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Thermophilic microbial diversity and physicochemical attributes of thermal springs in the Garhwal Himalaya

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Abstract

The study aim was assessment of physicochemical characteristics along with the microbial diversity of samples of water collected from three important thermal springs of Uttarakhand state in India for two continuous years (2015 – 2016) with two samples taken each year. Some parameters were assessed at the sampling sites and the others in the laboratory. Microbial diversity was explored by using morphological, biochemical, MALDI-TOF-mass spectrometry and molecular approaches. The α-diversity of Gauri Kund was 24 with 14 strains of bacteria, five strains of actinomycetes and five strains of fungi. The α-diversity of Gangnani Kund was 22 with 10 strains of bacteria, five strains of actinomycetes and seven strains of fungi. The α-diversity of Tapovan Kund was 18 with eight strains of bacteria, four strains of actinomycetes and six strains of fungi. The current study can be a good reference for further similar studies. The available data will also help to understand the reasons behind the curative properties of these hot water springs. It also helps the government and local administration to take preventive measures for the conservation and management of these hot water springs..

Key words: Garhwal Himalaya, Gauri Kund, Gangnani Kund, microbial diversity, Tapovan Kund, thermal springs.
Abbreviations: EC, electrical conductivity; DO, dissolved oxygen; TDS, total dissolved solids.

Introduction

Microorganisms can survive in every habitat on Earth. They can obtain nutrients for their existence and growth even from extreme environments. Extremophilic microorganisms are also known as extremotolerant microorganisms because they have the capability to tolerate and survive in unique and extreme environmental conditions including high and low temperature, pH, salinity, pressure, etc (Oarga 2009). If a microorganism can survive in more than one extreme condition then it is known as polyextremophile. Among these extremophiles, the thermophiles or the heat-loving microorganisms can survive at a very high temperature (Akmar et al. 2011). Thermophiles are only available in distinct habitats like hot water springs; geohot water silts, volcanic magma, etc (Rothschild, Manicineli 2001). Hot water springs have water temperature higher than the air temperature of the region where the thermal spring is located (Sen et al. 2010). Hot water springs are created due to tectonic activity and represent extreme environmental conditions, which are available across the hilly range of the Himalaya (Kumar et al. 2004). Hot water ponds are considered to be an important habitat for heat-loving microorganisms that can be utilized for various functions. Heat-loving or thermophilic microorganisms have enzymes that are useful and effective even at extremely high temperature, which makes these thermophilic microorganisms valuable for various industries including in the medical or pharmaceutical sector (Tekere et al. 2015).

Garhwal Himalayan region is especially rich in hot water springs. There is scientific literature available on various aspects of microorganisms and their diversity in hot water ponds of the Garhwal Himalaya, including: microbiological frequency in soil of two distinct hot water ponds in the Uttarakhand Himalaya (Kumar et al. 2004), identification and characterization of Geobacillus spp. isolated from Narad Kund (Sharma et al. 2008), identification of a novel heat loving bacterial strain from hot water pond (Akmar et al. 2011), microbial diversity in a thermal pond located at Unkeshwar (Bhusare, Wakte 2011), molecular characterization of the Manikaran hot spring microbial community (Sharma et al. 2012), isolation of esterolytic thermophilic bacterial strain from a thermal spring of India (Ghati et al. 2013), isolation of a bacterium from a thermal spring having tolerance against arsenate (Bandyopadhyay et al. 2013), diversity of bacteria dwelling in a sulphur pond (Rawat 2015), microorganisms and physicochemical properties of two Garhwal Himalayan thermal springs (Kumar, Sharma 2019), determination of microbial diversity and physicochemical characteristics of two hot water ponds near Badrinath Shrine in the Uttarakhand (Kumar, Sharma 2020). Similar studies have been performed on thermophiles from thermal springs in different countries: archaeal diversity in an Icelandic hot water spring (Kvist et al. 2007), thermal spring's bacterial diversity in the Tibetan Plateau (Huang et al. 2011), diversity of thermophilic actinomycetes of thermal springs in Tibet (Jiang et al. 2012), microorganisms of thermal springs in the Kamchatka Peninsula (Wemheuer et al. 2013), hot spring metagenomics (Lopez et al. 2013), geological chemistry and ecology of microorganisms in hot springs of Ambitle Island having alkaline pH in Papua New Guinea (Meyer-Dombard, Amend 2014), and bacterial diversity dwelling from some African hot water ponds (Tekere et al. 2015).

