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ORIGINAL ARTICLE

Thermophilic bacteria that tolerate a wide temperature and pH range colonize the Soldhar (95 °C) and Ringigad (80 °C) hot springs of Uttarakhand, India

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Abstract Twenty-eight bacterial cultures, isolated from hot springs in Uttarakhand, were characterized with particular reference to their wide temperature and pH tolerance and production of enzymes in the thermophilic range. All the bacterial isolates were observed as Gram-positive or variable rods in varied arrangement. Bacterial isolates exhibited tolerance to a wide temperature range (20–80 °C), from mesophilic (+11° to +45 °C) to thermophilic (+46 ° to +75 °C); few almost reached the hyperthermophilic range (+76 °C). The isolates also tolerated a wide pH range (4–14) and moderate salt concentration. The optimum growth of the bacterial isolates was observed at 55 °C and 7 pH. Out of 28 isolates, 25 produced lipase, 25 amylase, 24 cellulase, 22 protease and 13 xylanase at 55 and 65 °C. Tolerance to a wide temperature and pH range and the production of enzymes in a thermophilic temperature range can be considered as indicators of ecological competence of these bacterial isolates for colonizing the high temperature environment. On the basis of 16S rDNA similarity, 20 bacterial isolates belonged to *Bacillus licheniformis*, five to *Paenibacillus ehimensis* and one each to *Bacillus sonorensis*, *B. tequilensis*, and *Staphylococcus epidermidis*. Besides variation in phenotypic characters, strains of *B. licheniformis* and *P. ehimensis* showed varying 16S rDNA similarity between 97–99 % and 95–99 %, respectively. Consideration of temperature preferences in classifying microorganisms on the basis of their minimum, maximum, and optimum growth requirements is also discussed. The study has ecological relevance in the context of colonization of high temperature environments by thermophilic bacteria.

Keywords Thermophilic bacteria · Hot springs · Ecological competence

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Introduction

Hot springs have been recognized as natural environments that are suitable for the colonization of thermophiles. These environments vary with respect to geological features and physico-chemical properties including temperature gradients. The temperature in these sites is usually above 60 °C (limit of eukaryotic life), extending the opportunity for colonization by themophilic microorganisms, mainly bacteria and archaea. The early records on diversity and characterization of thermophiles came from Yellowstone National Park (Marsh and Larsen 1953). The diversity of thermophiles in these sites has been attributed to several factors such as temperature, pH and dissolved hydrogen sulphide levels (Purcell et al. 2007), biogeography (Whitaker et al. 2003; Valverde et al. 2012), and geological history (Takacs-Vesbach et al. 2008). Besides diversity and evolution, thermophiles have also been recognized for their capability to produce thermostable enzymes for biotechnological applications, the production of Taq DNA polymerase from *Thermus aquaticus* being the classical example (Chien et al. 1976).

Thermophiles have been extensively studied from hot springs around the world. In India, several low- as well as

high-temperature hot springs are located in various states; some of these sites, mainly in West Bengal, Himachal Pradesh, and Uttarakhand, have been taken into consideration for the study of thermophiles. The diversity of thermophilic bacteria from hot springs in Bakreshwar (West Bengal in Eastern India) has been studied with particular reference to phylogeny and biotechnological applications (Ghosh et al. 2003; Ghati et al. 2013). The geochemistry of thermal waters in the northwest Himalaya has been investigated (Cinti et al. 2009), as have the diversity and applications of thermophiles from Manikaran hot spring in Himachal Pradesh (Dwivedi et al. 2012; Kumar et al. 2013).

The Soldhar and Ringigad hot springs, located in Uttarakhand state under the Indian Himalayan Region (IHR), are being studied for the colonization of thermophiles. The state is prone to natural calamities, therefore, these hot springs and their associated microbial resources are considered important for conservation. A culture collection from these sites has been developed in order to study the diversity, applications, and ecological resilience associated with the thermophiles from these locations (Kumar et al. 2004; Trivedi et al. 2006; Sharma et al. 2009). The aim of the present study was to characterize and identify 28 bacterial isolates obtained from these two hot spring sites that survive at wide ranges of temperature and pH and produce enzymes in the thermophilic range.

