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ORIGINAL ARTICLE

Psychrophilic and psychrotrophic bacterial diversity of Himalayan Thajwas glacial soil, India

Saleem Farooq[1,2] · Ruqeya Nazir[2] · Bashir Ahmad Ganai[2] · Hina Mushtaq[1] · Gulam Jeelani Dar[2]

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Abstract

The current study deals with the diversity and distribution of culturable cold-adapted bacteria in Himalayan Thajwas glacial soil. The total bacterial count of the seven soil samples collected near glacier snout at different altitudes was investigated through a culture-dependent method. The highest cfu/g ($6.8\pm2 \times 10^7$) was recorded at site I, and the lowest cfu/g ($4.6\pm2 \times 10^3$) was recorded at site VII on nutrient agar (NA) media. The 31 isolated strains were identified using 16S rRNA gene sequencing. Based on their optimum growth at different temperatures, these bacterial isolates were categorized into two overlapping groups: psychrophiles (optimum growth ≥15°C) and psychrotrophs (optimum growth ≥20°C). Besides, these bacterial strains were screened for their cold-active enzyme activity. Further, the microbial diversity was affected by higher incubation temperature, which reduced the richness due to the selection of psychrotrophic microorganisms. Variation in microbial diversity may be related to soil physicochemical properties since a high positive correlation was observed with water content, total nitrogen and organic carbon. This is the first research to look at microbial communities from the Thajwas Glacier, and it adds to our knowledge of the microbial diversity found in Himalayan cold ecosystems.

Keywords Himalayan · Thajwas · Psychrophiles · Psychrotrophs · Cold-active enzyme

Abbreviations

BLAST	Basic Local Alignment Search Tool
CFU	Colony forming units
CMC	Carboxymethyl cellulose sodium salt
DNA	Deoxyribonucleic acid
MEGA	Molecular Evolutionary Genetic Analysis
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
OGT	Optimum growth temperature
PAST	PAleontological Statistics
PCR	Polymerase chain reaction
R2A	Reasoner's 2 agar
rRNA	Ribosomal Ribonucleic acid
SPSS	Statistical Package for the Social Sciences
USA	United States of America
WCA	Whole colony appearance

✉ Ruqeya Nazir
ruqeya.ku@gmail.com

[1] Department of Environmental Science, University of Kashmir, Jammu and Kashmir, India

[2] Microbiology Research laboratory, Centre of Research for Development (CORD), University of Kashmir, Hazratbal, Srinagar, Jammu and Kashmir, India

Introduction

The cryosphere is an important part of the global climate system and one of the earth's largest habitable ecosystems. From polar regions to non-polar mountain glaciers and deep oceans, the cold environments cover approximately 75% of the earth's surface (Margesin and Collins 2019; Bhatia et al. 2020). Cold habitats at permanently low temperatures (below 5°C) represent the largest portion of the earth's biosphere (Margesin and Collins 2019). Among these glacial soil, permafrost and glaciers exhibit very extreme weather conditions and harbor the oldest microorganisms on Earth (Willerslev et al. 2004). Between these cold environments, the non-polar and polar glaciers and ice sheets are significant, as they almost cover about 10% of the earth's surface (Anesio and Laybourn-Parry 2012) and host vast reservoir (9.61×10^{25} cells) of microorganisms (Priscu and Christner 2004; Priscu et al. 2007). Microbes that survive in these conditions near freezing point face several challenges, including persistently low temperatures, minimal nutrient availability, regular freeze-thaw cycles, desiccation, salinity fluctuations and different light conditions (Varin et al. 2012).

Glacial and high-altitude soils remain primarily understudied due to restricted accessibility and considerable

heterogeneity hampers the comparability of different alpine studies. Adaptation of psychrophilic microorganisms enables them to thrive in harsh environmental conditions of alpine environments. Microbes in glacial soil are thought to be pioneers in the initial build-up of biomass, with allochthonous deposition, input from landslides, avalanches, ancient organic matter deposited in the earth and autotrophic fixation as possible sources of C and N (Schmidt et al. 2008; Brankatschk et al. 2011). The potential source of nutrients, such as phosphorus and sulfur, could be the weathered bedrocks (Frey et al. 2010). However, these different sources' relative contributions remain a matter of ongoing debate for alpine glacier soils (Duc et al. 2009; Zumsteg et al. 2012; Frey et al. 2013; Rime et al. 2016; Donhauser and Frey 2018). The community structures of psychrophilic and psychrotrophic bacteria strongly correlate with climatic and soil properties and thus closely mirror the complexity and small-scale heterogeneity of alpine glacial soils (Srinivas et al. 2011; Donhauser and Frey 2018; Kumar et al. 2019).

Covering about 33,000 km^2, the Himalayas is home to many glaciers outside the polar region. These glaciers prove extremely inhospitable to every life form, as the temperature over here falls well below -70°C during winters and rarely exceeds 0°C during summers (Gangwar et al. 2009; Shivaji et al. 2011). This seasonal temperature shift makes the region more intense for microbes, including the psychrophilic and psychrotrophic bacteria. The persistent UV radiation, low nutrient availability and metabolite transfer further add to the region's environmental hostility (Johnson et al. 2007; Steven et al. 2007; Gilichinsky et al. 2008; Zhang et al. 2013). However, notwithstanding such extreme conditions, the psychrophiles, owing to their adaptive mechanisms, still inhabit the Himalayan glaciers and other cold environments like ice and sediments (Srinivas et al. 2011; Rafiq et al. 2017). Apart from mere isolation, these bacteria have also been investigated for extracellular hydrolytic enzymes like proteases, amylases, lipases, cellulases and esterases (Al-Ghanayem and Joseph 2020; Bhatia et al. 2020). More recently, these life forms have been extensively investigated using an amalgamation of culture-dependent and culture-independent methods (Nemergut et al. 2007; Shivaji et al. 2011; Pradhan et al. 2010; Rafiq et al. 2017; Lauro et al. 2011; Sułowicz et al. 2020). In this study, the psychrophilic and psychrotrophic bacterial diversity of soil samples collected near the Thajwas glacier in the Northwestern Himalayas, India, was determined using a culture-dependent approach. Besides, the cold-active enzyme activity of these bacterial isolates was also tested. Based on their optimum growth temperature (OGT), these microorganisms were classified (Morita 1975) into two groups *viz*., psychrophiles (OGT of ≥15°C) and psychrotrophs (OGT of ≥20°C).

