacterium koreense Slu-01T (AB166881)
P8s-31
Phenylobacterium lituiforme FaiI3T (AY534887)
P8s-253
Dongia mobilis LM22T (FJ455532)
P8s-105
Stella humosa DSM 5900T (AJ535710)
P8s-165
Acidisphaera rubrifaciens HS-AP3T (D86512)
Acidisoma sibiricum TPB606T (AM947653)
P8s-77
P8s-237
Neoasaia chiangmaiensis BCC 15763T (AB208549)
Alphaproteobacteria
P8s-319
P8s-108
Aquifex pyrophilus KoI5aT (M83548)
0.05

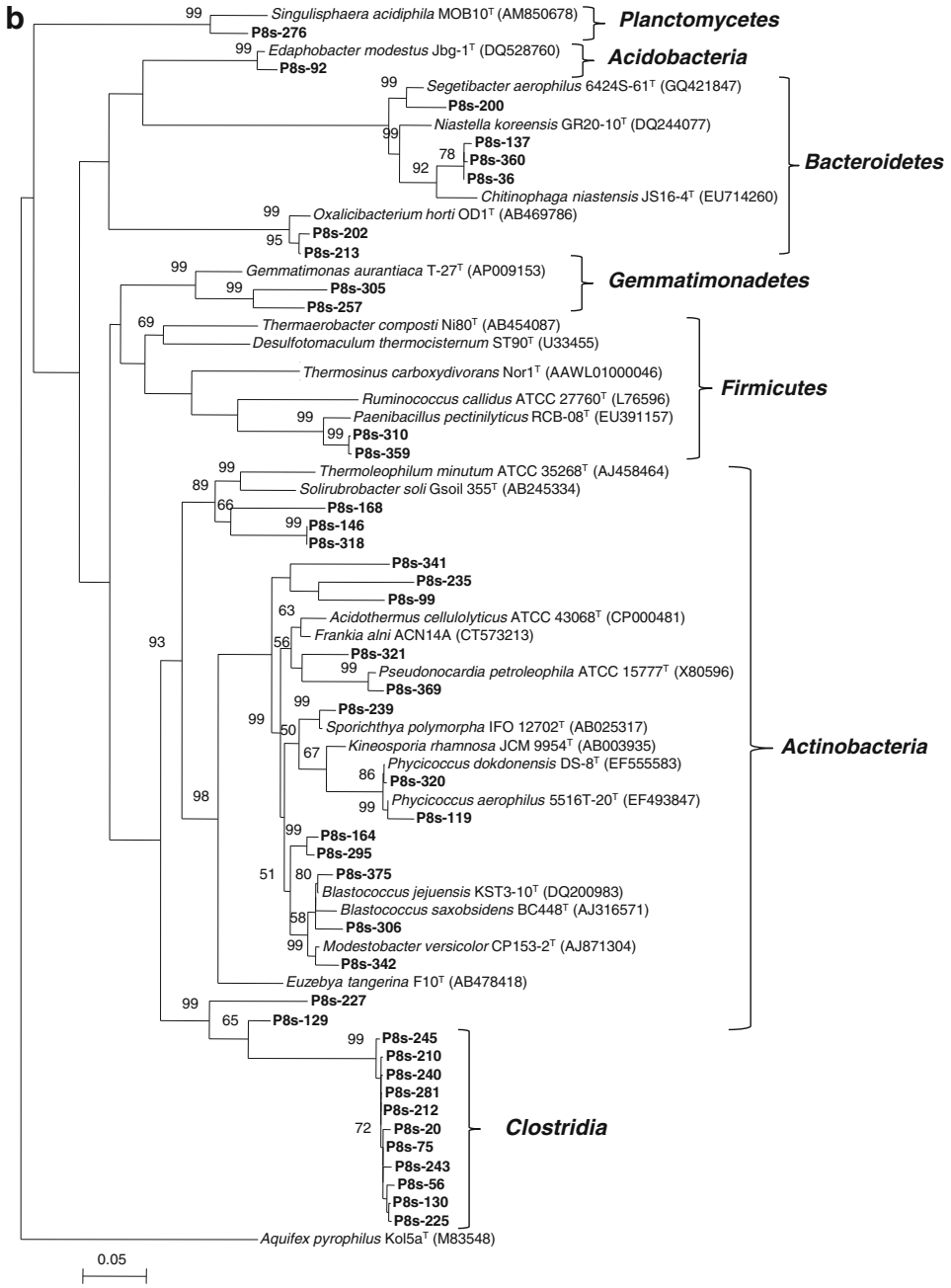


Fig. 5 continued

◀ **Fig. 5** Neighbour Joining phylogenetic tree of 16S rRNA gene clones from P8L library, showing the phylogenetic relationship of clones affiliated to *Proteobacteria* (**a**) and clones affiliated to *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes* and *Planctomycetes* (**b**). Out of 157 *Proteobacteria* related sequences 32 sequences were considered (based on 97% cut off) for phylogenetic analysis. *Numbers at nodes* are bootstrap values. The *bar* represents 0.05 substitutions per alignment position

studies have also indicated that coarse sediments are less favourable than fine sediments as a microbial substrate (Certini et al. 2004; Kastovská et al. 2005). This indeed may be the reason for the decreased diversity observed in the P4S which is coarse (sandy) compared to P1S and P8S soil samples which are fine (Table 1). An overall comparison indicated that the physical and chemical properties of the P4S soil sample and P1S and P8S 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). The total bacterial count in the soil samples collected near the Pindari glacier ranged from 2.2×10^8 to 8.7×10^8 cells/g of soil (Table 1) and was observed to be higher than