Materials and methods

Study sites

The sampling was done from two hot springs located in the Tapovan area in the Dhauliganga river valley (district Chamoli in the state of Uttarakhand, India). The area is known as a promising geothermal field in the IHR (Bhardwaj and Tiwari 2008). Several hot springs are found scattered in this region that maintain varied temperatures. The isolation of thermophilic bacteria was carried out from the sediment samples collected from two of these hot springs, Soldhar (latitude 39° 29′ 25″, longitude 79° 39′ 29″; altitude 1,900 m amsl) and Ringigad (latitude 30° 33′14″, longitude 79° 40′ 0.06″; altitude 1,850 m amsl). Both the hot springs are situated in the same biogeographic area and the temperature gradient along the slope remains between 90 and 95 °C and 75 and 80 °C at Soldhar and Ringigad, respectively. While Soldhar was devoid of any kind of vegetation, growth of luxuriant algal mats and a species of fern was recorded at Ringigad. The sites have slightly alkaline and low-nutrient properties (Kumar et al. 2004). The peculiar feature of these hot springs is that their high temperatures are maintained throughout the year, despite seasonal variations in the surroundings. The approximate area of both these sites was about 45 m^2 during initial samplings (the year 2002), but the Soldhar hot spring later reduced to 20 m^2 due to landslides and anthropogenic activities (Trivedi et al. 2006).

Bacterial cultures: isolation and culture collection

The 28 morphologically distinct bacterial isolates (16 from Soldhar and 12 from Ringigad) were initially isolated following a serial dilution technique on full to 1/8 strength of prescribed agar/broth media at a temperature range of 24–80 °C (Kumar et al. 2004). The pure cultures (100 ml each) were maintained in Tryptone Yeast extract (TY) broth in 250-ml flasks at 55 °C. When the broth came down to half of its original volume, fresh broth was poured into the flasks. Fresh bacterial cultures were raised for each of the experiments.

Morphological, physiological, and biochemical characterization of bacterial isolates

Colony morphology (size, shape, configuration, elevation, margin, and pigmentation) was determined by inoculating the cultures on TY agar plates and incubating at 55 °C for 24 h. Microscopic observations were taken following Gram reaction using a phase contrast microscope under oil emersion (Optiphot-2).

The physiological and biochemical characteristics of the isolates were determined using standard procedures. These experiments were performed following 24 h incubation at 55 °C, in triplicate. Temperature and pH requirements were determined by inoculating the cultures on TY agar/broth and incubating at a range of temperatures (15–90 °C, with an interval of 5 °C), and pH levels (3.5–14.0, with an interval of pH 0.5 unit) to record the minimum, optimum, and maximum temperature and pH range of all the bacteria. Salt tolerance was measured by growing the culture on TY agar containing different salt concentrations (0.5, 2.0, 5.0, 7.0, 10.0, 12.5, and 15 %). For oxygen requirements, the cultures were grown in TY broth and incubated. Catalase and oxidase activities were performed by taking observations on the formation of oxygen bubbles with 3 % hydrogen peroxide solution and the oxidation of TMPD (tetramethyl-*p*-phenylenediaminedi hydrochloride discs), respectively. Sugar utilization using four monosaccharides (arabinose, dextrose, fructose, and galactose), three disaccharides (lactose, sucrose, and trehalose), one trisaccharide (raffinose), and one polysaccharide (inulin) was evaluated in peptone water containing 0.1 % Andrade indicator. All the media and reagents used were from HiMedia Laboratories, Mumbai, India.

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

The extraction of DNA and its further purification was done following the procedure described by Yates et al (1997). The

16S rDNA was amplified with eubacterial-specific primers 27 F (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The polymerase chain reaction (PCR) cycling conditions included initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for one min, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min, followed by final extension for a period of 10 min at 72 °C. The amplified PCR product was checked on 1 % agarose in 1 X TAE buffer, and purified with a mixture of 20 % polyethylene glycol (PEG) and 2.5 M NaCl. Sequencing was done using 96 capillary 730 xl DNA nalyzer (Hitachi). The identity of the isolates was determined through a BLAST search. Sequences were aligned using CLUSTAL_X, version 1.81 algorithm (Thompson et al. 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0. The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei 1987), using the distance matrix from the alignment.

