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ORIGINAL ARTICLE

Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India

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Abstract The diversity of culturable, aerobic and heterotrophic *Bacillus* and *Bacillus*-derived genera (BBDG) was investigated in various extreme environments (including thermal springs, cold deserts, mangroves, salt lakes, arid regions, salt pans and acidic soils) of India. Heat treatment followed by enrichment in different media led to a total of 893 bacterial isolates. Amplified ribosomal DNA restriction analysis (ARDRA) using three restriction enzymes *Alu*I, *Msp*I and *Hae*III led to the clustering of these isolates into 12–74 groups for the different sites at 75 % similarity index, adding up to 559 groups. Phylogenetic analysis based on 16S rRNA gene sequencing led to the identification of 392 bacilli, grouped in two families, Bacillaceae (89.03 %) and Paenibacillaceae (10.97 %), and included 13 different genera with 75 distinct species. It was found that among the thirteen genera, nine (*Bacillus, Halobacillus, Lysinibacillus*, *Oceanobacillus*, *Pontibacillus, Salinibacillus, Sediminibacillus, Thalassobacillus and Virgibacillus*) belonged to Bacillaceae and four (*Ammoniphilus, Aneurinibacillus, Brevibacillus* and *Paenibacillus*) belonged to Paenibacillaceae. Novel isolates tolerant to low and high pH and temperature, salt and low moisture were identified. The major outcome of the present investigation was the identification of niche-specific species and also the ubiquitous presence of selected species of BBDG, which illustrate the diversity and pervasive nature of BBDG in extreme environments.

Keywords ARDRA · *Bacillus* · *Bacillus* derived genera · 16S rRNA gene · Extreme habitats

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Introduction

Extreme environments represent unique ecosystems that harbor novel biodiversity. India is one among 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world (Myers et al. 2000). Microbial communities are found in most diverse conditions, including extremes of temperature, salinity, water deficiency and pH. In order to survive under such extreme conditions, these organisms, referred to as extremophiles, have developed adaptive features that permit them to grow optimally under one or more environmental extremes, while poly extremophiles grow optimally under multiple conditions (Rothschild and Mancinelli 2001). These extremophiles can grow optimally in some of the earth's most hostile environments of temperature (−2 to 20 °C, psychrophiles; 60 to 115 °C, thermophiles), salinity (2–5 M NaCl, halophiles) and pH (< 4, acidophiles and > 9, alkaliphiles) (Van den Burg 2003). In an effort to understand the diversity and distribution of cultural *Bacillus* and *Bacillus*-derived genera (BBDG) in a diverse range of extreme environments, an investigation

involving isolation, identification and characterization employing biochemical and molecular techniques was undertaken. The niches explored included hot springs (Manikaran, Chumathang, Bakreshwar, Balarampur and Vashisht) and cold deserts (Leh and Rohtang Pass), areas with high salinity (Sambhar Lake, Chilka Lake and Rann of Kutch), acidity (Andaman and Nicobar Islands and Kollam), water deficient/drought stress (Jaisalmer), as well as mangroves (Bhitarkanika and Sunderbans) in India.

Thermal springs represent unique ecological niches and harbor both mesophilic and thermophilic members, especially those belonging to the genus *Bacillus* and related genera (Cihan et al. 2012). Phylogenetic characterization of microflora has been undertaken for geothermal springs in different parts of the world (Derekova et al. 2008). In India, Bakreshwar, Balarampur, Chumathang, Manikaran and Vashisht hot water springs represent an unusual niche with temperatures in the range of 60 to 100 °C; these springs can be potential sources of novel genes and microorganisms (Kumar et al. 2013). Prospecting cold habitats has led to the isolation of a great diversity of microorganisms (Sahay et al. 2013). Leh (Jammu and Kashmir) and Rohtang Pass (Himachal Pradesh) represent cold deserts and a niche for cold adapted microorganisms that have immense significance in the field of biotechnology, because of their distinct metabolism. Psychrophilic microorganisms are also potential sources of novel pigments (as food additives), enzymes, antifreeze compounds—which can be valuable in agriculture as inoculants (plant growth-promoting bacteria)—or bio control agents in extreme habitats (Khan and Patel 2007; Srinivas et al. 2009).

Extremes of high (alkaline) and low (acidic) pH also influence the buildup of microbial population, and in turn, soil productivity. If the soil is acidic, the availability of essential nutrients, particularly, phosphorus, calcium, magnesium, and molybdenum, is affected. Very few reports are available on the distribution and diversity of bacteria in acidic soils (Yadav et al. 2011; Verma et al. 2013). Another useful extreme environment is the mangrove ecosystem, which is mostly nutrient-deficient, especially in terms of nitrogen and phosphorus. In spite of this, mangroves can be highly productive, which can be attributed to microbial activity leading to major nutrient transformations. Sunderbans (West Bengal) and Bhitarkanika (Odisha) are important mangroves of India that represent salt tolerant, complex and dynamic ecosystems. Microbial research in saline environments has also attracted the interest of researchers due to various biotechnological applications (Sahay et al. 2011, 2012; Pandey et al. 2013). Soda lakes and deserts represent the most stable naturally occurring alkaline environments on Earth. Sambhar Lake (Rajasthan), Chilka Lake in Odisha, and the Great Rann of Kutch (Gujarat) are typical saline environments in India, and very few reports on microbial diversity in these habitats are available (Sahay et al. 2012). Low moisture conditions coupled with high temperatures in arid deserts lead to enrichment of microbial communities that can survive extreme variations in temperature and moisture. Such environments encompass typically poor soils with low organic content and limited amounts of bioavailable inorganic nutrients. The microbiota of desert ecosystems is not only responsible for the productivity, biogeochemical cycling of elements and ecosystem balance, but also for soil neogenesis and improvement of soil structure. Diversity of 16S rRNA and *nif*H genes derived from the rhizosphere of endemic drought tolerant grass *Lasiurus sindicus* has been reported from the samples collected from Jaisalmer (Rajasthan) (Chowdhury et al. 2009). However, the cultural diversity of bacteria in the Thar Desert has not been characterized.