Materials and methods

Sample collection

Soil samples were collected from the Thajwas glacier, located in the Northwestern Himalayan region of Kashmir, between altitudes of 2944-3909 m (Fig. S1). The soil samples were collected near glacier snout in sterile poly bags and plastic vials (Shivaji et al. 2011). The soil samples were transported to the laboratory and stored at -20°C until further analysis. Besides, with the mercury bulb soil thermometer's assistance, the temperature of the soil sample sites was recorded on site.

Physicochemical characteristics of soil

Standard methods were used for the physiochemical analysis of soil samples which included; soil moisture (Gardner 1986), soil pH (Watson and Brown 1998), total organic carbon (Walkley and Black 1934), and total Kjeldahl nitrogen (Bremner 1965). Each experiment was run in triplicates, and the average value was used.

Isolation of bacteria

Fourfold dilution was prepared in sterilized normal saline for every soil sample and three sets of triplicate were prepared for each dilution. The spread plate technique was used to spread 0.1 mL of inoculum on media plates. Each set of triplicate plates were incubated at three different temperatures (4, 20, and 37°C) on nutrient agar (NA) and Reasoner's 2 agar (R2A) for 2-30 days, growth was observed after every 24 h for the new bacterial colony (Zhang et al. 2013). The number of culturable bacteria was estimated by counting the average colony-forming units (cfu/g) individually on NA and R2A agar plates. Morphologically distinct colonies were purified by streaking and maintained on NA plates (Shivaji et al. 2011). For determining the optimum growth temperatures, the isolate was inoculated on pre-prepared nutrient media plates followed by incubation at five different temperatures (4, 10, 15, 20, 37°C) and growth was observed every 24 h (Zhang et al. 2013).

Morphological characterization

The colony was observed for macro morphological characteristics like whole colony appearance (WCA), margin, elevation and shape (Rafiq et al. 2017). After that, the pure isolates were stained by Grams Stain-Kit K001 (Himedia® India) to determine their Grams reaction and the cell

morphology was then studied under the light microscope (Olympus IX71).

Genomic DNA extraction and PCR amplification

The bacterial genomic DNA was extracted using the GenElute^{TM} bacterial genomic DNA kit (Sigma-Aldrich, USA). The molecular identification was done using 16S rRNA gene markers, F: 5'- AGA GTT TGA TCC TGG CTC AG -3'and R: 5'- GGT TAC CTT GTT ACG ACT T -3'. Amplification was done by polymerase chain reaction (PCR) as described previously by Shivaji et al. (2004).

Sequencing and phylogenetic analysis

Sequencing of the amplified PCR products was done at Agrigenome laboratory Kerala, India, using ABI 3730XL sequencer. The sequenced data thus obtained was compared with the database available in the NCBI database using BLASTn. The phylogenetic tree was constructed in MEGA 7.0 software (Kumar et al. 2016), employing the neighbor-joining method.

Screening for different hydrolytic enzymes

The 31 identified isolates were screened for their extracellular enzymatic activity on different media. The isolates were cultured on pre-prepared media plates containing NA with one of the following substrates; skim milk 1% (w/v), starch 0.2% (w/v), carboxymethyl cellulose (CMC) 1% (w/v), tributyrin 1% (v/v) and urea 1% (w/v), for protease, amylase, cellulase, lipase and urease respectively. The inoculated plates were incubated at 10, 15, and 20°C for 5-20 days (Alam and Singh 2002). After incubation, the colonies of isolates were examined for zones of hydrolysis (clear halos) on skim milk, tributyrin, CMC (after being flooded with congo-red solution) and starch (after being flooded with iodine solution) agar plates for protease, lipase, cellulase and amylase activities, respectively. Urease activity was determined by observing the development of pink color around the bacterial colonies.

Statistical analysis

The computation of Shannon's diversity index was done by using the PAST 4.03 statistical application (Hammer et al. 2001). SPSS software was used to determine the relationship between soil physicochemical parameters and bacteria diversity in glacial soil samples using the Pearson correlation (rp) matrix. Further, one-way ANOVA was used to calculate the significance of difference among different sites for physicochemical parameters.

GenBank accession numbers

The nucleotide sequences of the 16S rRNA gene were deposited at NCBI GenBank under accession numbers: MN102377 to MN102391, MT478140, MT478142 to MT478150, MT477765, MT478875 and MT495407 to MT495410.

Results

Isolation and enumeration of cultivable psychrophilic bacteria

Enumeration of viable bacteria was done after analyzing NA plate's growth at 4, 20 and 37°C, while as only at 4°C on R2A agar (Table 1). The highest bacterial count ($6.8\pm2 \times 10^7$ cfu/g) was recorded at site I at 4°C and the lowest bacterial count ($4.6\pm2 \times 10^3$ cfu/g) was recorded for site VII at 37°C (incubation temperature) on NA. The highest cfu/g ($5.2\pm2 \times 10^7$) on R2A agar was recorded at site I and the lowest ($1.8\pm1 \times 10^5$) at site VII. Isolates able to grow at 4°C preferred R2A media while NA was preferred at all three (4, 20, and 37°C) incubation temperatures. Besides, these bacterial strains showed growth at a different range of temperatures. Out of 31 isolates, 27 (79.41%) isolates were able to grow at 4°C while only 11 could grow at 37°C. All those isolates that could grow at 4°C also showed growth at 20°C. Based on their growth at different temperatures, these bacterial isolates were categorized into two overlapping groups: psychrophiles (optimum growth ≥15°C) and psychrotrophs optimum growth ≥20°C) (Supplementary table S1).