Enzyme assays

Qualitative bioassays for five enzymes (amylase, lipase, protease, cellulase, and xylanase) were performed in the thermophilic range (i.e., 55 and 65 °C). Starch (0.2 %) in Starch agar, tributyrin (0.1 %) in Tributyrin agar, skim milk (3.0 %) in Skim milk agar, cellulose (1.0 %) and xylan (1 %) in basal salt solution were used for performing amylase, lipase, protease, cellulase, and xylanase tests, respectively. The observations were recorded after 24 h of incubation. Three of these enzymes, namely amylase, xylanase and protease, were also quantified using a spectrophotometer (Ultrospec 2100 Pro) at 55 ° and 65 °C, following standard procedures. One enzyme unit was defined as the amount of

Table 1 Morphological characters of the bacterial isolates

S.No.	Isolate No.	Colony and cell morphology and oxygen requirement
1	GBPI_20	Off-white, smooth, convex and round colony (dia- 0.2–0.5 mm); Gram+ve single rods; facultative anaerobic
2	GBPI_21	Off-white, irregular, convex and round colony (dia- 0.2–1.0 mm); Gram+ve diplobacilli; facultative anaerobic
3	GBPI_22	Creamish-white, smooth, convex and round colony (dia- 2.0–10 mm); Gram variable rods in long chains; facultative anaerobic
4	GBPI_23	Off-white, smooth, convex-concentric colony (dia- 5.0–10.0 mm); Gram+ve rods in short chains; aerobic
5	GBPI_24	Off-white, irregular, convex and round colony (dia-11.0–13.0 mm); Gram+ve diplobacilli; facultative anaerobic
6	GBPI_25	Creamish-white, smooth, convex and round colony (dia-1.0–2.0 mm); Gram+ve rods in long chains; facultative anaerobic
7	GBPI_26	Creamish-white, irregular, convex-raised colony (dia- 3.0–5.0 mm); Gram+ve single rods; aerobic
8	GBPI_28	Creamish-white, smooth, flat-round colony (dia- 1.5–2.0 mm); Gram variable, single-diplobacilli; aerobic
9	GBPI_30	Off-white, smooth, convex-round colony (dia- 1.0–2.0 mm); Gram+ve rods in long chains; facultative anaerobic
10	GBPI_31	Creamish-white, irregular, convex-raised colony (dia- 4.0–5.0 mm); Gram+ve rods in long chains; facultative anaerobic
11	GBPI_33	Off-white, smooth, convex-raised colony (dia- 0.2–0.5 mm); Gram+ve diplobacilli; aerobic
12	GBPI_34	Off-white, smooth, convex-raised colony (dia- 6.0–7.0 mm); Gram+ve rods in long chains with palisade arrangement; facultative anaerobic
13	GBPI_35	Creamish-white, smooth, convex-round colony (dia- 1.0–1.2 mm); Gram-variable, single rods; facultative anaerobic
14	GBPI_36	Creamish-white, smooth, convex-concentric colony (dia- 2.0–3.0 mm); Gram-variable single rods; aerobic
15	GBPI_37	Creamish-white, smooth, convex-round colony (dia- 1.0–3.0 mm); Gram-variable single rods; aerobic
16	GBPI_38	Creamish-white, smooth, convex-round colony (dia- 0.2–0.5 mm); Gram+ve single rods; facultative anaerobic
17	GBPI_39	Creamish-white, smooth, convex-round colony (dia- 0.2–1.0 mm); Gram-variable single rods; aerobic
18	GBPI_40	Brownish-white, smooth, convex- round colony (dia- 5.0–6.0 mm); Gram-variable diplobacilli; aerobic
19	GBPI_41	Off–white, smooth, convex-round colony (dia- 0.5–1.0 mm); Gram-variable diplobacilli; aerobic
20	GBPI_42	Creamish-white, smooth, convex-round colony (dia- 3.0–5.0 mm); Gram+ve single rods; aerobic
21	GBPI_43	Creamish-white, irregular, convex-round colony (dia- 0.2–3.0 mm); Gram+ve single rods; aerobic
22	GBPI_44	Off-white, smooth, convex-round colony (dia- 0.2–0.5 mm); Gram+ve diplobacilli rods; aerobic
23	GBPI_45	Brownish-white, irregular, convex-round colony (dia- 0.5–1.0 mm); Gram+ve rods in short chains; aerobic
24	GBPI_46	Creamish-white, irregular, convex-raised colony (dia- 1.0–3.0 mm); Gram-variable rods in short chains; aerobic
25	GBPI_47	Off-white, smooth, convex-round colony (dia- 0.5–1.0); Gram-variable rods in short chains; aerobic
26	GBPI_48	Off-white, smooth, convex-round colony (dia-1.3–2.5 mm); Gram-variable diplobacilli rods; aerobic
27	GBPI_50	Off-white, smooth, convex-round colony (dia- 0.5–1.0 mm); Gram-variable rods in long chains; aerobic
28	GBPI_52	Brownish-white, irregular, convex-raised colony (2.0 mm); Gram-variable rods in shorts chain; aerobic