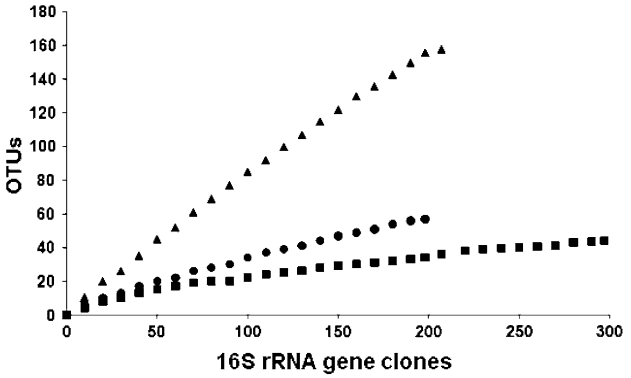


Fig. 6 Rarefaction curves of observed OTUs in the two soil samples collected in the vicinity of Pindari glacier (P1S and P4S) and a soil sample collected from the Pindari glacier (P8S). The symbols represent the rarefaction curves generated for the clone libraries from the three samples P1S (*filled triangle*), P4S (*filled square*) and P8S (*filled circle*), respectively

Table 3 Estimated numbers of phylotypes in the three 16S rRNA gene libraries (P1L, P4L and P8L) constructed using three soil samples (P1S, P4S and P8S) collected near the Pindari glacier, Himalayas, India, using different diversity indices

	P1L	P4L	P8L
Number of clones	207	298	198
Species richness (*S*)	158	44	57
Coverage (%)	23.7	85.2	71.2
Evenness	0.96	0.55	0.63
Shannon (H')	4.84	2.07	2.56
Simpson's (*D*)	0.99	0.69	0.81
Chao1	1,418.3	100.0	201.6
Diversity ratio	0.59	0.32	0.35

in most of the glaciers worldwide (Liu et al. 2009; Miteva et al. 2004; Pradhan et al. 2010; Skidmore et al. 2005; Xiang et al. 2006, 2009; Zhang et al. 2007). This could be attributed to the fact that Pindari glacier is located near human settlements and bacterial counts are known to be higher in nonpolar high altitude glaciers than in the polar glaciers, which are less influenced by anthropogenic activities (Zhang et al. 2007) with few exceptions like high biomass in the "silty" ice from the GISP 2 ice core (Miteva et al. 2004).

In addition when the biodiversity of the Pindari glacier soil samples was compared with that of other nonpolar cold habitats like alpine tundra wet meadow soil (Costello and Schmidt 2006; Zhou et al. 1997), surface snow (Segawa et al. 2005), alpine dry meadows soil (Lipson and Schmidt 2004), unvegetated deglaciated soil (Nemergut et al. 2007), periglacial soil and permafrost-affected soil (Wagner et al. 2009) it was observed that the present data is comparable in terms of bacterial diversity. *Betaproteobacteria* and

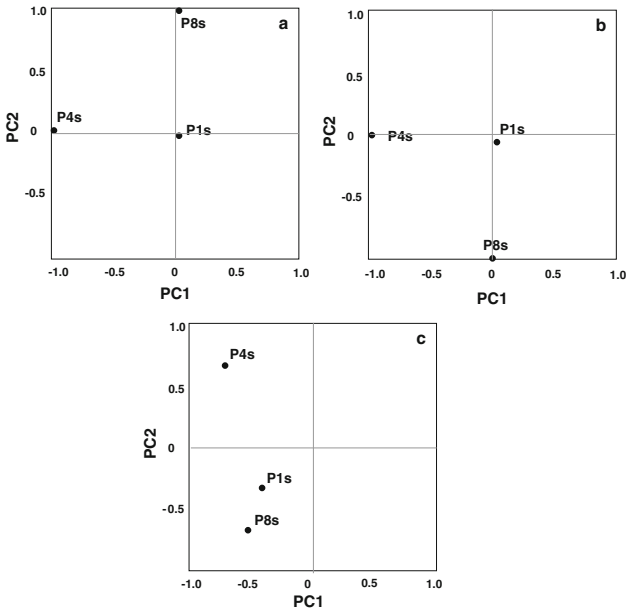


Fig. 7 Ordination diagrams based on principal component analysis using frequency tables obtained from the three 16S rDNA clone libraries (P1L, P4L and P8L) defined at different cutoff similarity values of 97% (**a**), 90% (**b**) and 80% (**c**) using Comm Cluster Software. *PC1* principal component analysis factor 1, *PC2* principal component analysis factor 2