Morphological and microscopic identification of bacterial strains

The isolated bacterial strains were first categorized based on morphological and physiological characteristics like

Table 1 Mean cfu/g of glacial soil (wet weight) on NA and R2A media at different temperatures

Sites	cfu/g			
	NA			R2A
	4°C	20°C	37°C	4°C
I	$6.8\pm2 \times 10^7$	$4.2\pm1 \times 10^5$	$4.0\pm1 \times 10^4$	$5.2\pm2 \times 10^7$
II	$5.9\pm2 \times 10^6$	$3.8\pm2 \times 10^5$	$1.5\pm2 \times 10^4$	$4.9\pm2 \times 10^6$
III	$6.1\pm2 \times 10^6$	$5.1\pm2 \times 10^5$	$3.2\pm2 \times 10^4$	$5.0\pm2 \times 10^6$
IV	$2.89\pm1 \times 10^7$	$3.7\pm3 \times 10^5$	$2.7\pm2 \times 10^4$	$1.0\pm1 \times 10^6$
V	$1.5\pm2 \times 10^6$	$3.2\pm2 \times 10^5$	$9.8\pm2 \times 10^4$	$1.1\pm2 \times 10^6$
VI	$1.0\pm2 \times 10^5$	$7.2\pm3 \times 10^4$	$6.7\pm1 \times 10^3$	$8.9\pm2 \times 10^5$
VII	$2.7\pm1 \times 10^5$	$5.9\pm2 \times 10^4$	$4.6\pm2 \times 10^3$	$1.8\pm1 \times 10^5$

Gram's stain, WCA, margin, elevation and shape (Supplementary table S1). Further, the bacterial isolates were placed in two distinct groups: Gram-positive and Gram-negative bacteria. Gram-positive were more prominent with 25 (81%) isolates than Gram-negative with only six isolates (19%). The psychrophilic and psychrotrophic bacterial diversity of Thajwas glacial soil comprised of 4 phyla *viz*., *Firmicutes*, ϒ-*Proteobacteria*, *Actinobacteria,* and β-*Proteobacteria*. *Firmicutes* dominated the overall bacterial diversity, followed by ϒ-*Proteobacteria*, *Actinobacteria* and β-*Proteobacteria* (Fig. 1a). In *Firmicutes* phyla, the maximum number of species was recorded at all seven sites that showed a slight decrease with increasing altitude. Three (*Firmicutes*, ϒ-*Proteobacteria,* and *Actinobacteria*) of the four phyla were present at all sites, while β-*Proteobacteria* was absent at sites IV, V, and VII (Fig. 1c). A gradual decline in the number of species from site I to site V was observed, with a rise in altitude.

Molecular characterization

The 16S rRNA nucleotide sequences of all the 31 isolates displayed close similarity (100-98%) with respective species after BLASTn search at NCBI. Phylogenetic analysis of 31 isolates based on 16S rRNA gene nucleotide sequences revealed substantial similarities with other highly homologous species by sharing the same branch in the phylogenetic tree. Each similar specie's strain name is in front of the species name (Figs. 2 and 3).