enzyme that catalyzed formation of 1 μmole of end product in 1 min under experimental conditions.

Results and discussion

The bacterial isolates showed varied colony as well as cell morphology. The colonies on the agar plates were observed as white to cream, none showing pigment. All were observed as rods (single or arranged in short or long chains) under microscope. The cultures that were observed as light purple are referred as Gram-variable. In culture tubes, the bacteria were observed as aerobic or facultative anaerobic. Morphological characteristics and the oxygen requirement of the bacterial isolates are presented in Table 1. The bacterial isolates varied in terms of utilization of carbon sources, 25 utilized raffinose, 24 galactose and inulin (both), and 23 utilized dextrose and lactose (both). Sucrose was the least utilized sugar (by four isolates). Twenty isolates were positive for catalase and oxidase (detailed data not presented).

Out of 28 bacterial isolates, 11 could grow between 20 and 80 °C, 12 between 20 and 70 °C, and five between 20 and 65 °C; none could grow at 15 or 85 °C. The optimum temperature preference for all the isolates was observed between 55 to 65 °C. The cultures also showed their ability to tolerate a wide range of pH, i.e., from 4–5 to 14. While all the isolates could grow without NaCl in the medium, 14 isolates could grow in up to 12.5 % and the other 14 in up to 10.0 % NaCl. Organisms based on their temperature range, in general, are classified as psychrophilic (−1 ° to +10 °C), mesophilic (+11 ° to +45 °C), and thermophilic (+46 to +75 °C), and the organisms that could grow above +75 °C are referred as hyperthermophilic. Organisms in the present study were observed to grow in temperatures ranging from mesophilic to thermophilic, many almost reaching the hyperthermophilic

Table 2 Temperature and pH range and enzymatic activities of the bacterial isolates

S.No.	Isolate No.	Temperature (°C)	pH	Enzymatic activities				
				Amylase	Protease	Lipase	Cellulase	Xylanase
1	GBPI_20	20 to 80	5–14	+	+	+	–	+
2	GBPI_21	20 to 80	5–14	+	+	+	+++	–
3	GBPI_22	20 to 70	4–14	+++	+	+	+++	–
4	GBPI_23	20 to 65	5–14	+	+	+	+	–
5	GBPI_24	20 to 70	4–14	+	+	+	+	–
6	GBPI_25	20 to 70	5–14	+	+	+	+++	+++
7	GBPI_26	20 to 70	5–14	+	+	+	+	+
8	GBPI_28	20 to 65	5–14	–	–	–	+	–
9	GBPI_30	20 to 70	5–14	+	–	+	+	–
10	GBPI_31	20 to 80	5–14	+	+	+	+	–
11	GBPI_33	20 to 65	5–14	+	–	+	+	–
12	GBPI_34	20 to 70	5–14	+	+	+	++	–
13	GBPI_35	20 to 80	5–14	+	+	+	+	–
14	GBPI_36	20 to 70	5–14	+	+	+	+	+
15	GBPI_37	20 to 70	5–14	+	+	+	+	–
16	GBPI_38	20 to 70	5–14	+	+	+	++	+
17	GBPI_39	20 to 70	5–14	+	+	+	+	–
18	GBPI_40	20 to 70	4–14	+	–	+	+	+
19	GBPI_41	20 to 80	4–14	+	+	+	+	–
20	GBPI_42	20 to 80	5–14	+	+	+	+	+
21	GBPI_43	20 to 80	5–14	+	+	+	+	+
22	GBPI_44	20 to 65	5–14	+	+	+	–	–
23	GBPI_45	20 to 80	5–14	+	+	+	–	+
24	GBPI_46	20 to 80	5–14	+	+	+	+	+
25	GBPI_47	20 to 65	5–14	+	–	+	+	+
26	GBPI_48	20 to 70	5–14	+	+	+	+	+
27	GBPI_50	20 to 80	5–14	–	–	–	–	–
28	GBPI_52	20 to 80	4–14	–	+++	–	+	+