Bacteroidetes were the most predominant except that *Betaproteobacteria* were absent in the permafrost-affected soil (Wagner et al. 2009). *Chlorobi*, *Planctomycetes* and *Verrucomicrobia* were comparable with most of the habitats except in snow. The other phyla present in P1S, P4S or P8S were not very common and were seen in only a few specific habitats like *Acidobacteria* in alpine soils (Costello and Schmidt 2006; Zhou et al. 1997; Lipson and Schmidt 2004), *Actinobacteria* and *Firmicutes* in permafrost soil, snow and tundra soils (Segawa et al. 2005; Wagner et al. 2009; Costello and Schmidt 2006; Zhou et al. 1997). Further, the low abundance phyla like *Chloroflexi*, *Fibrobacteres*, *Gemmatimonadetes, Nitrospirae* and candidate phylum TM7 observed in Pindari glacier were not observed in most of the habitats and *Chlamydiae* and *Dictyoglomi* were absent in any of the above habitats. Similarly this study differed with the above studies in the absence of *Cyanobacteria* (present in alpine tundra wet meadow soil, alpine dry meadows soil, unvegetated deglaciated soil and periglacial soil), *Deinococcus* (present in unvegetated deglaciated soil), *Spirochaetes* (present in tundra soil), *Themomicrobia* (present in permafrost-affected soil) and candidate divisions OS-K and OP10 (present in alpine tundra wet meadow soil).

The bacterial diversity of the Pindari glacier was also compared with that of various polar habitats like lake accretion ice like (Christner et al. 2001; Mosier et al.

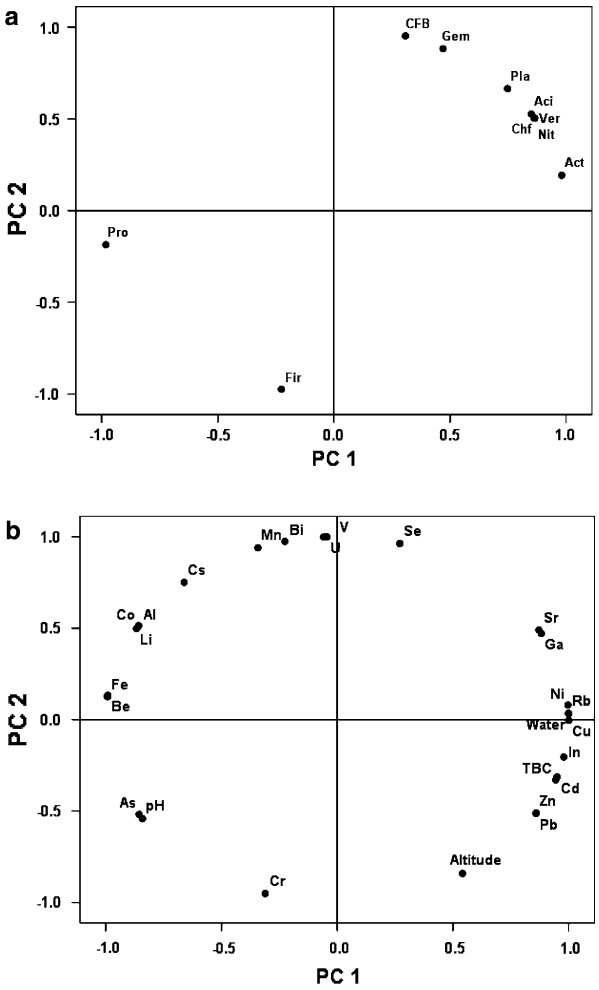


Fig. 8 **a** Principal component analysis based on percentage of specific OTUs from three 16S rRNA gene clone libraries (*PC1* 82.457%, *PC2* 17.543%). **b** PCA plot based on biogeochemical properties (altitude, total bacterial count, pH value and micronutrients Li, Be, Al, V Cr, Mn Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, In, Cs, Pb, Bi and U) of three soil samples (*PC1* 83.318%, *PC2* 11.682%)

2007), ancient glacial ice (Christner et al. 2003), sea ice (Brinkmeyer et al. 2003), seafloor basalt (Lysnes et al. 2004), deep sea sediments (Reed et al. 2006; Li et al. 2008; Srinivas et al. 2009), glacial sediments (Cheng and Foght 2007), deep glacier horizons (Abyzov et al. 1998), supraglacial runoff of proglacial lakes (Mindl et al. 2007; Foght et al. 2004; Vardhan Reddy et al. 2009), permafrost sample (Steven et al. 2007) and cyanobacterial mat samples (Brambilla et al. 2001). The bacterial diversity in these polar habitats as anticipated was very different compared to the glaciers in the Himalayas in terms of distribution of bacterial taxa like *Janthinobacterium*, *Myxococcus*, *Pseudomonas*, *Psychrobacter*, *Sphingobacterium*, *Arthrobacter*, *Bacillus*, *Cryobacterium*, *Hymenobacter*, *Micrococcus*, and *Planococcus* (Shivaji et al. 2004; Vardhan Reddy et al. 2009) which are dominant in these polar habitats**.** The observed variation in diversity could be attributed to the pristine environment of the polar regions, to the climatic conditions which are not as severe as in the polar regions and to anthropogenic activities.