The genus *Bacillus* is a heterogeneous collection of aerobic or facultative anaerobic endospore-forming bacteria that are ubiquitous in many environments. The sequencing of 16S rDNA led to the identification of five phylogenetically distinct groups within the genus *Bacillus* (Ash et al. 1991). Since 1990, several new genera, such as *Amphibacillus* (Niimura et al. 1990), *Paenibacillus* (Ash et al. 1991), *Alicyclobacillus* (Wisotzkey et al. 1992), *Aneurinibacillus*, *Brevibacillus* (Shida et al. 1996), *Virgibacillus* (Heyndrickx et al. 1998), *Gracilibacillus, Salibacillus* (Waino et al. 1999), *Geobacillus* (Nazina et al. 2001), *Filobacillus* (Schlesner et al. 2001), *Jeotgallibacillus*, *Marinibacillus* (Yoon et al. 2010) and *Ureibacillus* (Fortina et al. 2001), have been sorted out. *Bacillus* species are phenotypically and genotypically heterogeneous (Claus and Berkeley 1986). Members of the families Bacillaceae and Paenibacillaceae are widely used in agriculture as plant growth-promoting and disease-suppressing agents, besides their use in industry as a source of enzymes and in medicine. The importance of this group is reflected in the large number of studies targeting their phenotypic and genotypic diversity in different ecological niches (Suihko and Stackebrandt 2003, Sass et al. 2008, Ettoumi et al. 2009, Yadav et al. 2011; Kumar et al. 2013) in food (Oguntoyinbo et al. 2010) and industry waste (Freitas et al. 2008). *Bacillus thuringiensis* is currently used in the biological control of insects (Perez et al. 2007).

The present investigation was undertaken towards exploring the diversity and pervasive nature of BBDG in extreme environments and identifying niche-specific species, using a combination of culture-based, biochemical and molecular approaches.

Material and methods

Sampling sites

Water, soil and sediment samples were collected from different extreme environments of India (Fig. 1), whose details are

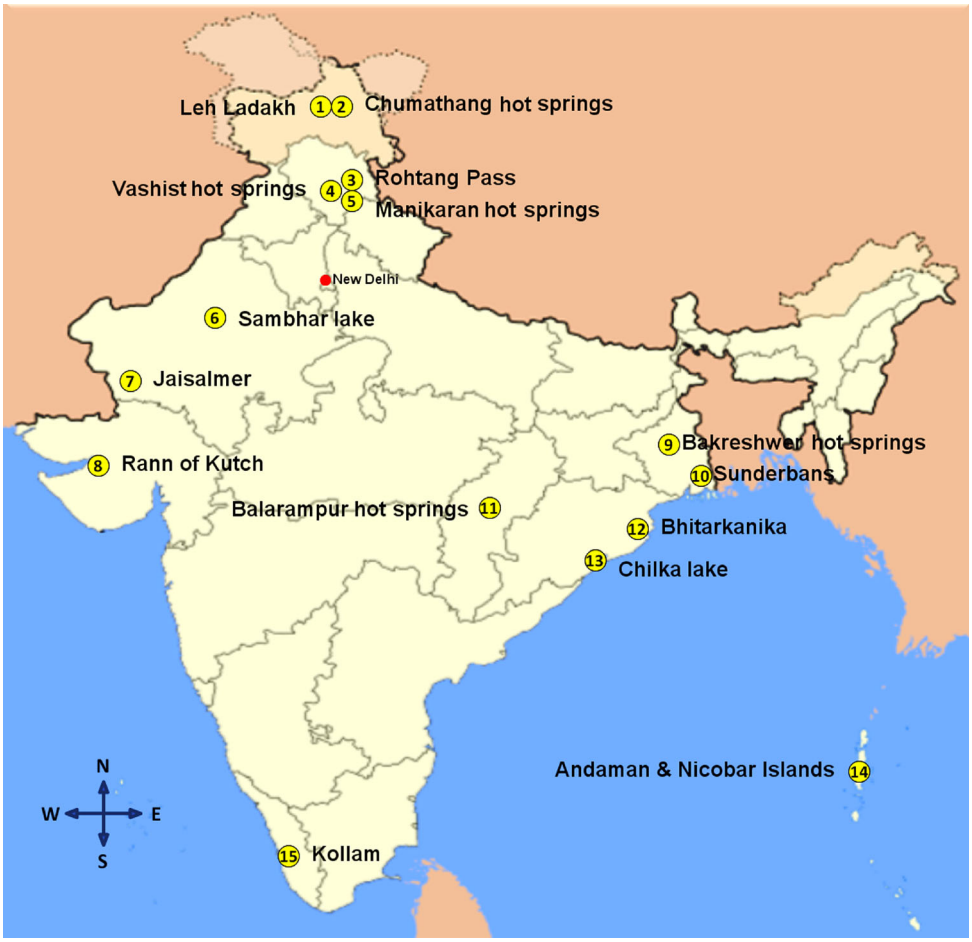


Fig. 1 Map of India depicting sampling locations

given in Table 1. A total of 152 samples were collected from fifteen sites comprising various habitats. The sites surveyed included those exhibiting extreme conditions of pH, temperatures, salinity and drought. Samples were collected in sterile polythene bags/bottles, labeled, transported on ice and stored at 4 °C until analysis. The pH and conductivity of the samples was recorded on site.