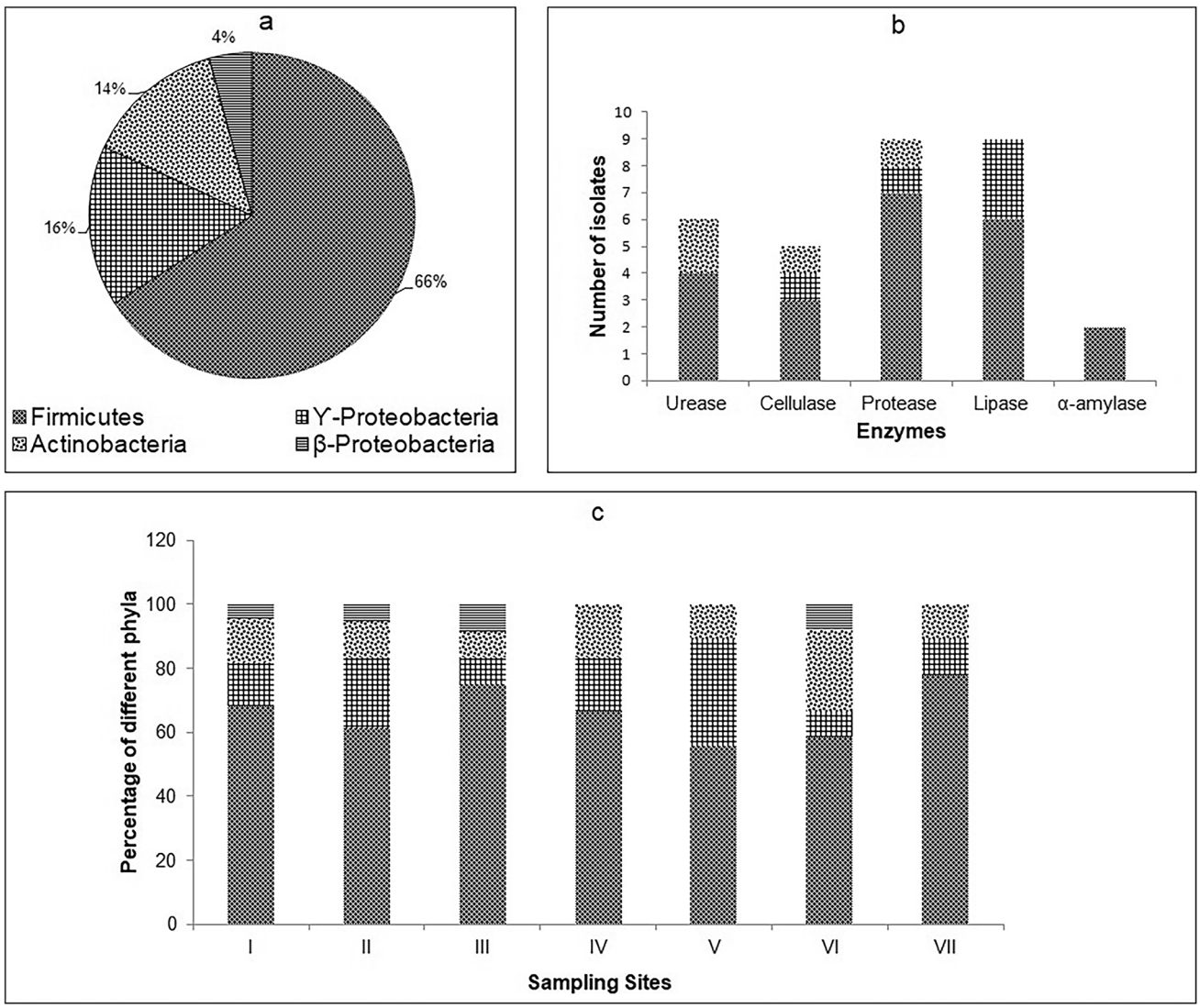

Fig. 1 **a** Overall distribution of phyla **b** Screening of bacterial phyla for their cold-active enzymes **c** Site wise distribution of phyla.

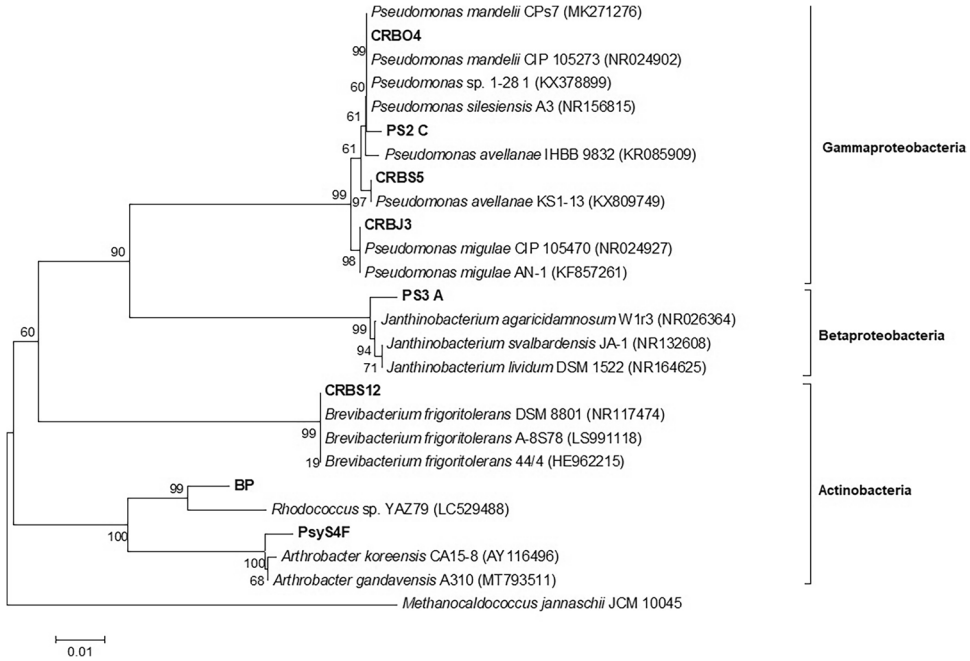

Fig. 2 16S rRNA phylogenetic tree (Neighbor-joining) showing the phylogenetic relationship of *Proteobacteria* affiliated species, *Actinobacteria* affiliated species and bootstrap values were 1000

Screening of psychrophilic and psychrotrophic bacteria for their cold-active enzyme activity

The enzymatically positive species were dominated by *Firmicutes*, which exhibited activity for all 5 enzymes tested (Fig. S2). It was followed by species belonging to ϒ-*Proteobacteria*, which were found positive for protease, lipase and cellulose at their respective optimal temperatures. The species of phyla *Actinobacteria* showed the protease and urease activity while no enzyme activity was observed in β-*Proteobacteria* phyla, which consisted of only single species. Proteases and lipases were found to be equally dominant (Fig. 1b).

Shannon-Wiener diversity index

Shannon-Wiener bacterial diversity index of seven sampling sites (Fig. 4a) showed the highest diversity (2.95) at site I followed by site II (2.77) and the lowest diversity was observed at site V (1.99). As shown in Table 2, temperature-dependent diversity indices showed low Shannon diversity and evenness at 37°C and highest at 20°C, so it could be concluded that higher temperatures restricted the growth of psychrophilic microorganisms (unable to develop above 20°C) and only the psychrotrophic portion of the population was active at 37°C, resulting in lower diversity at 37°C.

Bacterial diversity influencing factors

The number of psychrophilic and psychrotrophic bacterial species isolated from glacial soil showed a high positive correlation with water content (0.841), total nitrogen (0.918) and organic carbon (0.898), respectively. Temperature showed a medium positive correlation (0.406); however, pH showed a negative correlation (-0.772) with bacterial diversity of glacial soil (Fig. 4b).