Based on culture-dependent studies, a total of 20 bacterial strains were isolated and were observed to be phylogenetically related to species from 12 genera, namely *Advenella*, *Arthrobacter*, *Bacillus*, *Brachybacterium*, *Flavobacterium*, *Lysinibacillus*, *Microterricola*, *Paenisporosarcina*, *Pseudomonas*, *Rhodococcus*, *Sporosarcina* and *Viridibacillus*. All strains were psychro-, halo- and alkalitolerant and majority of the strains resembled the nearest phylogenetic neighbour in these characteristics. For instance strains PA2, PN9, PA1, PN10, PON2, PON13, PA4, PN8 and PN11 are psychrotolerant and were similar to their nearest phylogenetic neighbours *Bacillus cecembensis* PN5^T (Reddy et al. 2008b), *Arthrobacter alpinus* S6-3^T, *Arthrobacter psychrochitiniphilus* GP3^T, *Flavobacterium psychrolimnae* LMG 22018^T, *Arthrobacter sulfonivorans, Pseudomonas azotoformans*, *Rhodococcus qingshengii*, respectively (Srinivas et al. 2009; Vardhan Reddy et al. 2009). A few strains such as those affiliated to *Brachybacterium tyrofermentans*, *Lysinibacillus fusiformis*, *Microterricola viridarii* and *Paenisporosarcina quisquiliarum* have been reported to be mesophilic and psychrotolerant strains have not been reported. Noticeably, neither enteric nor pathogenic bacteria were detected in this study 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). The limited number of colonies seen in the present study may reflect the extreme conditions prevailing in the Pindari glacier soil samples. This also may be reason that we have observed limited diversity in the 16S rRNA gene clonal libraries, especially with respect to P4L.

Psychrophilic bacteria have attracted the attention of the scientific community (Feller and Gerday 2003) due to their ability to produce cold-active enzymes, with potential applications in a broad range of industrial, agricultural and medical processes. Comparison of the enzymatic activities of the present isolates with those of their nearest phylogenetic neighbour indicated that only strains PA8 and PN11 were similar to their phylogenetic neighbours *Lysinibacillus fusiformis* and *Rhodococcus qingshengii*, respectively with respect to urease activity. All the remaining strains showed properties which did not match their nearest phylogenetic relatives. For instance strains PA1, PA2, PN7, PA8 and PON4 exhibited amylase activity but not protease activity like the corresponding phylogenetic neighbour *Arthrobacter psychrochitiniphilus*. Strain PA2 and PN7 exhibited amylase activity but did not exhibit protease activity like the corresponding phylogenetic neighbours *Bacillus cecembensis* and *Bacillus psychrodurans*, respectively. Surprisingly, none of the strains produced extracellular proteases and lipases.

Table 4 Growth characteristics and activities of amylase, lipase, protease and urease of the 20 bacterial strains isolated from three soil samples (P1S, P4S and P8S), collected near the Pindari glacier, Himalayas, India

Serial number	Strain number	Phenotypic characteristics			Enzymatic activity			
		Temperature range (°C)	NaCl tolerance (M)	pH range	Amylase		Urease	
					4°C	20°C	4°C	20°C
1	PN8	4–30	1	6–11	−	+++	+	+
2	PN1	4–37	0.5	6–11	+++	+++	−	+
3	PON2	4–37	0.5	6–11	++	−	+	−
4	PON4	4–37	1	6–11	+++	−	+	+
5	PN9	4–37	0.5	6–11	+++	+++	+	+
6	PA1	4–37	1	7–11	+++	+++	+	+
7	PA7	4–37	1	6–11	+++	+++	+	+
8	PA3	4–37	1	6–11	+++	++	+	+
9	PN7	4–30	0.5	7–11	−	+	−	−
10	PN2	4–30	1	7–11	−	−	−	+
11	PN4	4–30	0.5	6–11	−	+++	−	+
12	PA2	4–37	0.5	7–11	+++	+++	−	+
13	PA8	4–37	0.5	7–11	+	+	+	+
14	PON5	4–37	0.5	6–11	−	+++	−	−
15	PN11	4–37	1	6–11	−	+++	+	+
16	PON13	4–30	0.5	6–11	−	+++	−	+
17	PN10	4–37	0.5	6–11	++	++	−	+
18	PON14	4–30	2	6–11	+++	+++	+	+
19	PA4	4–30	1	6–11	++	+++	−	+
20	PON8	4–37	2	6–11	++	+++	+	+

All isolates were negative for lipase and protease activities

+ positive; − negative

Table 5 Identification of the 20 bacterial strains isolated from the three soil samples (P1S, P4S and P8S) collected near the Pindari glacier, Himalayas, India, based on BLAST analysis of the 16S rRNA gene sequences

Serial number	Strain number	Nearest phylogenetic neighbour (NCBI-BLAST/EzTaxon) based on 16S rRNA gene sequence similarity [%]
Bacteroidetes		
1	PN-10	*Flavobacterium psychrolimnae* LMG 22018^T AJ585428 [99.4]
Proteobacteria		
2	PON-8	*Advenella kashmirensis* WT001^T AJ864470 [99.8]
3	PA-4	*Pseudomonas azotoformans* IAM1603^T D84009 [99.5]
Actinobacteria		
4	PN-9	*Arthrobacter alpinus* S63^T GQ227413 [98.4]
5	PA-1	*Arthrobacter psychrochitiniphilus* GP3^T AJ810896 [99.2]
6	PON-2	*Arthrobacter sulfonivorans* ALL^T AF235091 [99.2]
7	PON-13	*Arthrobacter sulfonivorans* ALL^T AF235091 [99.4]
8	PON-14	*Brachybacterium tyrofermentans* CNRZ 926^T X91657 [99.2]
9	PN-1	*Microterricola viridarii* KV-677^T AB282862 [99.3]
10	PN-8	*Rhodococcus qingshengii* djl6^T DQ090961 [99.9]
11	PN-11	*Rhodococcus qingshengii* djl-6^T DQ090961 [100]
Firmicutes		
12	PA-2	*Bacillus cecembensis* PN5^T AM773821 [98]
13	PON-5	*Bacillus mycoides* DSM 2048^T ACMU01000002 [98.6]
14	PN-7	*Bacillus psychrodurans* DSM 11713^T AJ277984 [99.5]
15	PA-8	*Lysinibacillus fusiformis* NBRC 15717^T [98.9]
16	PN-2	*Paenisporosarcina quisquiliarum* SK 55^T DQ333897 [99.5]
17	PA-3	*Sporosarcina aquimarina* SW28^T AF202056 [99.2]
18	PA-7	*Sporosarcina aquimarina* SW28^T AF202056 [99.5]
19	PON-4	*Sporosarcina aquimarina* SW28^T AF202056 [99]
20	PN-4	*Viridibacillus arvi* LMG 22165^T AJ627211 [99.1]