Uttarakhand is the home of many important hot water springs, including Gauri Kund, Gangnani Kund and Tapovan Kund. These springs are a rich source of sulfur and used for balneotherapy due to its healing properties that include treating skin infections such as rashes and eczema. No relevant information is available on the diversity of microorganisms and physicochemical characteristics of these three important thermal springs, but several researchers have published their research articles on the Ringigad site, erroneously claiming that it is Tapovan Kund (Ranawat, Rawat 2017). The aim of the present study was to provide baseline information and relevant data on the diversity of thermophilic microorganisms and physicochemical features of hot water of these hot water springs (Gauri Kund, Gangnani Kund and Tapovan Kund).

Materials and methods

The study area

Gauri Kund (Fig. 1) is located at the Gauri Kund village enroute to the Kedarnath temple. This place became famous after the Kedarnath flash flood disaster in the year 2013 during which this place was entirely damaged. It is located at elevation 1982 m above sea level in the Rudraprayag District (30°1'55" N, 79°39'18" E). It is situated on the bank of Mandakini River. The maximum in situ water temperature recorded at Gauri Kund is 50 °C. Gangnani is sometimes referred to as Rishikund Tirth. The Gangnani thermal spring (Fig. 1) lies along the road enroute to Gangotri temple in Uttarkashi district of Uttarakhand. It is situated at elevation 1960 m above sea level (30°55'15.73" N, 78°48'41.99" E). The maximum in situ water temperature of Gangnani Kund recorded is 57 °C. The Tapovan thermal spring (Fig. 1) is situated at 1868 m above sea level elevation in the Chamoli District (30°29.503' N, 79°38.336' E). The approximate area of this thermal spring is about 30 m^2. The maximum in situ water temperature recorded at Tapovan Kund is 52 °C.

Water sampling

Hot water samples were taken in an autoclaved thermosteel flask from all three sampling sites and analyzed in two years (2015; 2016) with two samples taken each year. The sampling was conducted during June and August, due to the locations of these hot water springs at high elevations. Hot water springs and hot water ponds both have the hot water. The only major difference is that ponds have stagnant water within cemented boundary and springs have continuous water flow. Water samples were stored in an ice box filled with dry ice. The collected samples of water were analyzed at the Laboratory of Environmental Microbiology and Biotechnology, Department of Environmental Sciences, H.N.B. Garhwal University, Srinagar Garhwal, Uttarakhand, India within 24 h. Water temperature, pH, free CO_2, the concentration of dissolved oxygen were measured at the sampling sites. The remaining parameters were analyzed at the Laboratory following the approved methodology outlined in APHA (2012). Microorganisms were isolated and identified by using the methodology given in Morello et al. (2003).

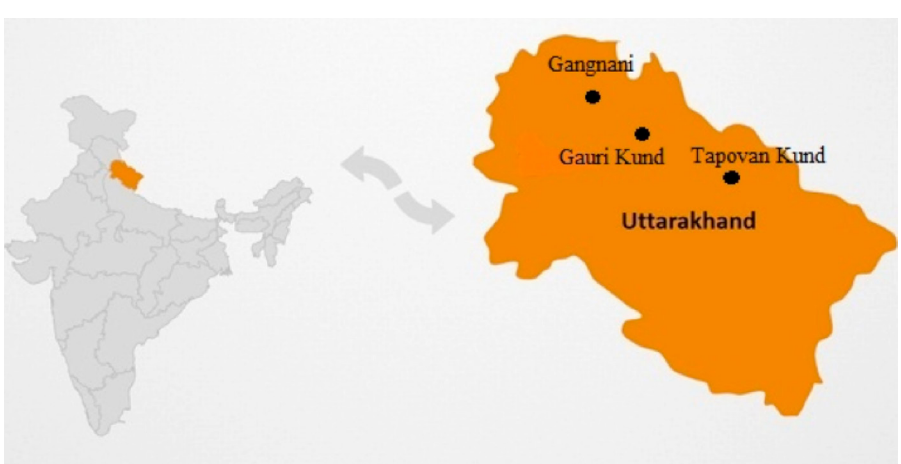

Fig. 1. Map showing location of Uttarakhand in India (left) and location of the three studied springs in Uttarakhand (right).

Physical and chemical parameters

Samples of water from all three hot water springs were collected for analysis of a predefined set of fifteen physicochemical parameters. The temperature of water was recorded by submerging a digital centigrade thermometer (temperature range –50 to +300 °C) into water at depth 10 cm. A Handy pH meter (Electronics India Model No. 7011) was used to record the pH at the sampling sites. A benchtop Multiparameter Analyzer (Toshcon, Model No. TPC-17) was used at the laboratory. A modified Winkler's method was used to estimate concentration of dissolved oxygen at the sampling sites. Electric conductivity, salinity and amount of total dissolved solids (TDS) were also measured using the Multiparameter Analyzer. The total alkalinity, total hardness, and concentration of chlorides, calcium and magnesium were estimated using the methodology available in APHA (2012). An UV-VIS Spectrophotometer (Systronic, Model No. 117) was used for estimation of nitrate, sulfate and phosphate concentration.