Soil physicochemical characteristics

The average on-site temperature for the seven sampling sites varied between 0-1.3°C. The lowest average temperature of 0°C was recorded at site III, while the highest on-site average temperature of 1.3°C was recorded at sites II, V and VI (Fig. 5a). The physicochemical characteristics of seven glacial soil samples showed variation except for the soil pH, where a slight variation was observed. The pH value ranged from 7.44 to 8.04 (Fig. 5b). The highest pH was recorded for site VII (8.04) and the lowest was recorded for site II (7.35). The water content showed a greater variation between 61.37 to 11.44%. It was recorded highest for sites I, II and V; lowest for sites III, IV, VI and VII (Fig. 5c). Total nitrogen and organic carbon assessment showed that the soil overall had a lower content. Further, the total nitrogen in the glacial soil samples from higher altitudes was found below the detection level (Fig. 5d). Organic carbon concentration was comparatively higher for low altitude sites, which decreased swiftly with increasing altitude (Fig. 6). Physiochemical parameters were analyzed for seven different sampling sites of Thajwas glacial soil by one-way ANOVA. pH ($p<0.005$), water content (%) ($p<0.001$), total nitrogen (%) ($p<0.001$), organic carbon ($p<0.001$) showed significant difference among different sites however, temperature ($P>0.1$) was not significantly different among all sites (Supplementary table S2).

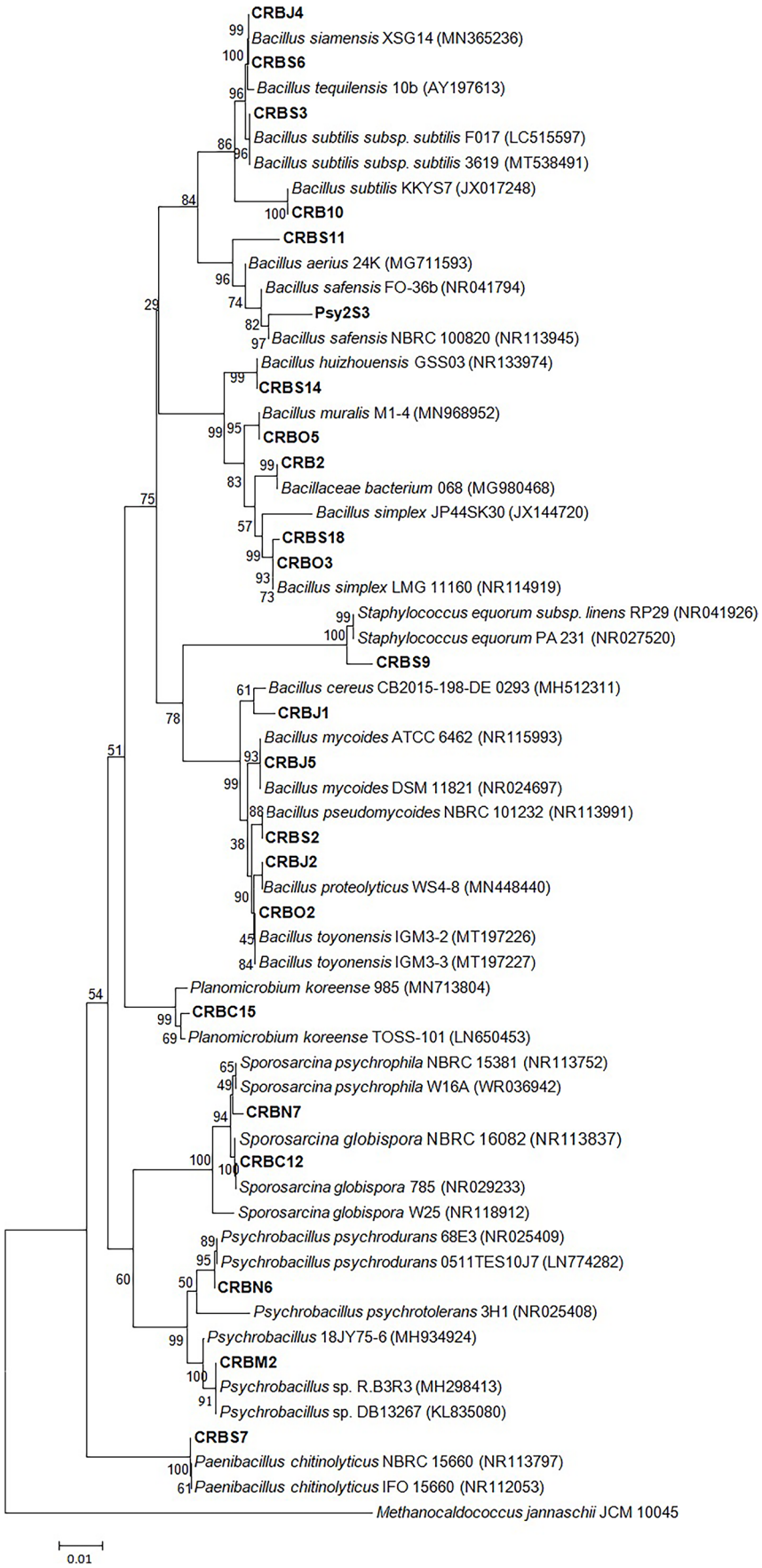

Fig. 3 16S rRNA phylogenetic tree (Neighbor-joining) showing the phylogenetic relationship of phyla *Firmicutes* affiliated species and bootstrap values were 1000