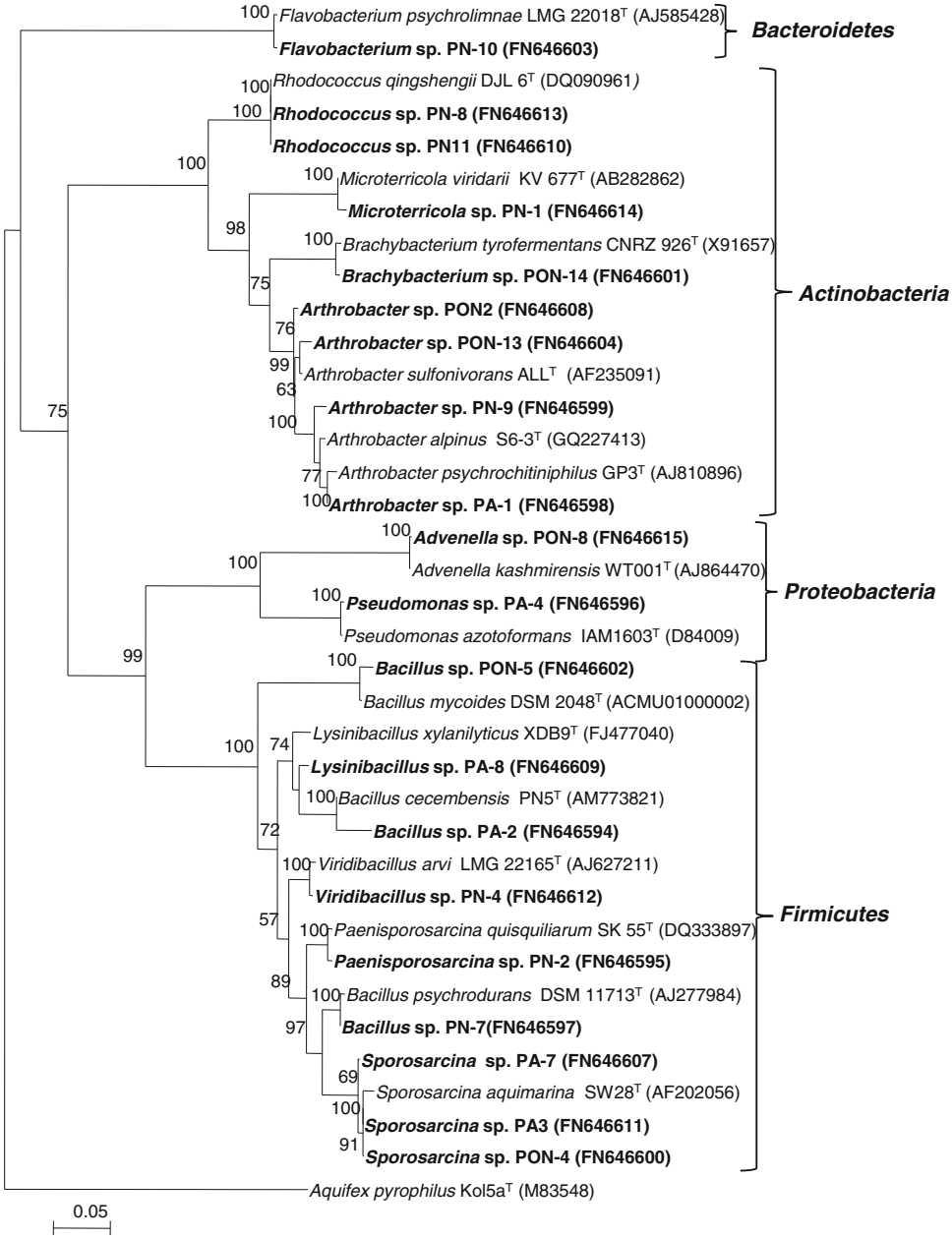


Fig. 9 Phylogenetic tree based on the 16S rRNA gene sequences of the 20 strains isolated from the soil samples (P1S, P4S and P8S) collected near Pindari glacier, Himalayas, India, with their nearest phylogenetic relatives. Phylogenetic trees were constructed by Maximum likelihood method. *Numbers at nodes* are bootstrap values. The *bar* represents 0.05 substitutions per alignment position

The synthesis of unsaturated fatty acids is very vital for survival at low temperatures (Nishida and Murata 1996). In addition, polyunsaturated fatty acids from psychrophiles could be used as a dietary supplement (Russell 1998; Gomes and Steiner 2004). With this in view the fatty acid composition of the 20 isolates was determined. In accordance with previous studies medium chain fatty acids with 8–12 carbons, unsaturated fatty acids, branched (including the iso- and anteiso-fatty acids), methyl, cyclic and the *cis* fatty acids that maintain the membrane in a fluid state and which are common in psychrophilic bacteria (Chintalapati et al. 2004; Shivaji et al. 2007) were present in the Pindari isolates.