Enrichment and isolation

The population of BBDG in the water, sediments and soil samples were enumerated through enrichment using the standard serial dilution plating technique. One gram of sediment/soil or 1 ml of water sample was added to 9 ml of sterile distilled water and heated in a hot water bath at 80 °C for 15 min to kill the vegetative cells. The samples were diluted and appropriate dilutions were spread on nutrient agar (NA) plates amended with methyl red (0.02 %). In addition to NA medium (HiMedia Laboratories, Mumbai, India), other media and conditions were used for the isolation of different categories of extremophiles (details given in Table 2). Selective isolation of *Bacillus thuringiensis* was done using sodium acetate (0.25 and 0.75 M) buffer (HiMedia Laboratories, Mumbai, India), followed by Luria broth enrichment and heat treatment at 80 °C for 15 min. The samples were then diluted and appropriate dilutions were spread on nutrient agar and T3 agar medium plates (Travers et al. 1987).

The nutrient agar and soil extract agar plates having pH of 3–5 and 9–11 were used to isolate acidophilic and alkaliphilic BBDG, and were incubated at 30 °C for 2–3 days. To isolate psychrophilic and thermophilic BBDG, plates were incubated at 4 °C and 45 °C, and populations were counted after 15–20 days (Larkin and Stokes 1966) and 2–3 days, respectively. For isolation of halophilic BBDG, aerobic enrichment cultures were prepared by adding 1.0 g of each sample into separate Erlenmeyer flasks and 100 ml of chemically defined medium or halophilic medium, followed by incubation on a shaker (Kuhner LT-X, Dinkelbergstrasse 1, Switzerland) at 30 °C. An aliquot of 100 μL from each enriched sample was spread-plated on four different media listed in Table 2. Plates were incubated at 30 °C for a maximum period of 5–7 days. Colonies that appeared were purified by repeated re-

Table 1 Geographic details and physico-chemical characteristics of collection sites

Habitat	Sampling location	Latitude	Longitude	No. of samples	Collection date	Temp.(°C)	pH	Conductivity (mS cm^{-1})
High temperature								
	Chumathang, Jammu and Kashmir	33° 18′ 00″ N	78° 24′ 00″ E	LC (07)	March 2010	65–72	6.5–8.4	0.486–0.552
	Vashisht, Himachal Pradesh	32° 16′ 00″ N	77° 10′ 56″ E	VH (05)	April 2010	43–55	6.5–7.4	0.116–0.118
	Manikaran, Himachal Pradesh	32° 02′ 00″ N	77° 20′ 48″ E	MH (8)	April 2010	88–94	7.5–7.8	0.118–0.210
	Bakreshwar, West Bengal	23° 52′ 48″ N	87° 22′ 12″ E	BW (13)	July 2011	55–80	7.8–8.2	0.112–0.114
	Balrampur, Chhattisgarh	23° 60' 67" N	83° 62' 03" E	BC (10)	Dec 2011	90–92	7.2–7.8	0.124–0.128
Low temperature								
	Leh, Ladakh, Jammu and Kashmir	34° 08′ 31″ N	77° 34′ 11″ E	LL (11)	March 2010	−10−+15	6.8–8.6	0.078–0.875
	Rohtang Pass, Himachal Pradesh	32° 22′ 17″ N	77° 14′ 47″ E	RP (11)	Sept 2011	−10−+18	7.2–8.2	0.030–0.232
Mangroves								
	Bhitarkanika, Odisha	20° 30′ 10″ N	86° 45′ 00″ E	BO (12)	Dec 2009	34–36	7.8–8.4	2.90–4.30
	Sunderbans, West Bengal	21° 55′ 13″ N	88° 44′ 46″ E	SW (10)	Nov 2010	30–32	7.8–8.6	1.80–3.40
Salinity								
	Sambhar Lake, Rajasthan	26° 58′ 37″ N	75° 05′ 00″ E	SL (14)	April 2011	35–37	8.2–9.8	2.78–16.7
	Rann of Kutch, Gujarat	24° 05′ 11″ N	70° 38′ 16″ E	KG (11)	Jan 2010	32–35	8.2–8.8	46.9–48.5
	Chilka Lake, Odisha	19° 39′ 56″ N	85° 09′ 56″ E	CL (14)	Oct 2009	28–30	7.8–8.8	12.2–42.2
Drought								
	Jaisalmer, Rajasthan	26° 55′ 00″ N	70° 54′ 00″ E	JR (13)	March 2011	35–37	7.8–8.2	0.39–0.49
Acidic								
	Andaman and Nicobar Islands	09° 09′ 55″ N	92° 46′ 26″ E	AN (08)	May 2011	30–32	4.2–5.4	0.425–0.862
	Kollam, Kerala	11° 26′ 36″ N	75° 40′ 60″ E	KK (05)	May 2011	32–35	4.4–5.2	0.384–0.392

streaking to obtain isolated colonies using respective medium plates. The pure cultures were maintained at 4 °C as slant and glycerol stocks (20 %) at −80 °C for further use.

PCR amplification of 16S rDNA and amplified rDNA restriction analysis (ARDRA)

Genomic DNA was extracted by the modified Pospiech and Neumann method (1995). The primers pA (5′-AGAGTTTG ATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATC CAGCCGCA-3′) were used for the amplification of 16S rDNA (Edwards et al. 1989). The amplification was carried out in a 100 μl volume, and amplification conditions as described earlier were used (Sahay et al. 2011). After amplification, the PCR products were resolved by electrophoresis in 1.2 % agarose gel in 1X TAE buffer (Bangalore Genei, Bangalore, India). Gels were stained with ethidium bromide (10 mg ml^{-1}), (Hengstmann et al. 1999), visualized on a gel documentation system (Alpha-Imager), and gel images were digitalized. PCR-amplified 16S rDNA products were purified with a Quiaquick purification kit (Qiagen). Aliquots of purified 16S rDNA PCR products were digested separately with three restriction endonucleases, *Alu*I, *Hae*III and *Msp*I (Bangalore Genei, Bangalore, India), in 25 μl reaction volumes, using the manufacturer's recommended buffer and temperature. Restricted DNA was analyzed by horizontal electrophoresis in 2.5 % agarose gels. The gels were visualized and gel images were digitalized. Strong and clear bands were scored for similarity, and clustering analysis was undertaken using the software, NTSYS-2.02e package (Numerical taxonomy analysis program package, Exeter software, USA). Similarity among the isolates was calculated by Jaccard's coefficient (Jaccard 1912) and dendrogram was constructed using the UPGMA method (Nei and Li 1979).