Microbial isolation and enumeration

Bacterial colonies were isolated on Nutrient Agar media (HiMedia), commonly known as NA Media. The fungal strains were isolated using the Sabaroud Dextrose Agar media (HiMedia). To avoid bacterial contamination during the isolation of fungal strains, the media was enriched with ampicillin and streptomycin at 50 mg L^{-1} each. Actinomycetes Isolation Agar media (HiMedia) was used to isolate Actinomycetes. pH of each culture medium was adjusted to the pH recorded at the sampling site. After the enumeration of microorganisms, the isolated colonies were streaked on separate suitable media plates to obtain pure cultures (Clesceri et al. 1998).

Morphological and biochemical characteristics

A total of 27 morphological and biochemical characteristics were analyzed during the study period. During analysis, pure cultures of microorganisms were carefully examined by unaided eyes and under a phase contrast microscope (Nikon, Eclipse TS100) to describe the morphology of the bacterial strains. The morphological assessment included shape, size, margin, elevation, colour, cell shape, motility, spore formation, flagellation, and gram staining. Various biochemical tests were performed to identify a microbial strain up to the lowest possible taxon (Rohomania et al. 2015; Ayitso, Onyango 2016). The tests included the assessment of catalase, citrate, urease, fructose, methyl red and Voges-Proskauer, indole test, maltose, etc.

Extraction of DNA and amplification of gene

For isolates whose identification was not successful using the MALDI-TOF-Mass Spectrometry technique, DNA was extracted using a HiPurA Bacterial Genomic DNA Extraction Kit (HTBM008) for bacteria and GSure Fungal DNA Extraction Kit (G45331) for fungal isolates. Amplification of the 16S rRNA gene was performed by PCR using specific universal primers, forward primer 8F (AGAGTTTGATCCTGGCTCAG) and reverse primer 1492R (TACGGYTACCTTGTTACGACTT) (Takahashi et al. 2014) whereas, the 18S rRNA gene amplification was performed by PCR using specific universal ITS primers, forward143primerITS1F(TCCGTAGGTGAACCTGCGG) and ITS 4R (TCCTCCGCTTATTGATATGC) (Pryce et al. 2003; Raja et al. 2017).

Identification of microbial isolates

Pure cultures of each isolate were sent for final confirmation to the National Centre for Microbial Resources, Pune for testing using the MALDI-TOF-Mass Spectrometry technique. In addition, several isolates were sent for 16S rRNA and 18S rRNA sequencing at the same facilitation centre. The centre provided an accession number only to those microbial strains whose identification was done using the gene sequencing method. The accession numbers of these microbial strains are given in Table 1. Later, after obtaining the sequence from the facilitation centre in Fasta format, a phylogenetic tree was constructed by using Bioinformatical software MEGA 6.

Statistical treatment of data

MS Office 2013 was used for statistical treatment of data (minimum; maximum; mean; standard deviation) obtained from the physicochemical characteristics of water samples.

Results

Physicochemical characteristics of water samples

Results for the obtained physicochemical parameters

Table 1. Summary of the closest neighbour for the samples sent for identification to the sequencing facility centre

Isolate No.	Closest neighbour	Accession No.	Similarity (%)
1	*Brevibacillus borstelensis* NRRL NRS-818(T)	D78456	99.74
2	*Bacillus licheniformis* ATCC 14580(T)	AE017333	100.00
3	*Bacillus subtilis* subsp. *spizizenii* NRRL B-23049(T)	CP002905	99.87
4	*Bacillus aryabhattai* B8W22(T)	EF114313	99.26
5	*Bacillus paralicheniformis* KJ-16(T)	LBMN01000156	99.50
6	*Aspergillus costaricaensis* CBS 115574	NR_103604.1	100.00
7	*Bacillus tequilensis* KCTC 13622(T)	AYTO01000043	99.64

Table 2. Physicochemical properties of thermal springs located in the Garhwal Himalaya