Optimum temperature and pH for all the bacterial isolates: 55–65 °C and 7, respectively; +++=strong (>5.0 mm); ++=moderate (2.0–5.0 mm); + weak (<2.0 mm)

range (Table 2), hence broadly referred to as mesophilic-thermophiles.

Temperature is considered to be one of the most important factors in the determination of microbial communities in a given ecological niche. It can influence the morphological, physiological and molecular characteristics and cause compositional as well as functional shifts in microbial communities (Zheng and Wu 2010; Tobler and Benning 2011). The two hot springs under study from same biogeographic region with varied temperature have different reported microbial compositions. From Soldhar, strains of *Geobacillus stearothermophilus* and *G. kaustophilus* possessing a temperature tolerance between 40 and 85 °C and pH tolerance between 4 and 11 (Sharma et al. 2009), along with an amylolytic mycelial yeast identified as *Saccharomycopsis fibuligera* (Kumar et al. 2005) and few algae (Trivedi et al. 2006) have been reported. During initial observations, several cultures were also obtained in a viable but not culturable (VBNC) state (Kumar et al. 2004). On the contrary, the Ringigad hot spring was observed with an exceptional growth of cyanobacteria with prominent blue, green and brown pigments. More than 35 species of cyanobacteria have been reported from this site (Bhardwaj et al. 2011). It is interesting to note that the distinct variation in microbial colonization in these two sites was due to the difference of about 10 °C in temperature. The relatively higher temperature at Soldhar restricted the algal growth. The bacterial isolates, considered in the present study, possessed the ability to survive the wide temperature range (20–80 °C) in comparison to the other colonizers.

The microorganisms have been classified as mesophiles, psychrophiles, and thermophiles based on their upper and lower temperature limits and the optimal conditions for growth (Wiegel 1990). These traditional definitions are now being revisited in view of the knowledge on the increasing number of microorganisms mainly belonging to the extreme environments. The ability of microorganisms to grow at wide temperature ranges is one of the main strategies for their survival under extreme temperature (low as well as high) environments (Rothschild and Mancinelli 2001). In a more

Table 3 Similarity of the bacterial isolates with reference strains from database and their accession numbers