16S rDNA sequencing and phylogenetic analysis

The nucleotide sequences of purified 16S rDNA were dideoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Bio systems) and run in a 3130xl Applied Bio systems ABI prism automated DNA sequencer. The DNA sequence was double-checked by sequencing both strands using primers pA and pH for forward and reverse reactions, respectively. The partial 16S rDNA sequences of the isolated strains were compared with those available in the databases. Identification at the species level was determined using a 16S rRNA gene sequence similarity of ≥97 % with that of a prototype strain sequence in the GenBank (Benson et al. 2010). Sequence alignment and

Table 2 Media and the conditions employed for isolation of different categories of extremophiles

Category	Media and conditions
Acidophilic	Nutrient agar (NA): 5 g peptone; 5 g NaCl; 3 g beef extract; 20 g agar (pH 3–5)
	Soil extract agar: 2 g glucose; 1 g Yeast Extract; 0.5 g K_2HPO_4; 100 ml Soil extract[a]; 20 g agar; (pH 3–5)
Alkaliphilic	Nutrient agar (pH 8–11)
	Soil extract agar (pH 8–11)
Thermophilic	Nutrient agar
	Soil extract agar
	Tryptic soy agar: 17 g tryptone; 3 g soya meal; 2.5 g dextrose; 5 g NaCl; 2.5 g K_2HPO_4; 20 g agar; pH 7.2
Psychrophilic	NA, NA diluted 10 times, 100 times
	Soil extract agar
	Tryptic soy agar
Halophilic	NA (with 5,10, 15 and 20 % NaCl concentration)
	Soil extract agar
	Chemically defined medium: 5 g casamino acids; 5 g yeast extract; 1 g sodium glutamate; 3 g tri-sodium citrate; 20 g $MgSO_4$; 2 g KCl; 100 g NaCl; 36 mg $FeCl_2$; 0.36 mg $MgCl_2$; 20 g agar; pH 7.0–7.2
	Halophilic medium: 100 g NaCl; 2 g KCl; 1 g $MgSO_4 \cdot 7H_2O$; 0.36 g $CaCl_2 \cdot 2H_2O$; 0.23 g NaBr; 0.06 g $NaHCO_3$; 5 g protease-peptone; 10 g yeast extract; 1 g glucose; trace FeCl3; 20 g agar; pH 7.2–7.4

[a] Soil extract: 250 g soil from sampling site+1 L H_20, autoclave and filter

comparison was performed, using the program CLUSTAL W (Thompson et al. 1994) with default parameters and the data converted to PHYLIP format. Minor modifications were done manually on the basis of conserved domains, and positions containing more than 50 % gaps were removed. One sequence from each group was selected as a representative operational taxonomic unit (OTU). The phylogenetic tree was constructed on the aligned data sets using the neighbor-joining method (Saitou and Nei 1987) implemented in the program MEGA 4.0.2 (Tamura et al. 2007). Bootstrap analysis on 1,000 random samples taken from the multiple alignments was performed as described by Felsenstein (1981).

The partial 16S rDNA sequences were submitted to NCBI GenBank (Benson et al. 2010), and accession numbers were assigned as given in Table 3.

Tolerance of isolates to extremes of pH, temperature, salinity and drought

The screening of all the isolates for pH tolerance was carried out by spot inoculating the cultures onto nutrient agar medium with pH ranging from 3 to 11, with an increment of 1. The pH in the medium was adjusted by addition of acid phthalate buffer (pH 3–4), neutralized phthalate buffer (pH 5.0), 50 mM phosphate buffer (pH 6.0–8.0)], bicarbonate buffer (pH 9.2–10.6) or sodium hydroxide/potassium chloride buffer (pH 12.0–13.0) (HiMedia Laboratories, Mumbai, India). The isolates from saline habitats (Sambhar Lake, Chilka Lake, Rann of Kutch, Bhitarkanika and Sunderbans) were screened for tolerance to salt (5 to 25 % NaCl concentration) by spot inoculating each of the isolates onto nutrient agar plate supplemented with NaCl to total concentrations of 5, 10 15, 20 and 25 % (w/v). Isolates from drought habitat were screened for tolerance to low water potential on polyethylene glycol (PEG-8000)-infused plates of −0.5, 0.7 and −1.2 MPa water potential, following the procedure described by Verslues and Bray (2006). The isolates were screened for temperature tolerance by incubating the culture spot inoculated plates at different temperatures of 5, 15, 20 and 25 °C (for low temperature tolerance) and at 40, 45, 50, 55 and 60 °C (for high temperature tolerance) for 72–96 h. The cultures tolerant to 60 °C were screened further by inoculating in broth and incubating in shaker at 150 rpm for 72 h at 75 and 90 °C. The optical density of the broth was measured at 600 nm and compared with the growth at 37 °C.

Statistical analysis

In order to compare the bacterial diversity among fifteen different habitats, the 16S rRNA gene sequences of the isolates showing ≥ 97 % sequence similarity were grouped into the same OTU (phylotype). The software Shannon–Wiener Diversity Index/Shannon Entropy Calculator (http://www.changbioscience.com/genetics/shannon.html) and Rarefaction Calculator (http://www2.biology.ualberta.ca/jbrzusto/rarefact.php) were used to calculate Shannon index (H), Evenness (J) and the Simpson's index (D).