Physicochemical parameter	Gauri Kund			Gangnani Kund			Tapovan Kund		
	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.	Mean ± SD
Water temperature (°C)	50.0	53.0	51.3 ± 1.5	56.4	58.2	57.3 ± 0.8	51.0	53.0	52.0 ± 0.8
Dissolved oxygen (mg L^{-1})	2.0	2.4	2.3 ± 0.2	3.0	3.2	3.2 ± 0.1	1.6	1.6	1.6 ± 0.0
pH (units)	6.80	7.20	7.10 ± 0.20	9.30	9.60	9.40 ± 0.15	6.50	6.70	6.60 ± 0.10
Conductivity (mS cm^{-1})	12.00	12.70	12.40 ± 0.29	14.10	14.30	14.30 ± 0.1	3.11	3.19	3.16 ± 0.04
Free CO_2 (mg L^{-1})	145.2	154.0	149.6 ± 5.1	88.0	88.0	88.0 ± 0.0	17.6	22.0	20.9 ± 2.2
Salinity $^{(SAL)}$	6.5	6.8	6.7 ± 0.2	7.6	7.8	7.7 ± 0.1	1.3	1.5	1.4 ± 0.10
TDS (mg L^{-1})	6.60	6.80	6.70 ± 0.10	7.10	7.40	7.20 ± 0.13	1.64	1.70	1.70 ± 0.03
Chloride (mg L^{-1})	41.18	49.70	44.40 ± 4.08	82.36	89.46	84.85 ± 3.36	9.74	12.62	10.80 ± 1.40
Total alkalinity (mg L^{-1})	205.0	230.0	216.3 ± 11.1	295.0	352.0	315.5 ± 25.8	170.0	185.0	177.5 ± 6.5
Nitrate (mg L^{-1})	0.204	0.210	0.207 ± 0.000	0.102	0.109	0.106 ± 0.000	0.271	0.293	0.284 ± 0.010
Sulfate (mg L^{-1})	0.248	0.253	0.251 ± 0.000	0.451	0.463	0.459 ± 0.010	0.389	0.407	0.401 ± 0.010
Phosphate (mg L^{-1})	0.007	0.008	0.008 ± 0.000	0.031	0.034	0.033 ± 0.000	0.021	0.025	0.023 ± 0.000
Total hardness (mg L^{-1})	310.0	320.0	316.0 ± 4.90	92.0	96.0	94.0 ± 1.63	180.0	186.0	183.0 ± 2.58
Calcium (mg L^{-1})	83.2	87.0	85.4 ± 1.7	23.2	27.2	25.2 ± 1.7	40.8	42.4	41.4 ± 0.8
Magnesium (mg L^{-1})	24.95	25.44	25.10 ± 0.23	6.36	8.31	7.58 ± 0.84	19.06	19.55	19.42 ± 0.24

of Gauri Kund, Gangnani Kund and Tapovan Kund are presented in Table 2. Water temperature showed significant difference between Gangnani Kund and that in other springs, with mean values of 51.3, 57.3 and 52 °C at Gauri Kund, Gangnani Kund and Tapovan Kund, respectively. The mean value of water pH ranged from 6.8 to 7.2 for Gauri Kund, indicating slightly acidic to neutral water; 9.3 to 9.6 for Gangnani Kund indicating highly alkaline water, and 6.5 to 6.7 for Tapovan Kund representing slightly acidic water. The mean value of dissolved oxygen in the hot water springs was relatively similar within a range of 1.6 to 3.2 mg L^{-1}. Electrical conductivity (EC) was relatively high in Gauri Kund and Gangnani Kund (12.0 to 14.3 mS cm^{-1}), and significantly lower in Tapovan Kund (3.16 mS cm^{-1}). Salinity measurements confirmed this difference: 6.7 in Gauri Kund, 7.7 in Gangnani Kund, and only 1.4 in Tapovan Kund (in SAL units). The same relationship was evident for amount of TDS in water with mean values 6.7 and 7.2 mg L^{-1} in Gauri Kund and Gangnani Kund, and only 1.7 mg L^{-1} in Tapovan Kund. Extremely large differences in mean values of free CO_2 concentration were evident between the three springs, with the highest level in Gauri Kund (149.6 mg L^{-1}), followed by Gangnani Kund (88.0 mg L^{-1}), and Tapovan Kund (20.9 mg L^{-1}). Similarly, total hardness of water was very high in Gauri Kund (316.0 mg L^{-1}) and very low in the water of Gangnani Kund (94 mg L^{-1}). Relatively smaller differences were evident for bivalent cation (Ca and Mg) concentration in water between the springs, with a decreasing trend from Gauri Kund to Tapovan Kund, and with the lowest level in Gangnani Kund. In contrast, the situation was different in respect to anion concentration, as water in Gangnani Kund had the highest concentration of chlorides, sulfates and phosphates, and the lowest of nitrates. Nitrate concentration was the highest in water of Tapovan Kund.

Microbial diversity

All microbial strains isolated from the water samples were carefully examined to study their morphological and biochemical characteristics. Due to limited space, detailed biochemical and morphological characteristics of a few isolates selected randomly are given in Table 3. The identification report was generated using the EzBioCloud Database maintained at the facilitation center at Pune (Yoon et al. 2017). All microbial isolates identified using the 16S rRNA/18S rRNA sequencing facility are presented in Table 1 along with their accession number and percentage of similarity to its closest neighbor.