Conclusions

1. Bacterial diversity of three soil samples (P1s, P4S and P8S) collected near Pindari glacier based on 16S rRNA gene sequence analysis led to the identification of *Actinobacteria, Firmicutes* and *Proteobacteria* as the common bacterial phyla.
2. In P4S bacterial diversity was limited and only clones affiliated to *Actinobacteria, Firmicutes* and *Proteobacteria* were detected.
3. PCA indicated that altitude, pH, As and Cr are the key parameters that influence the heterogeneity observed in the bacterial diversity.

4. Comparison of the bacterial diversity of Pindari glacier with that of the bacterial diversity in other Himalayan cold habitats, other nonpolar high altitude habitats and with that of various polar habitats indicated that the diversity in the former two habitats was different from that of the polar habitats.
5. As anticipated all the bacterial isolates obtained in this study were alkali, halo and psychrotolerant and majority of the strains resembled the nearest phylogenetic neighbours. A few strains such as those affiliated to *Brachybacterium tyrofermentans*, *Lysinibacillus fusiformis*, *Microterricola viridarii* and *Paenisporosarcina quisquiliarum* have only been reported to be mesophilic unlike the psychrotolerant strains identified in this study.
6. Cold-active enzymes and fatty acids isolated from bacterial strains of Pindari glacier might offer novel opportunities for biotechnological exploitation.

Acknowledgments We would like to thank the Department of Biotechnology and Council of Scientific and Industrial Research, Government of India, for financial support to Dr. S. Shivaji. T.N.R.S. acknowledges the CSIR, Government of India, for the award of Research Associateship. We would like to thank Dr. S.M. Singh, Scientist, from National Centre for Antarctic Ocean Research, Goa, for providing the data on the soil chemical characteristics.

References

Abyzov SS, Mitskevich IN, Poglazova MN (1998) Microflora of the deep glacier horizons of central Antarctica. Microbiology 67:66–73

Ageta Y, Kadota T (1992) Predictions of changes of glacier mass balance in the Nepal Himalaya and Tibetan Plateau: a case study of air temperature increase for three glaciers. Ann Glaciol 16:89–94

Blakemore LC, Searle PL, Daly BK (1987) Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report 80

Booth C (1978) Introduction to general methods. In: Booth C (ed) Methods Microbiol. Academic Press, New York, pp 57–91

Brambilla E, Hippe H, Hagelstein A, Tindall BJ, Stackebrandt E (2001) 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles 5:23–33

Brinkmeyer R, Knittel K, Jürgens J, Weyland H, Amann R, Helmke E (2003) Diversity and community structure of bacterial communities in Arctic versus Antarctic sea ice. Appl Environ Microbiol 69:6610–6619

Certini G, Campbell CD, Edwards AC (2004) Rock fragments in soil support a different microbial community from the fine earth. Soil Biol Biochem 36:1119–1128

Chaturvedi P, Shivaji S (2006) *Exiguobacterium indicum* sp. nov., a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. Int J Syst Evol Microbiol 56:2765–2770

Chaturvedi P, Reddy GSN, Shivaji S (2005) *Dyadobacter hamtensis* sp. nov., from Hamta glacier, located in the Himalayas, India. Int J Syst Evol Microbiol 55:2113–2117

Cheng SM, Foght JM (2007) Cultivation-independent and -dependent characterization of bacteria resident beneath John Evans glacier. FEMS Microbiol Ecol 59:318–330

Chintalapati S, Kiran MD, Shivaji S (2004) Role of membrane lipid fatty acids in cold adaptation. Cell Mol Biol 50:631–642

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2001) Isolation of bacteria and 16S rDNAs from Lake Vostok accretion ice. Environ Microbiol 3:570–577

Christner BC, Mosley-Thompson E, Thompson LG, Reeve JN (2003) Bacterial recovery from ancient glacial ice. Environ Microbiol 5:433–436

Costello EK, Schmidt SK (2006) Microbial diversity in alpine tundra wet meadow soil: novel *Chloroflexi* from a cold, water-saturated environment. Environ Microbiol 8:1471–1486

Dancer SJ, Shears P, Platt DJ (1997) Isolation and characterization of coliforms from glacial ice and water in Canada's High Arctic. J Appl Microbiol 82:597–609

Dong H, Zhang G, Jiang H, Yu B, Chapman LR, Lucas CR, Fields MW (2006) Microbial diversity in sediments of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. Microbiol Ecol 51:65–82

Dyurgerov MB, Meier MF (1997) Mass balance of mountain and subpolar glaciers: a new global assessment for 1961–1990. Arct Alp Res 29:379–391

Eichorst SA, Breznak JA, Schmidt TM (2007) Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov. in the phylum *Acidobacteria*. Appl Environ Microbiol 73:2708–2717

Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1:200–208

Foght J, Aislabie J, Turner S, Brown CE, Ryburn J, Saul DJ, Lawson W (2004) Culturable bacteria in subglacial sediments and ice from two southern hemisphere glaciers. Microbiol Ecol 47:329–340