Results

Abundance and diversity analyses of BBDG

The population of BBDG was enumerated in different samples collected from fifteen sites of India having extreme environmental conditions (Table 1). The abundance of BBDG in the samples varied from 4.5×10^3 to 4.0×10^6 CFU g^{-1} sediment or ml^{-1} water, with the highest values in samples from Chilka Lake, followed by Kollam, Kerala (9.5×10^5 CFU g^{-1} sediment or ml^{-1} water), and the lowest values in were in samples from Sunderbans, followed by Chumathang (1.1×10^4 CFU g^{-1} sediment or ml^{-1} water) (Table 3). The diversity of morphotypes was highest at Sunderbans (99) and Rann of Kutch (99), and lowest (24) at Kollam (Table 3).

Table 3 Enumeration of isolates using standard techniques, Gen Bank Accession numbers and classification based on 16S rDNA-PCR–RFLP profiles

Location name	Average CFU ml^{-1} or g^{-1}soil	No. of isolates	No. of clusters[a]	Accession number	No. of BBDG	Niche-specific BBDG
Chumathang, Jammu and Kashmir	1.1×10^4	32	18	JX312603, JX312607-8, JX312611, JX312612	5	
Vashisht, Himachal Pradesh	6.7×10^4	26	12	JN411368, JN411370, JN411372, JN411374-75	5	*Brevibacillus*
Manikaran, Himachal Pradesh	1.3×10^4	58	45	JF343230, JF343233, JF343235, JN411328-31, JN411334-39, JN411341, JN411343, JX312613-16, JX312621, JX312624, JX312625-26, JX312628, JX312631- JX312633-34, JX312636-39	31	*Aneurinibacillus danicus, Bacillus beijingensis, B. vireti, Brevibacillus agri, Lysinibacillus xylanilyticus, Paenibacillus pabuli, P. tylopili*
Bakreshwar, West Bengal	4.1×10^4	41	23	JN411315-18, JN411321-25, JN411327, JX312592, JX312594-97, JX312599, JX428951	17	*Bacillus fusiformis*
Balrampur, Chhattisgarh	5.3×10^4	38	24	JX312575- JX312583, JX312585-88	13	*Brevibacillus* sp.
Leh, Ladakh, Jammu and Kashmir	1.5×10^4	90	74	JF343177-78, JF343180-81, JF343183, JF343185-86, JF343189-90, JF343192-93, JF343195, JF343197-98, JF343201-02, JF343204-5, JF343207, JF343212-14, JN411432-34, JN411438-42, JN411448, JN411459-65, JN411467, JX428996- 98, JX429001-02, JX429004, JN411300- 302, JN411304-314	59	*Bacillus muralis, Lysinibacillus fusiformis, Paenibacillus terrae*
Rohtang Pass, Himachal Pradesh	1.3×10^4	55	32	JX429005-06, JX429008-13, JX428965, JX428967, JX460851	11	*Bacillus psychrosaccharolyticus, Paenibacillus lautus*
Bhitarkanika, Odisha	6.5×10^5	85	49	JN411376- JN411381, JN411384-85, JN411388-97, JX460806- JX460810, JX460812- JX460814-16, JX460818-22, JX428950	33	*Bacillus atrophaeus, B. bombysepticus, B. methylotrophicus*
Sunderbans, West Bengal	4.5×10^3	99	56	JN411476-83, JN411487-88, JN411491-93, JN411495, JX460823-24, JX460828-29, JX460831, JX460835-36, JX460839-43 JX460845-48, JX428969-70	32	*Bacillus alcalophilus, B. aquimaris B. cibi, B. barbaricus, B. horikoshii, B. hwajinpoensis, B. nanhaiensis, B. vietnamensis, Halobacillus litoralis, Paenibacillus amylolyticus, P. tundra, P. taichungensis*
Sambhar Lake, Rajasthan	4.0×10^4	51	35	JX428971- JX428977, JX428981, JX428983, JX428985-86, JX428988- JX428994, JX645202-13, JX645218, JX645221, JX645226-27, JX645231	35	*Ammoniphilus* sp. *Bacillus decolorationis, B. halodurans, B. methanolicus, B. safensis, B. vallismortis, Halobacillus dabanensis, H. trueperi, Oceanobacillus manasiensis*
Rann of Kutch, Gujarat	1.5×10^4	99	53	JN411344-54, JN411356, JN411358, JN411360, JN411363, JN411365, JN411367, JX428957-58 JF802166- JX518269	61	*Bacillus aerophilus, B. foraminis, B. sonorensis, Salinibacillus aidingensis, Sediminibacillus halophilus, Thalassobacillus devorans, Virgibacillus salarius*
Chilka Lake, Odisha	4.0×10^6	91	57	JF343134-43, JF343146-49, JF343154-56, JF343159-61, JF343164, JF343166, JF343168,	35	*Pontibacillus* sp.

Table 3 (continued)

Location name	Average CFU ml^{-1} or g^{-1}soil	No. of isolates	No. of clusters[a]	Accession number	No. of BBDG	Niche-specific BBDG
Jaisalmer, Rajasthan	8.8×10^4	56	34	JF343171-75, JN411468-69, JN411471-72, JN411474, JX428952, JX428956 JN411400- JN411414, JN411416-17, JN411419-22, JX441879- JX441881	24	*Bacillus endophyticus*
Andaman and Nicobar Islands	6.9×10^4	48	32	JN411276- JN411288, JN411290- JN411298	22	*Bacillus pseudomycoides*
Kollam, Kerala	9.5×10^5	24	15	JN411423- JN411431	9	*Bacillus amyloliquefaciens*
		893	559		392	

[a] No of clusters after digestion with three restriction enzymes: -*Alu*I, *Hae*III and *Msp*I

BBDG–*Bacillus* and *Bacillus*-derived genera

PCR amplification of 16S rDNA and amplified rDNA restriction analysis (ARDRA)

PCR amplification of 16S rDNA followed by ARDRA with three restriction endonucleases was carried out to look for the species variation among the morphotypes selected. The 16S rDNA amplicons were digested with restriction enzymes, which generated profiles having 3 to 7 fragments ranging in size from 100 to 820 base pairs. ARDRA results revealed that among the restriction endonucleases, *Alu*I was more discriminatory as compared to *Msp*I and *Hae*III. A combined dendrogram was constructed for each sampling site to determine the percent similarity among the isolates. At a level of 75 % similarity (Table 3), the isolates were grouped into clusters; and the number of clusters ranged from 12 (for Vashisht thermal springs) to 74 (for Leh cold desert). The total number of clusters was 559, summed up for all the sites.