A phylogenetic tree was constructed for *Bacillus aryabhattai* and *Aspergillus costaricaensis* by using the sequence provided by the facilitation center in FASTA format (Fig. 2 and Fig. 3). During the study period, the α-diversity of microorganisms in Gauri Kund was 24 with 14 species of bacteria, five species of actinomycetes and five species of fungi. The α-diversity of microorganisms in the Gangnani Kund was 22 with 10 species of bacteria, five species of actinomycetes and seven species of fungi. The α-diversity of microorganisms in Tapovan Kund was 18 with eight species of bacteria, four species of actinomycetes and six species of fungi (Table 4).

Discussion

Usually water temperature is directly proportional to the air temperature, but it is not the case for thermal springs. High temperatures significantly affect chemical composition of water in thermal springs. For example, concentration of DO in water is inversely related to temperature: at high water temperature, the amount of DO decreases (Rana et al. 2018). Also, solubility of other ions change with temperature, and therefore, differences in chemical composition between

Table 3. Morphological and biochemical characterization of microbial isolates. +, positive; –, negative; v, variable

Characteristic	*Bacillus licheniformis*	*Lysinibacillus sphaericus*	*Streptococcus thermophilus*	*Geobacillus stearothermophilus*	*Bacillus tequilensis*	*Pseudomonas putida*	*Bacillus sonorensis*	*Brevibacillus parabrevis*	*Streptomyces rangoon*	*Actinobacillus hominis*	*Bacillus subtilis*	*Streptomyces clavifer*
Shape	Round	Round	Round	Circular	Circular	Oval	Irregular	Round	Round	Round	Round	Round
Size	4 mm	3 mm	2 mm	2 – 3 mm	2 – 4 mm	1 – 3 mm	3 mm	2 mm	1 – 3 mm	1 mm	2 – 3 mm	1 – 3 mm
Margin	Undulate	Regular	Entire	Entire	Smooth	Entire	Smooth	Smooth	Entire	Regular	Lobate	Entire
Elevation	Semi-raised	Raised	Convex	Convex	Convex	Convex	Slightly convex	Flat	Centrally raised	Flat	Flat	Centrally raised
Color	White	Pale-white	Cream	Cream	Yellow	Yellowish brown	Yellowish cream	Yellowish grey	Cream	White	White	Sandy yellow
Cell shape	Straight rods	Rod	Cocci in chain	Rod	Rod	Rod	Rod	Rod	Rod	Pleomorphic	Rod	Rod
Spore formation	+	+	–	+	+	–	+	+	+	–	–	+
Motility	Motile	Motile	Non-motile	Motile	Motile	Motile	Motile	Motile	Motile	Non-motile	Motile	Motile
Grams staining	+	+	+	+	+	–	+	v	+	–	+	+
Flagella	Peritrichous	Peritrichous	Atrichous	Monotrichous	Peritrichous	Multitrichous	Peritrichous	Peritrichous	Peritrichous	Atrichous	Peritrichous	Peritrichous
Catalase	+	+	+	–	+	+	+	+	–	+	+	–
Citrate	–	+	+	v	+	+	+	v	+	–	+	+
Urease	+	–	+	–	–	+	+	–	–	+	–	–
Methyl red	–	–	–	–	–	–	–	–	–	–	–	–
Voges Proskauer	–	–	–	–	+	–	+	–	+	–	+	+
Fructose	–	–	+	–	+	+	+	–	+	–	+	+
Indole test	–	–	–	–	+	–	+	–	–	–	–	–
Raffinose	–	–	–	–	+	–	v	–	–	+	+	–
Ribose	+	–	–	+	+	+	+	+	–	+	+	–
Sorbitol	+	–	–	–	+	–	+	–	+	–	+	+
Sucrose	+	–	+	–	+	+	+	v	+	+	+	+
Xylose	–	–	–	+	+	+	+	–	–	+	+	–
Trehalose	+	–	–	–	+	+	+	+	–	–	+	–
Mannose	+	–	+	–	+	+	+	–	–	–	+	–
Mannitol	+	+	–	+	+	+	+	+	+	+	+	–
Lactose	+	–	+	–	+	–	+	–	–	–	v	–
Maltose	+	-	-	-	+	+	+	+	–	–	+	–