Fushimi H, Ikegami K, Higuchi K, Shankar K (1985) Nepal case study: catastrophic floods. In: Young GJ (ed) Techniques for prediction of runoff from glacierized areas (publication 149). International Association of Hydrological Sciences, Wallingford, UK, pp 125–130

Gangwar P, Alam SI, Bansod S, Singh L (2009) Bacterial diversity of soil samples from the western Himalayas, India. Can J Microbiol 55:564–577

Gomes J, Steiner W (2004) The biocatalytic potential of extremophiles and extremozymes. Food Technol Biotechnol 42:223–235

Good IJ (1954) The population frequencies of species and the estimation of population parameters. Biometrica 40:237–264

Hur I, Chun J (2004) A method for comparing multiple bacterial community structures from 16S rDNA clone library sequences. J Microbiol 42:9–13

Jackson ML (1967) Soil chemical analysis. Prentice–Hall of India, New Delhi

Kastovská K, Elster J, Stibal M, Santrůcková H (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). Microbiol Ecol 50:396–407

Kishore KH, Begum Z, Pathan AA, Shivaji S (2010) *Paenibacillus glacialis* sp. nov., isolated from Kafni glacier of Himalayas, India. Int J Syst Evol Microbiol (in press)

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–147

Lefauconnier B, Hagen JO, Rudant JP (1994) Flow speed and calving rate of Kongsbreen glacier, Svalbard, using SPOT images. Pol Res 10:56–65

Li Y, Li F, Zhang X, Qin S, Zeng Z, Dang H, Qin Y (2008) Vertical distribution of bacterial and archaeal communities along discrete