S.No.	Isolate No.	Closest match	Similarity (%)	Accession no. of the isolates
1	GBPI_20	*Bacillus licheniformis* (AE017333)	99.74	KF862005
2	GBPI_21	*B. licheniformis* (AE017333)	99.19	KF862006
3	GBPI_22	*B. licheniformis* (AE017333)	98.97	KF862007
4	GBPI_23	*B. licheniformis* (AE017333)	98.46	KF862008
5	GBPI_24	*Paenibacillus ehimensis* (AY116665)	95.47	KF862009
6	GBPI_25	*B. tequilensis* (HQ223107)	99.22	KF862010
7	GBPI_26	*B. licheniformis* (AE017333)	99.58	KF862011
8	GBPI_28	*P. ehimensis* (AY116665)	99.91	KF862036
9	GBPI_30	*P. ehimensis* (AY116665)	98.32	KF862013
10	GBPI_31	*P. ehimensis* (AY116665)	99.82	KF862014
11	GBPI_33	*B. licheniformis* (AE017333)	99.76	KF862016
12	GBPI_34	*Staphylococcus epidermidis* (L37605)	98.88	KF862017
13	GBPI_35	*P. ehimensis* (AY116665)	98.16	KF862018
14	GBPI_36	*B. licheniformis* (AE017333)	97.78	KF862019
15	GBPI_37	*B. sonorensis* (AF302118)	97.94	KF862020
16	GBPI_38	*B. licheniformis* (AE017333)	99.60	KF862021
17	GBPI_39	*B. licheniformis* (AE017333)	99.84	KF862022
18	GBPI_40	*B. licheniformis* (AE017333)	99.55	KF862023
19	GBPI_41	*B. licheniformis* (AE017333)	99.83	KF862024
20	GBPI_42	*B. licheniformis* (AE017333)	99.84	KF862025
21	GBPI_43	*B. licheniformis* (AE017333)	99.82	KF862026
22	GBPI_44	*B. licheniformis* (AE017333)	99.39	KF862027
23	GBPI_45	*B. licheniformis* (AE017333)	99.41	KF862028
24	GBPI_46	*B. licheniformis* (AE017333)	99.70	KF862029
25	GBPI_47	*B. licheniformis* (AE017333)	99.78	KF862030
26	GBPI_48	*B. licheniformis* (AE017333)	99.35	KF862031
27	GBPI_50	*B. licheniformis* (AE017333)	99.68	KF862033
28	GBPI_52	*B. licheniformis* (AE017333)	98.16	KF862035

recent study, Pesciaroli et al. (2012) described the preferences of 52 bacterial cultures, isolated from seawater, being different from those reported in literature for the same species. The optima were found to be at lower temperatures and, sometimes, with broader ranges, indicating the adaptation of these microorganisms to the wide temperature in a peculiar environment. In this study, while *Pseudomonas* is reported to be the most abundant genus, the bacterial strains are referred to as mesophilic-psychrotolerants. Psychrotolerant species of *Pseudomonas* have also been reported from low temperature environments in the Indian Himalaya (Pandey and Palni 1998; Pandey et al. 2006; Pandey and Palni 2007). Tolerance to a wide range of pH and moderate salt concentration are other important characteristics of these bacterial isolates on similar lines.

Out of 28 isolates, 25 produced lipase, 25 amylase, 24 cellulase, 22 protease and 13 xylanase at 55 and 65 °C (Table 2), indicating that these isolates could be promising sources of thermostable enzymes. The activity estimated for the three enzymes at an optimal growth temperature was recorded up to 0.5 U/ml. While thermophilic bacteria have been recognized for production of a range of thermostable enzymes for biotechnological applications (Niehaus et al. 1999; Haki and Rakshit 2003), production of these enzymes and other chemicals has been taken into consideration by the scientific community in view of their role in adaptation and survival under extreme environments. DNA and protein-based studies have been performed on extremophiles, including thermophiles, in ecological contexts (Georlette et al. 2003; Wolferen et al. 2013). In a recent study, production of thermostable enzymes (amylase and lipase) has been associated with the coping mechanism of a resilient strain of *G. stearothermophilus*, recovered from the autoclaved sediments from Soldhar hot spring (Pandey et al. 2013). Production of enzymes in lower quantities for longer periods has been recognized as a feature of ecological significance under extreme temperature environments (Rinu and Pandey 2010, 2011; Dhakar et al. 2014). Additionally, the ability of thermophiles to produce thermostable enzymes has opened up new possibilities for commercial applications (Pennisi 1997; Bouzas et al. 2006).

Based on 16S rDNA similarity, 20 bacterial isolates belonged to *Bacillus licheniformis*, five to *Paenibacillus ehimensis* and one each to *B. sonorensis*, *B. tequilensis*, and *Staphylococcus epidermidis* (Table 3, Fig. 1). The bacterial isolates belonging to *B. licheniformis* and *P. ehimensis* are referred to as different strains on the basis of their similarity that ranged from 97 to 99 % and 95 to 99 %, respectively. These isolates also exhibited variations in terms of morphological, physiological, and biochemical characters. Representation of these strains in the phylogenic tree is restricted with respect to their 16S rDNA similarity index. Variation in temperature tolerance mainly at higher degrees