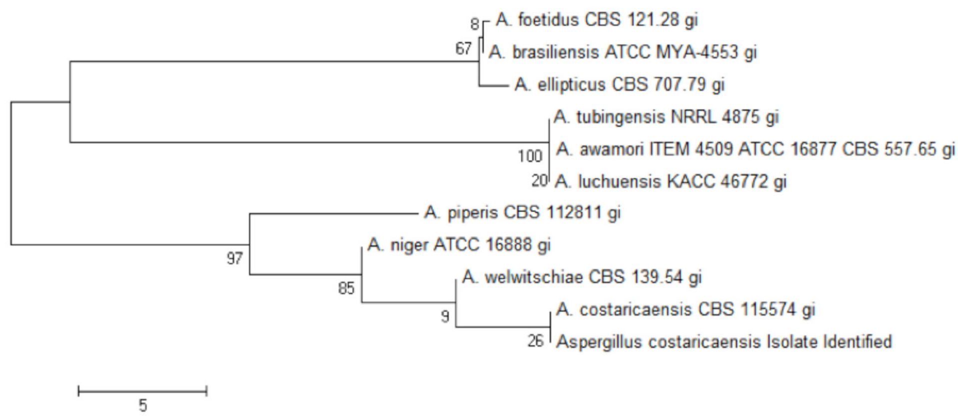

Fig. 2. Phylogenetic tree constructed for the isolated strain (*Aspergillus costaricaensis*).

study sites can be in part related to differences in water temperature.

In the present study, pH values of water samples were between slightly acidic to highly alkaline. The presence of cyanobacteria may be the reason for high alkalinity. The blue-green algae effectively use carbonate and bicarbonate ions, consequently increasing the pH or acidity of water. The acidic nature of the hot water spring of the Kullu district in Himachal Pradesh was observed (Kumar et al. 2013). However, also highly alkaline hot water of Uttarakhand was recorded (Kumar, Sharma 2019). Total alkalinity is a measurement of the water's ability to resist change in pH. Alkalinity was the highest in water of Gangnani Kund, high in Gauri Kund, and the lowest in Tapovan Kund. High total alkalinity within a range of 270 to 320 mg L^{-1} was found in the Ringigad and Saldhar hot water springs of Garhwal Himalaya in the Uttarakhand (Kumar, Sharma 2019). Moderately high alkalinity (196 mg L^{-1}) was also observed in the hot water ponds of Himachal Pradesh (Kumar et al. 2013).

Low dissolved oxygen concentration (2.52 mg L^{-1}) occurred in the hot water springs located in Kullu district of Himachal Pradesh (Kumar et al. 2013), but it was even lower in water of Tapovan Kund. Dissolved oxygen concentration between 3.25 to 3.57 mg L^{-1} was observed in thermal springs of Iran (Fazlzadeh et al. 2017).

Electrical conductivity (EC) reflects the capacity of water to conduct electrical current and is directly related to the concentration of all ions dissolved in water. EC within the same range as found in the present study was recorded in hot water springs located in Ethiopia (Haki, Gezmu 2012) and hot water ponds of the Itoigawa Shizuoka Tectonic Line in Japan (Homma, Tsukahara, (2008). As total salinity also is a measure of the quantity of salts dissolved in water, there was a strong relationship between EC and salinity across the three hot springs in the present study. High salinity was also observed in thermal springs of Malaysia (Hamzah et al. 2013).

Concentration of total dissolved solids (TDS) characterizes the total amount of mobile charged ions,

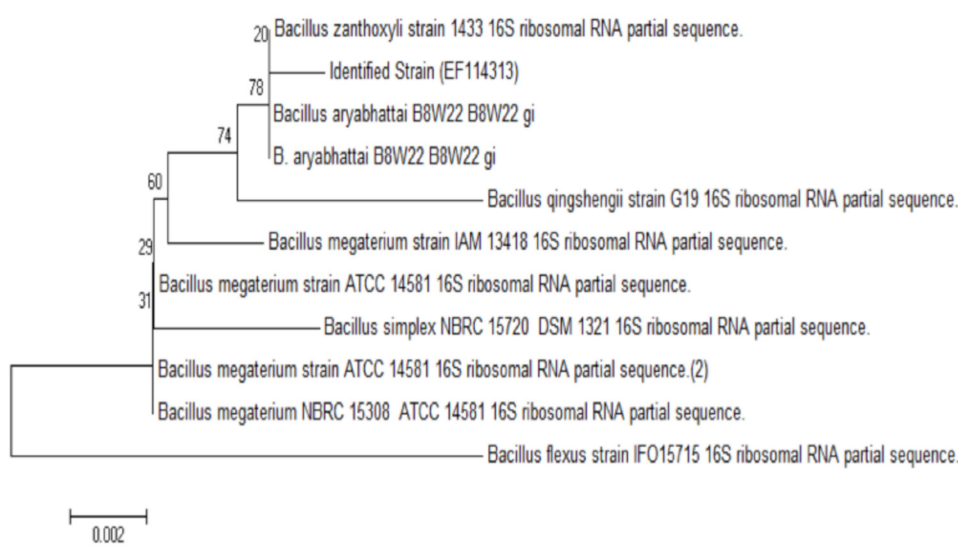

Fig. 3. Phylogenetic tree constructed for the isolated strain (*Bacillus aryabhattai*).