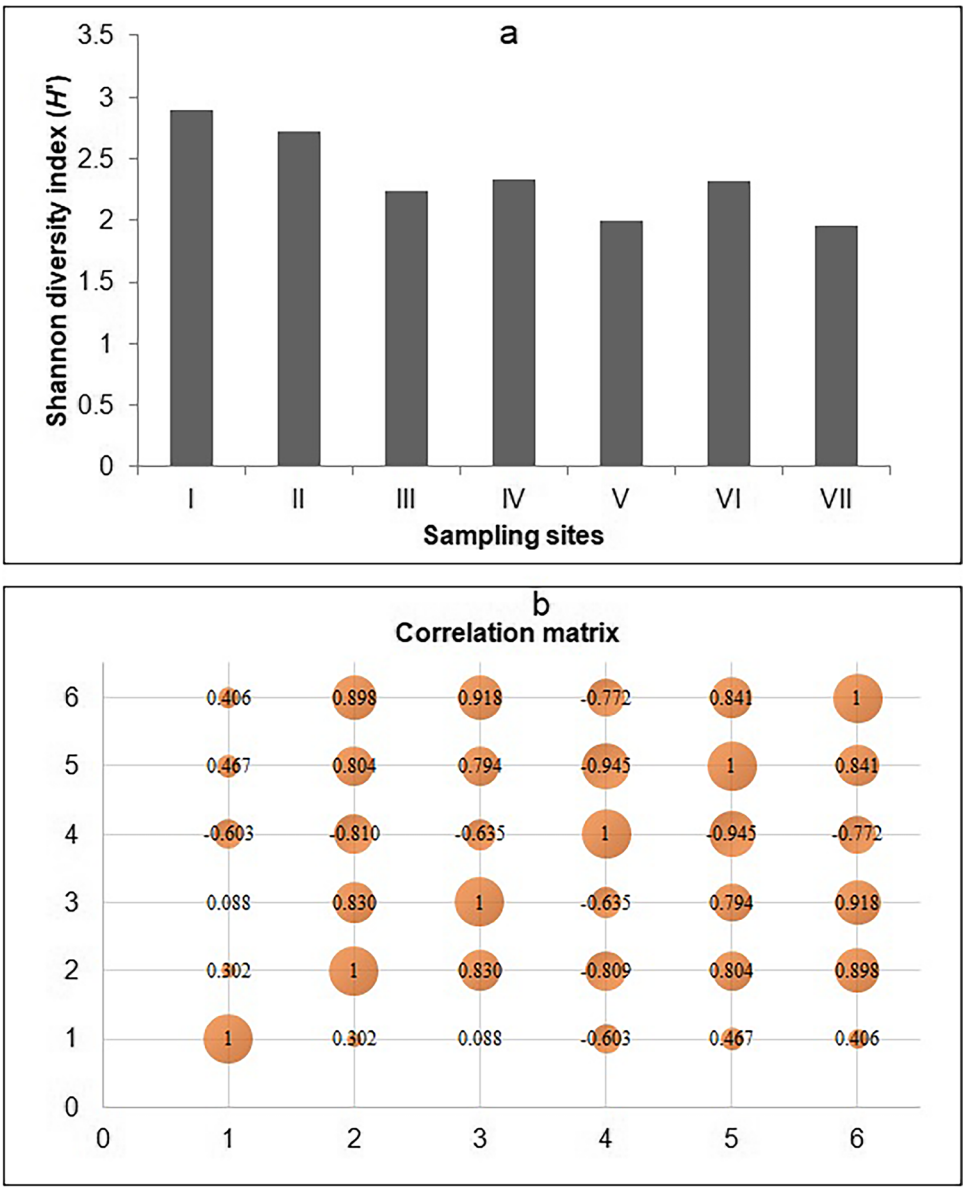

Fig. 4 **a** Shannon-Wiener diversity index of seven sampling sites **b** Correlation between different physicochemical parameters and bacterial count obtained from glacial soil of Thajwas glacier where size and numerical values inscribed inside the bubble determines the type of correlation (numerical values inscribed on horizontal and vertical axis designate 1: Temperature; 2: Organic carbon; 3: Total nitrogen; 4: pH; 5: Water content; 6: Number of species)

Table 2 Temperature-wise diversity and evenness of bacterial isolates

	Temperature		
	4°C	20°C	37°C
Species (number of individuals)	24	30	11
Dominance (*D*)	0.05759	0.04409	0.1034
Shannon diversity index (*H'*)	2.968	3.219	2.327
Shannon evenness (*E*)	0.8103	0.8335	0.9317

Discussion

The bacterial abundance of Himalayan glacial soils based on culture-independent technique is reported in the range 0.9×10^7 to 30.71×10^8 bacteria/g (Pradhan et al. 2010; Shivaji et al. 2011; Srinivas et al. 2011). Based on the culture-dependent technique, the viable count is reported in the range 2.0×10^1 to 1.53×10^9 cfu/g (Gangwar et al. 2009; Rafiq et al. 2017; Sherpa et al. 2018). The soil samples of the Thajwas glacier had a viable count ranged from $6.8 \pm 2 \times 10^7$ to $4.6 \pm 2 \times 10^3$ cfu/g at 4 and 37°C, respectively. When using a culture-dependent technique, the cfu/g varies with incubation temperature; for example, a high viable count was recorded below 20°C, which decreased above 20°C. So far, the number of culturable species found in the soils of Himalayan glaciers has been limited. Some studies have reported 20 (Shivaji et al. 2011), 11 (Srinivas et al. 2011) and 24 (Rafiq et al. 2017), ours is the highest with 31 different species. Further, among 4 genera (*Firmicutes*, ϒ-*Proteobacteria*, β-*Proteobacteria,* and *Actinobacteria*),