16S rRNA gene sequencing and phylogenetic analysis

16S rRNA gene sequencing and phylogenetic analysis of a representative isolate from each cluster revealed that all the isolates showed > 97 to 100 % similarity with the sequences within GenBank (Table 3). A total of 392 isolates were identified as BBDG. The remaining 167 isolates (which included genera such as *Desemzia, Exiguobacterium, Jeotgalicoccus Planococcus, Sporosarcina*, and *Staphylococcus*) showed no similarity with BBDG; hence, these were excluded from this study. One sequence from each group was selected as a representative operational taxonomic unit (OTU), and all the isolates were classified into 75 OTUs using a ≥ 97 % sequence similarity cut-off value. The phylogenetic tree of 75 identified species of BBDG was constructed to determine their affiliations (Fig. 2). Analysis of the 16S rRNA sequences revealed that 61 strains belonged to Bacillaceae, whereas 14 strains belonged to Paenibacillaceae. The 61 Bacillaceae members were further distributed into 16 clusters, the major clusters being the *Bacillus cereus* group (*Bacillus cereus*, *Bacillus anthracis*, *B. thuringiensis*, *B. mycoides*, *B. bombysepticus* and *B. pseudomycoides*) and the *B. subtilis* group (*B. altitudinis*, *B. pumilus*, *B. safensis*, *B. amyloliquefaciens*, *B. subtilis*, *B. vallismortis*, *B. mojavensis*, *B. licheniformis* and *B. tequilensis*). *Halobacillus, Pontibacillus, Oceanobacillus manasiensis* and *Virgibacillus* sp. representing halophilic bacilli isolated from salt lake were present in one cluster; while moderately salt tolerant bacilli including *B. baekryungensis, B. barbaricus, B. decolorationis, B. halodurans, B. hwajinpoensis* and *B nanhaiensis* were present as a separate cluster. *B. aryabhattai*, *B. megaterium*, *B. flexus*, *B. horikoshii*, *B. simplex* and *B. psychrosaccharolyticus*, despite being salt tolerant bacilli, were present in a separate cluster. Fourteen strains of Paenibacillaceae were distributed in three

different clusters, three species of *Brevibacillus* constituting one cluster, 11 species of *Paenibacillus* formed the second, and one species each of *Aneurinibacillus* and *Ammoniphilus* formed the third cluster.

Diversity analyses

The species-wise distribution of the BDDG isolates from the present investigation is given in Fig. 3, and in Table 4 in terms of their abundance in the different environments. *Bacillus cereus* was among the most commonly recorded species. Two typing methods (ARDRA profiling and 16S rRNA gene sequence analysis) adopted for examining the diversity of BBDG provided a similar level of resolution. Shannon's Diversity index was highest (H′=3.04) for Sunderbans, followed by Manikaran hot springs (H′=2.73), whereas Kollam recorded the lowest value (H′=1.73). The lowest species richness was recorded in BBDG of Chumathang and Vashisht hot springs, while Sunderbans recorded the highest species richness (Table 5). The individual rarefaction curves for all the samples of fifteen sites indicated that the bacterial populations were the least diverse in Chumathang hot springs followed by Vashisht hot springs, and most diverse in Sunderbans followed by Rann of Kutch (Fig. 4).