Table 4. Microbial diversity of thermal springs located in the Garhwal Himalaya

Sample No.	Microbial taxon	Gauri Kund	Gangnani Kund	Tapovan Kund
Bacteria				
1	*Bacillus subtilis*	+	+	+
2	*Brevibacillus borstelensis* (D78456)	+	+	–
3	*Bacillus pumilus*	+	+	–
4	*Brevibacillus reuszeri*	–	+	–
5	*Bacillus licheniformis* (AE017333)	+	+	–
6	*Bacillus circulans*	–	+	–
7	*Geobacillus stearothermophilus*	–	+	–
8	*Lysinibacillus sphaericus*	–	+	–
9	*Streptococcus thermophilus*	+	+	+
10	*Actinobacillus seminis*	+	+	+
11	*Anoxybacillus flavithermus*	–	–	+
12	*Geobacillus thermodinitrificans*	–	–	+
13	*Brevibacillus parabrevis*	+	–	+
14	*Actinobacillus hominis*	–	–	+
15	*Bacillus sonorensis*	+	–	+
16	*Pseudomonas putida*	+	–	–
17	*Bacillus thermocatenula*	+	–	–
18	*Bacillus aryabhattai* (EF114313)	+	–	–
19	*Bacillus tequilensis* (AYTO01000043)	+	–	–
20	*Bacillus subtilis* subsp. *spizizenii* (CP002905)	+	–	–
21	*Bacillus paralicheniformis* (LBMN01000156)	+	–	–
Actinomycetes				
1	*Streptomyces clavifer*	–	+	–
2	*Streptomyces rangoon*	–	+	+
3	*Thermobifida fusca*	–	+	–
4	*Streptomyces lydicus*	–	+	–
5	*Streptomyces erumpens*	–	+	+
6	*Streptomyces ruber*	+	–	+
7	*Streptomyces albus*	–	–	+
8	*Nocardiopsis prasina*	+	–	–
9	*Streptomyces albidoflavus*	+	–	–
10	*Streptomyces canescens*	+	–	–
11	*Thermoactinomyces candidus*	+	–	–
Fungi				
1	*Aspergillus fumigates*	–	+	+
2	*Aspergillus flavus*	–	+	–
3	*Aspergillus terreus*	–	+	–
4	*Cladosporium fulvum*	+	+	+
5	*Trichoderma viride*	+	+	–
6	*Thermoascus aurantiacus*	–	+	+
7	*Aspergillus costaricaensis* (NR_103604.1)	–	+	–
8	*Aspergillus terreus*	–	–	+
9	*Thermomyces lanuginosus*	–	–	+
10	*Aspergillus tubingensis*	+	–	+
11	*Aspergillus nidulans*	+	–	–
12	*Aspergillus awamori*	+	–	–
Total number of microorganisms		24	22	18

including minerals, salts or metals dissolved in a given volume of water. TDS is made up of inorganic salts, as well as a small amount of organic matter. Common inorganic salts that can be found in water include both cations (calcium, magnesium, potassium and sodium) and anions (carbonates, nitrates, bicarbonates, chlorides and sulfates).

The concentration of TDS showed high variation between water of Gauri Kund and Gangnani Kund, and Tapovan Kund. A high range of TDS was also observed in the thermal springs of Malaysia (Hamzah et al. 2013). Total hardness of water is a measurement of both calcium and magnesium. It is expressed as $CaCO_3$. Total hardness within a range of 124 to 192 mg L^{-1} was recorded in hot water ponds located near Badrinath Shrine in the Uttarakhand (Kumar, Sharma 2020). As water moves through soil and rock, it dissolves very small amounts of minerals and holds them in solution. Calcium and magnesium dissolved in water are the two most common minerals that make water "hard". The degree of hardness becomes greater as the calcium and magnesium content increases and is related to the concentration of multivalent cations dissolved in water (Oram 2018). In the hot water ponds of Jharkhand and West Bengal, cation concentration was found to be between 12.60 to 15.62 mg L^{-1} (Singh et al. 2015).

Chloride is a naturally occurring element that is common in most natural waters. Chloride concentration within a range of 106.5 to 140.58 mg L^{-1} was found in the hot water ponds of Uttarakhand (Kumar, Sharma 2020). Sulfate usually occurs as a principal ion in water. Under anaerobic conditions in which bacteria persist in the oxidation of biological material, hydrogen sulfide is produced. Orthophosphate and various organic phosphates are the most important phosphorous compounds in water. Phosphates and nitrates are heavily consumed in the upper surface of the water. Highly variable nitrate concentration between 0.089 to 0.256 mg L^{-1} was found in the Ringigad and Saldhar hot water springs of Garhwal Himalaya in the Uttarakhand (Kumar, Sharma 2019), which was similar to the levels in the present study. However, a high concentration of sulfates and nitrates in the hot water ponds of Sikkim was reported (Sherpa et al. 2013).