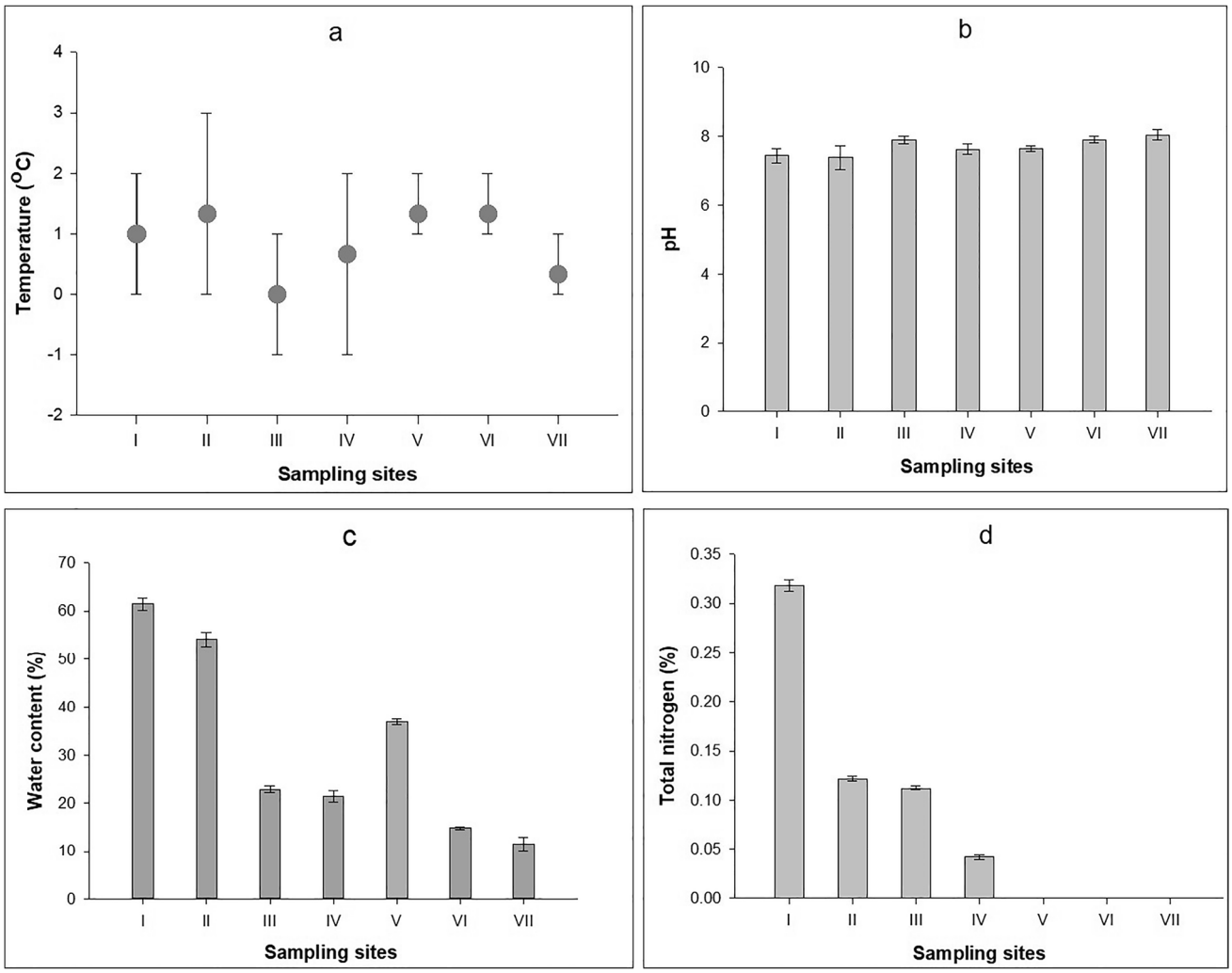

Fig. 5 Site-wise average **a** On-site temperature **b** pH **c** Water content (%) **d** Total nitrogen (%) in the Thajwas glacial soils

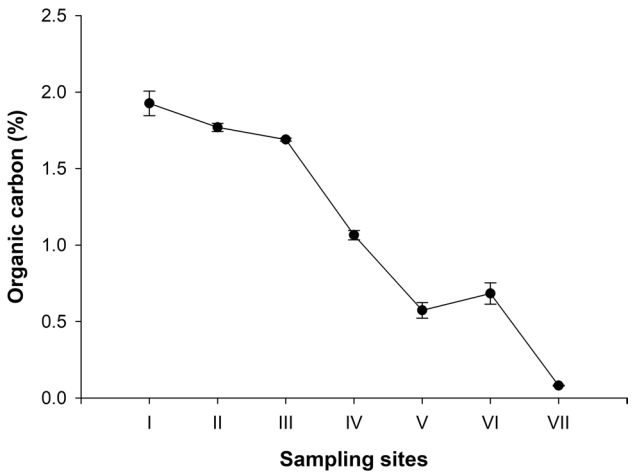

Fig. 6 Site-wise mean organic carbon (%) content of glacial soil samples

Firmicutes and *Proteobacteria* were seen to be dominant and similar observations were made in other culture-based studies on Himalayan glacial soils (Gangwar et al. 2009; Shivaji et al. 2011; Srinivas et al. 2011; Rafiq et al. 2017).

Studies on psychrophilic and psychrotrophic bacterial isolation from cold-habitats show that NA and R2A are the more suitable media for their isolation (Zhang et al. 2013; Rafiq et al. 2017; Hassan et al. 2020). Incubation on R2A at lower temperatures has already been shown to facilitate the development of rare, slow-growing bacteria, resulting in higher levels of diversity (Reasoner and Geldreich 1985; Wernicke et al. 1990; Reasoner 2004; Zhang et al. 2013; Gensberger et al. 2015). Because the bacterial count on R2A plates at 20 and 37°C was too low to count (TFTC), only the 4°C counts were considered. Furthermore, Reasoner and Geldreich (1985) developed R2A, which was categorized as low nutrient media since it contained lower carbon concentration and ionic strength (Reasoner and Geldreich 1985;

Allen et al. 2004) than high nutritional medium (e.g., NA). This could also explain why some isolates were unable to grow at higher temperatures on R2A.