Tolerance of isolates to extremes of pH, temperature, salinity and drought

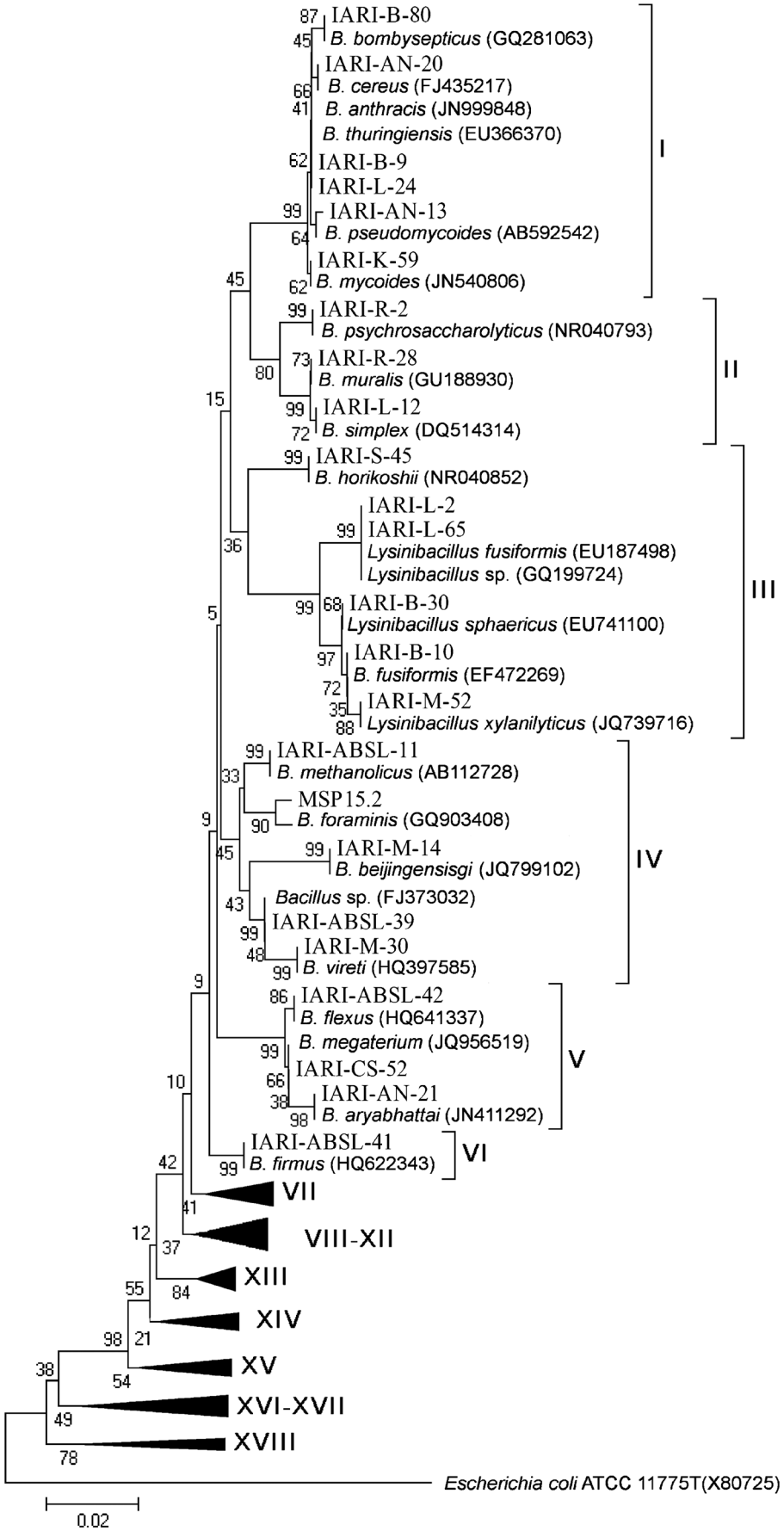
All the isolates were screened for tolerance to a range of salinities, pH, temperatures and PEG-mediated water deficit (drought) (Fig. 4). All the 196 bacterial isolates from Chilka Lake, Sambhar Lake, Rann of Kutch, Bhitarkanika, and Sunderbans were tolerant to 5 % NaCl, while 87, 72 and 31 isolates could tolerate 10, 15 and 20 % NaCl, respectively (Fig. 5a). Two isolates, *Virgibacillus halodenitrificans* ABK-2 and *Bacillus marisflavi* AB-18 from Rann of Kutch could grow even at 30 % NaCl concentration. In general, the isolates obtained from Sambhar Lake and Rann of Kutch, showed tolerance to higher levels of salinities (20 % NaCl or more). Among the representative isolates from each cluster, all except CS-39 were able to tolerate 10 % NaCl concentration, 25 were able to tolerate 15 %, whereas eight isolates could tolerate 20 % NaCl concentration. Three isolates, *Bacillus halodurans* ABSL-8, *B. methanolicus* ABSL-11 and *Ammoniphilus* sp. ABSL-12, were obtained from Sambhar Salt Lake; one isolate from Bhitarkanika mangrove soil, *Bacillus mojavensis* AB-10, and two isolates from Sunderbans mangrove soil, *Halobacillus litoralis* S-91 and *Bacillus hwajinpoensis* S-93, were able to grow at 20 % NaCl and pH 9.0. Eight isolates, *Bacillus* sp. NSP22.2, *Bacillus* sp. SB47, *Thalassobacillus devorans* strain MSP14, *Bacillus* sp. NSP10, *Bacillus* sp. MSP18.3, *Salinibacillus aidingensis* strain MSP4 and *Bacillus licheniformis* strain NSP4 obtained from Rann of Kutch, were obligate halophiles, as they required 5 % NaCl in the medium for their growth. All isolates from Andaman and Nicobar Island and Kollam were acidotolerant and could grow at pH 5 (Fig. 5b). Seventy-one bacterial isolates obtained from the thermal springs of Manikaran, Bakreshwar, Vashisht, Balrampur and Chumathang were tolerant to temperatures of 55 °C or more (Fig. 5c). Isolates from Manikaran thermal spring could grow at temperatures ranging from 45 to 90 °C. Only two isolates, *Paenibacillus pabuli* M-10 and *Paenibacillus tylopili* M-18 from Manikaran hot springs, could tolerate temperatures as high as 90 °C. Most of isolates from the hot water springs were alkalitolerant and could grow at pH 9.

All the isolates obtained from Leh and Rohtang Pass were psychrotolerant and could grow at 10 °C (Fig. 6a). Two isolates, *Paenibacillus xylanexedens* L-76 and *Paenibacillus terrae* L-102 from Leh, and one isolate, *Bacillus psychrosaccharolyticus* R-2 obtained from Rohtang Pass, grew well at 4 °C and are psychrophilic, as they could not grow beyond 20 °C. Interestingly, isolate R-2 could tolerate 5 % NaCl and a pH of 5.0. Among the 24 isolates obtained from Jaisalmer, 17 were able to grow on PEG-infused plates with water potential of −0.5 Mpa (Fig. 6b). Three representative isolates, *Bacillus mycoides* J-4, *B. megaterium* J-5 and *B. endophyticus* J-13 obtained from Jaisalmer, were tolerant to moisture stress (−0.5 Mpa and 10 % NaCl).