layers of a deep-sea cold sediment sample at the East Pacific Rise (approximately 13 degrees N). Extremophiles 12:573–585
Lipson DA, Schmidt SK (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl Environ Microbiol 70:2867–2879
Liu Y, Yao T, Jiao N, Kang S, Zeng Y, Huang S (2006) Microbial community structure in moraine lakes and glacial meltwaters, Mount Everest. FEMS Microbiol Lett 265:98–105
Liu Y, Yao T, Jiao N, Kang S, Xu B, Zeng Y, Huang S, Liu X (2009) Bacterial diversity in the snow over Tibetan Plateau Glaciers. Extremophiles 13:411–423
Lysnes K, Thorseth IH, Steinsbu BO, Øvreås L, Torsvik T, Pedersen RB (2004) Microbial community diversity in seafloor basalt from the Arctic spreading ridges. FEMS Microbiol Ecol 50:213–230
Magurran AE (1996) (ed) Ecological diversity and its measurement. Chapman–Hall, London, pp 274–286
Marcy Y, Ouverney C, Bik EM, Losekann T, Ivanova N, Martin HG, Szeto E, Platt D, Hugenholtz P, Relman DA, Quake SR (2007) Dissecting biological 'dark matter' with single-cell genetic analysis of rare and uncultivated TM7 microbes from the human mouth. Proc Natl Acad Sci USA 104:11889–11894
Mayilraj S, Prasad GS, Suresh K, Saini HS, Shivaji S, Chakrabarti T (2005) *Planococcus stackebrandtii* sp. nov., isolated from a cold desert of the Himalayas, India. Int J Syst Evol Microbiol 55:91–94
Mindl B, Anesio AM, Meirer K, Hodson AJ, Laybourn-Parry J, Sommaruga R, Sattler B (2007) Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacier. FEMS Microbiol Ecol 59:307–317
Miteva VI, Sheridan PP, Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 70:202–213
Mosier AC, Murray AE, Fritsen CH (2007) Microbiota within the perennial ice cover of Lake Vida, Antarctica. FEMS Microbiol Ecol 59:274–288
Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microbiol Ecol 53:110–122
Nishida I, Murata N (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Annu Rev Plant Physiol Plant Mol Biol 47:541–568
Pindi PK, Kishore HK, Reddy GSN, Shivaji S (2009) Description of *Leifsonia kafniensis* sp. nov. and *Leifsonia antarctica* sp. nov. Int J Syst Evol Microbiol 59:1348–1352
Pradhan S, Srinivas TNR, Pindi PK, Kishore KH, Begum Z, Singh PK, Singh AK, Pratibha MS, Yasala AK, Reddy GS, Shivaji S (2010) Bacterial biodiversity from Roopkund glacier, Himalayan mountain ranges, India. Extremophiles 14:377–395
Priest FG (1977) Extracellular enzyme synthesis in the genus *Bacillus*. Bacteriol Rev 41:711–753
Priscu JC, Christner BC (2003) Microbial diversity and bioprocessing. In: Bull AT (ed) Earth's icy biosphere. ASM Press, Washington DC, pp 130–145
Reddy GSN, Aggarwal RK, Matsumotto GI, Shivaji S (2000) *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolate from a pond in McMurdo Dry Valley, Antarctica. Int J Syst Evol Microbiol 50:1553–1561
Reddy GSN, Prabagaran SR, Shivaji S (2008a) *Leifsonia pindariensis* sp. nov., isolated from the Pindari glacier of the Indian Himalayas, and emended description of the genus *Leifsonia*. Int J Syst Evol Microbiol 58:2229–2234
Reddy GSN, Uttam A, Shivaji S (2008b) *Bacillus cecembensis* sp. nov., isolated from the Pindari glacier of the Indian Himalayas. Int J Syst Evol Microbiol 58:2330–2335
Reddy GSN, Pradhan S, Manorama R, Shivaji S (2010) *Cryobacterium Pindariense* sp. nov., a psychrophilic bacterium from a Himalayan glacier. Int J Syst Evol Microbiol 60:866–870
Reed AJ, Lutz RA, Vetriani C (2006) Vertical distribution and diversity of bacteria and archaea in sulfide and methane-rich cold seep sediments located at the base of the Florida Escarpment. Extremophiles 10:199–211
Rhoades JD (1982) Soluble salts. In: Page AL (ed) Methods of soil analysis. Part 2, 2nd edn. Agronomy monograph No. 9. American Society of Agronomy, Madison, WI, pp 167–179
Russell NJ (1998) Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications. Adv Biochem Eng Biotechnol 61:1–21
Segawa T, Miyamoto K, Ushida K, Agata K, Okada N, Kohshima S (2005) Seasonal change in bacterial flora and biomass in mountain snow from the Tateyama Mountains, Japan, analyzed by 16S rRNA gene sequencing and real-time PCR. Appl Environ Microbiol 71:123–130
Shivaji S, Shyamala Rao N, Saisree L, Sheth V, Reddy GSN, Bhargava PM (1989) Isolation and identification of *Pseudomonas* species from Schirmacher Oasis, Antarctica. Appl Environ Microbiol 55:767–771
Shivaji S, Reddy GSN, Aduri RP, Kutty R, Ravenschlag K (2004) Bacterial diversity of a soil sample from Schirmacher Oasis, Antarctica. Cell Mol Biol 50:525–536
Shivaji S, Kiran MD, Chintalapati S (2007) Perception and transduction of low temperature in bacteria. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 194–207
Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD (2005) Comparison of microbial community composition in two subglacial environments reveals a possible role for microbes in chemical weathering processes. Appl Environ Microbiol 71:6986–6997
Srinivas TNR, Nageswara Rao SSS, Vishnu Vardhan Reddy P, Pratibha MS, Sailaja B, Kavya B, Hara Kishore K, Begum Z, Singh SM, Shivaji S (2009) Bacterial diversity and bioprospecting for cold-active lipases, amylases and proteases, from culturable bacteria of Kongsfjorden and Ny-Ålesund, Svalbard, Arctic. Curr Microbiol 59:537–547
Steven B, Briggs G, McKay CP, Pollard WH, Greer CW, Whyte LG (2007) Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. FEMS Microbiol Ecol 59:513–523
Treves DS, Xia B, Tiedje JM (2003) A two-species test of the hypothesis that spatial isolation influences microbial diversity in soil. Microbiol Ecol 45:20–28
Tsai YL, Olson BH (1991) Rapid method for direct extraction of DNA from soil and sediments. Appl Environ Microbiol 57:1070–1074
Tsai YL, Olson BH (1992) Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Appl Environ Microbiol 58:2292–2295
Vardhan Reddy VP, Nageswara SSS, Pratibha MS, Sailaja B, Kavya B, Ruth Manorama R, Singh SM, Srinivas TNR, Shivaji S (2009) Bacterial diversity and bioprospecting for cold-active enzymes from culturable bacteria associated with sediment of melt water stream of Midtre Lov′enbreen glacier, an Arctic glacier. Res Microbiol 160:538–546
Wagner D, Kobabe S, Liebner S (2009) Bacterial community structure and carbon turnover in permafrost-affected soils of the Lena Delta, northeastern Siberia. Can J Microbiol 55:77–83
Xiang SR, Yao TD, An LZ, Xu BQ, Li Z, Wu GJ, Wang YQ, Ma S, Chen XR (2004) Bacterial diversity in Malan ice core from the Tibetan Plateau. Folia Microbiol 49:269–275

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

Xiang SR, Yao TD, Wu GJ, Chen Y, Shang TC, Pu LL, An LZ (2006) Deposition properties of bacterial populations in the Muztag Ata ice core. Quat Sci 26:185–191

Xiang SR, Shang TC, Chen Y, Jing ZF, Yao T (2009) Dominant bacteria and biomass in the Kuytun 51 glacier. Appl Environ Microbiol 75:7287–7290

Yamada T (1991) Preliminary report on glacier lake outburst flood in the Nepal Himalayas, Report 4/1/291191/1/l, Seq. No. 387. Water and Energy secretariat, HMG, Kathmandu, Nepal

Yamada T, Shiraiwa T, Iida H, Kadota T, Watanabe T, Rana B, Ageta Y, Fushimi H (1992) Fluctuations of the glaciers from the 1970s to 1989 in the Khumbu, Shorong and Langtang regions, Nepal Himalayas. Bull Glaciol Res 10:11–19

Zhang X, Tandong Y, Lizhe A, Lide T, Shijian X (2006) A study on the vertical profile of bacterial DNA structure in the Puruogangri (Tibetan Plateau) ice core using denaturing gradient gel electrophoresis. Ann Glaciol 43:160–166

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

Zhang X, Yao T, Tian L, Xu S, An L (2008) Phylogenetic and physiological diversity of bacteria isolated from Puruogangri ice core. Microbiol Ecol 55:476–488

Zhou J, Davey ME, Figueras JB, Rivkina E, Gilichinsky D, Tiedje JM (1997) Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA. Microbiol 143:3913–3919