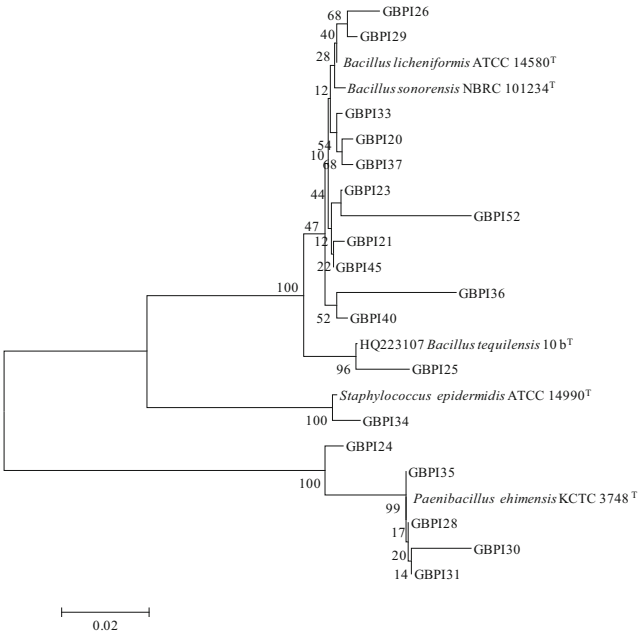

Fig. 1 The phylogenetic tree of all the bacterial isolates and their reference strains using MEGA 4.0 with bootstrap value 1,000

(65–80 °C), pH tolerance up to 14, and production of enzymes are important phenotypic characteristics that need attention at the biochemical and molecular level in future studies. Bacterial isolate GBPI24, which showed 95 % similarity with *P. ehimensis*, can be taken into consideration for further investigation related to its novelty. The dominance of *Bacillus* and *Paenibacillus* in the hot springs under study can be attributed to the endospore-forming ability of these bacteria that represents a successful strategy for their survival (Nicholson et al. 2000). While all the bacterial isolates are being maintained in the microbiology laboratory of the GB Pant Institute of Himalayan Environment and Development, Almora, India, the nucleotide sequences have been accessioned in NCBI.

Species of *Bacillus*, including *B. licheniformis*, *B. sonorensis*, and *B. tequilensis*, have been interesting from the perspective of their importance in evolutionary relationships. Both phenotypic traits, such as colony and cell morphology, salt, pH and temperature tolerance, pigmentation and antibiotic resistance, and genotypic characteristics have been considered in distinguishing these species from each other (Palmisano et al. 2001; Earl et al. 2008; Madslien et al. 2012). Species of *Bacillus* and *Paenibacillus* have also been taken into consideration for their application in agriculture, medicine, and industry (Pandey et al. 1997; Aktuganov et al. 2008; Pandey et al. 2011; Karatas et al. 2013; van Dijl and Hecker 2013; Naing et al. 2014). These species are likely to be the natural thermophilc colonizers of high temperature environments. *S. epidermidis* has been reported as an accidental pathogen (Otto 2009) and probably found space in the Soldhar hot spring due to the anthropogenic activities.

Conclusion

From the present study and earlier reports from these sites, it is possible to conclude that the tolerance to wide temperature and pH ranges could be considered an indicator of ecological resilience held by the natural colonizers of hot springs. Species of *Bacillus*, as in the present case, are likely to be the most competent colonizers of the high-temperature hot springs in comparison with other communities, such as *Geobacillus* and cyanobacteria, which grow in relatively narrow temperature ranges. The earlier findings in the literature are on the microbes that survive a wide temperature range, either in psychrophilic to mesophilic, or mesophilic to thermophilic ranges. Similarly, in the case of pH, the microbes have been reported to survive in either wide acidic or wide alkaline ranges. The widest temperature and pH range over which a single organism can grow is likely to be an important consideration in ecological as well as biotechnological applications. In view of the natural calamities and disasters that occur in the IHR, these sites are important for both bioprospecting and conservation. Further investigations from such sites with varied physico-chemical properties will help in deriving comparative assessments on the colonization abilities of microbial communities in extreme environments.

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