The majority of isolated and identified bacteria were gram-positive and such bacteria are known to be comparatively stress-resistant and long-range migrants, especially the Actinobacteria (Cerritos et al. 2011; Kumar et al. 2014). During the study period, the authors recorded a total of 44 species of microorganisms, including 21 species of bacteria, 11 species of actinomycetes and 12 species of fungi in the hot water springs. In the water sample of Gauri Kund there were 14 species of bacteria, five species of actinomycetes and five species of fungi. In the water samples of Gangnani Kund, 10 species of bacteria, five species of actinomycetes and seven species of fungi were recorded. There were eight species of bacteria, four species of actinomycetes and six species of fungi in the water sample of in Tapovan Kund. *Bacillus subtilis, Streptococcus thermophiles, Actinobacillus seminis* and *Cladosporium fulvum* were common species of microorganisms found in water samples of all three identified sampling sites. Presence of *Bacillus subtilis, Brevibacillus borestelensis, Brevibacillus parabrevis, Geobacillus stearothermophilus, Streptomyces albus, Streptomyces canescens* and *Aspergillus tubingensis* were also reported in the water samples of Ringigad and Saldhar thermal springs of Uttarakhand (Kumar, Sharma 2019). In water samples of hot water ponds located near Badrinath shrine of Uttarakhand, the species *Brevibacillus reuszeri, Bacillus licheniformis, Lysinibacillus sphaericus, Actinobacillus seminis, Streptomyces clavifer, Streptomyces lydicus, Streptomyces erumpens, Streptomyces ruber, Nocardiopsis prasina, Aspergillus flavus, Thermoascus aurantiacus*, and *Thermomyces lanuginosus* were reported (Kumar, Sharma 2020).

Thermophilic microbial communities can be used as cell factories for the preparation and manufacturing of unusual compounds as well as for treatment of contaminated soils and wastewaters. The biomolecules, mainly proteins and enzymes, found in hot adapted microorganisms have already found useful applications in molecular biology, medicine, beverage industries, soaps and beauty creams. Enzymes obtained from heat loving microorganisms are of paramount importance as they act as biocatalysts for various industrial applications. Such enzymes can work effectively even at high temperature (Lopez et al. 2013). There are many thermophiles that have industrial applications and there are various thermophilic polymer-degrading enzymes, such as amylases, chitinases and cellulases, which have great stability and activity at an extreme temperature. The bacterial species *Bacillus subtilis* that was isolated from Gauri Kund, Gangnani Kund and Tapovan Kund act as an antidiarrhoeal microorganism, which has an ability to treat diarrhea. This species is also used to produce fermented soybeans, yougurt, ice cream, milk and cheese. The bacterial species *Streptococcus thermophilus* that was isolated from all the three hot water springs has a property of boosting the immunity of a human body and can also be used in the fermentation of cheese. *Bacillus licheniformis, Bacillus megaterium, Actnobacillus hominis, Lysinibacillus sphaericus, Paenibacillus alvei, Bacillus simplex, Actnobacillus seminis, Paenibacillus fragii* and *Staphylococcus cohnii* belong to firmicutes and gamma proteobacteria produce of various hydrolytic enzymes of paramount importance, which can be degraded at an extreme temperature, i.e. caseinase, gelatinase, lipase, etc. Similar results were also reported in other studies with thernophilic bacteria isolated from hot springs (Pathak, Rathod 2014).

Conclusions

The current study on the physicochemical characteristics of Gauri Kund, Gangnani Kund and Tapovan Kund revealed that the water samples of two of the three hot water springs (Gauri Kund and Tapovan Kund) were slightly acidic or near to neutral in nature. However, the water of Gangnani Kund was highly alkaline, which may be due to the presence of cyanobacteria. All of the three hot water springs are also a rich source of thermophilic microorganisms. The α-diversity of microorganisms in Gauri Kund was 24 species and the α-diversity of microorganisms in Gangnani Kund was 22 species. The α-diversity of microorganisms in Tapovan Kund was 18 species. *Bacillus subtilis, Actinobacillus seminis* and *Streptococcus thermophilus* were the common bacterial species present in the water samples of all hot water springs. Almost all bacterial species were gram positive in nature. The current study can provide baseline data for further studies of the hot springs and provide useful characteristics of indigenous thermophilic microbial isolates that produce thermostable enzymes. After considering the available data, the decision makers can take necessary and preventive measures for conservation and management of these three thermal springs.

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