Besides, Gram-positive bacteria are more dominant than Gram-negative bacteria (Gangwar et al. 2009; Zhang et al. 2009; Pradhan et al. 2010; Shivaji et al. 2011) in Himalayan glacial soils. Since the survival of psychrotrophic bacteria in extremely stressful circumstances is attributable to the development of spores in Gram-positive bacteria (Miteva and Brenchley 2005; Rafiq et al. 2017). These results support our research as psychrotrophic and spore formers were the majority of the Gram-positive bacteria in our study. The development of spores may help resolve low-temperature stress conditions (Nicholson et al. 2000; Rafiq et al. 2017).

Although diversity was lower at 37°C (incubation temperature) in our samples, as shown by the Shannon diversity index and evenness (Table 2), it could therefore be inferred that the lower diversity observed at 37°C is explained by lower richness, i.e., lesser number of microorganism functional classes. Since higher temperatures limit the growth of psychrophilic (cold-adapted) microorganisms that are unable to grow above 20°C, only a small portion of the psychrotrophic (cold-tolerant) population was active at 37°C, resulting in lower diversity (Pessi et al. 2012). Besides, because of the maximum decrease in diversity observed at 37°C, it can be inferred from these results that the populations examined here are predominantly composed of psychrotrophic microorganisms since higher diversity was observed at 20°C than 4°C (Table 2), which tends to be the important criteria of classification for bacterial communities of the Himalayan and other polar glaciers (Yergeau and Kowalchuk 2008; Shivaji et al. 2011; Srinivas et al. 2011; Pessi et al. 2012). In cold environments, the prevalence of psychrotrophic microorganisms may be associated with an increase in soil temperature through solar radiation, especially during the summer season, when soil temperatures may rise above 9-15°C (Möller and Dreyfuss 1996; Liu et al. 2009).

The results also indicated low species richness and decreased bacterial count with increasing altitude, and this might be due to low nitrogen and organic carbon in the glacial soil. Similar observations were also made previously in several studies that showed that nutrient availability (nitrogen and carbon content) greatly influences microbial diversity and composition (Fierer et al. 2003; LaMontagne et al. 2003; Fierer et al. 2007; Pradhan et al. 2010; Pessi et al. 2012). The decreased bacterial diversity with increased altitude can be related to the fact that the glacial soil at higher altitudes lacks traceable amounts of carbon and nitrogen (Gangwar et al. 2009), which could otherwise provide the suitable media constituents for their growth.

Himalayan glaciers offer a vast resource of unexplored cold-habitats that are rich in psychrophilic and psychrotrophic bacterial species and are exposed to one of the world's harshest cold environments. Under such high-stress conditions, these psychrophilic and psychrotrophic bacteria produce extracellular cold-active enzymes to get acclimatized to such environment (Shivaji et al. 2011; Srinivas et al. 2011; Rafiq et al. 2017). These enzymes can thus be explored for their industrial application since they are active even at low temperatures and can decrease industrial energy consumption thereby reducing the environmental pollution. Among these enzymes, the cold-active proteases, lipases, amylases, urease and cellulase are vital for industrial uses because they possess high catalytic activity at lower temperatures (Gangwar et al. 2011; Kuddus and Ramteke 2012; Singh et al. 2014; Farooq et al. 2021) and have gained much attention in the effort to decrease the industrial energy costs and pollution. Cold-active enzymes are emerging as the first choice for several industrial applications particularly in the detergent, dehairing, food and pharmaceutical industry as these enzymes can be exploited to replace the ones derived from mesophilic organisms since the former have more economic benefits by decreasing the industrial energy expenditures, pollution and costs than the later (Al-Ghanayem and Joseph 2020). Previous studies have also used skim milk (Farooq et al. 2021; Gangwar et al. 2011; Alam and Singh 2002), tributyrin (Maharana and Ray 2015; Salwoom et al. 2019; Gangwar et al. 2011), starch (Gangwar et al. 2011; Kuddus et al. 2012; Singh et al. 2014), CMC (Gangwar et al. 2011; Singh et al. 2014) and urea (Singh et al. 2014) as substrates and methods for screening for protease, lipase, amylase, cellulase and urease activity respectively, in psychrophilic and psychrotrophic bacterial species.

Conclusion

Firmicutes were the common bacterial phyla besides organic carbon and nitrogen were probably the key parameters that influenced the bacterial diversity. Comparing the Thajwas glacial soil's bacterial diversity with the diversity of other cold ecosystems in the Himalayas showed that the taxa distribution and abundance differ. However, the similarity was noticed for Gram-positive psychrotrophic dominance. The discovery of these psychrotrophic/psychrophilic and their genetic engineering will offer novel opportunities for biocatalysis and biotransformation in biotechnology. Cold-adapted enzymes screened from these bacterial isolates can be exploited to replace the enzymes derived from mesophilic organisms as the former has more economic benefits by decreasing the industrial energy expenditures and costs than the latter.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11756-021-00915-6.

Author contribution SF framed the work design, collected, compiled and interpreted the data. Further, conducted experiments, wrote the manuscript and designed the figures. RN designed the research work, evaluated the data and did the revision of the manuscript. BAG and HM did the critical revision of the manuscript. GJD helped in statistical analysis. Both RN and BAG helped in designing the experiments and supervised the overall work. All the authors helped in writing the manuscript and provided critical feedback.

Data availability Data about the 16S rRNA gene sequences have been deposited at NCBI GenBank under the accession numbers: MN102377-91, MT478140, MT478142-50, MT477765, MT478875, and MT495407-10.

Code availability Not applicable.

Declarations

Ethical statement The authors in the current study carried out no animal or human studies. Authors further confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

Consent to participate Consent was obtained from all the authors for the submission of the manuscript to the journal.

Consent for publication The publication of this manuscript has been approved by all the authors.

Conflict of interest The authors report no conflict of interest.

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