Fig. 2 Phylogenetic tree showing the relationships among 75 BBDG, 16S rRNA gene sequences with reference sequences obtained through BLAST analysis. The sequence alignment was performed using the CLUSTAL W program and trees were constructed using Neighbor joining with algorithm using MEGA4 software (Tamura et al. 2007). One thousand bootstrap replicates were performed. Bootstrap values are indicated on the branches. The tree was rooted using *E. coli* as the outgroup

Discussion

Microorganisms represent the richest gamut of molecular and chemical diversity in nature, as they comprise the simplest, yet dynamic, forms of life. Interest in the exploration of microbial diversity has been spurred by the fact that microbes are essential for life as they perform numerous functions integral to the sustenance of the biosphere, including nutrient cycling and environmental detoxification, which involve processes such as augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture. Microorganisms abound in all kind of habitats, viz. with extremes of pH, temperature, salinity and water stress. More recently, this largely unexplored reservoir of resources has become the


IARI-B-80
B. bombysepticus (GQ281063)
IARI-AN-20
B. cereus (FJ435217)
B. anthracis (JN999848)
B. thuringiensis (EU366370)
IARI-B-9
IARI-L-24
IARI-AN-13
B. pseudomycoides (AB592542)
IARI-K-59
B. mycoides (JN540806)
I
IARI-R-2
B. psychrosaccharolyticus (NR040793)
IARI-R-28
B. muralis (GU188930)
IARI-L-12
B. simplex (DQ514314)
II
IARI-S-45
B. horikoshii (NR040852)
IARI-L-2
IARI-L-65
Lysinibacillus fusiformis (EU187498)
Lysinibacillus sp. (GQ199724)
IARI-B-30
Lysinibacillus sphaericus (EU741100)
IARI-B-10
B. fusiformis (EF472269)
IARI-M-52
Lysinibacillus xylanilyticus (JQ739716)
III
IARI-ABSL-11
B. methanolicus (AB112728)
MSP15.2
B. foraminis (GQ903408)
IARI-M-14
B. beijingensisgi (JQ799102)
Bacillus sp. (FJ373032)
IARI-ABSL-39
IARI-M-30
B. vireti (HQ397585)
IV
IARI-ABSL-42
B. flexus (HQ641337)
B. megaterium (JQ956519)
IARI-CS-52
IARI-AN-21
B. aryabhattai (JN411292)
V
IARI-ABSL-41
B. firmus (HQ622343)
VI
VII
VIII-XII
XIII
XIV
XV
XVI-XVII
XVIII
Escherichia coli ATCC 11775T(X80725)
0.02

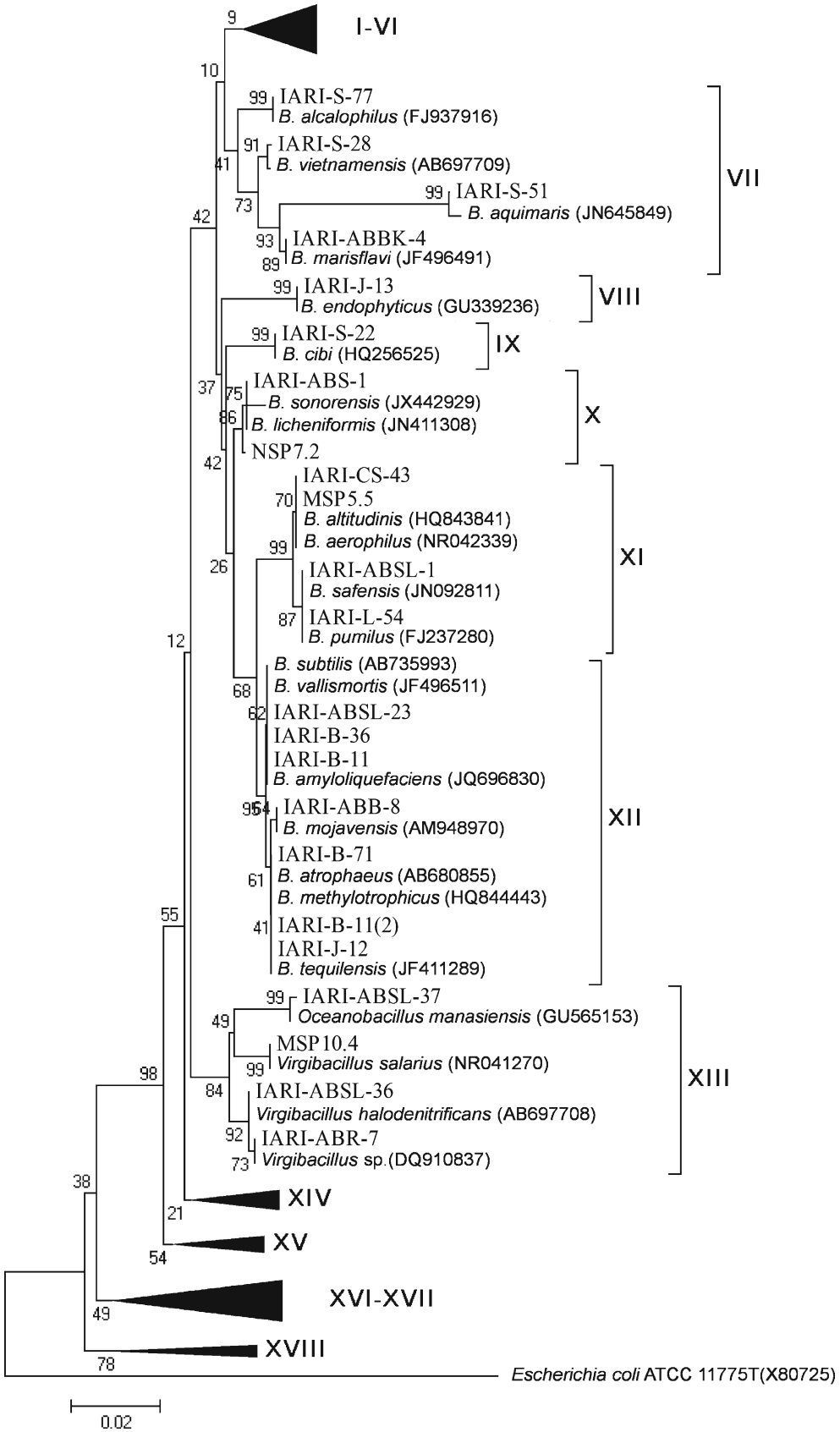


Fig. 2 (continued)

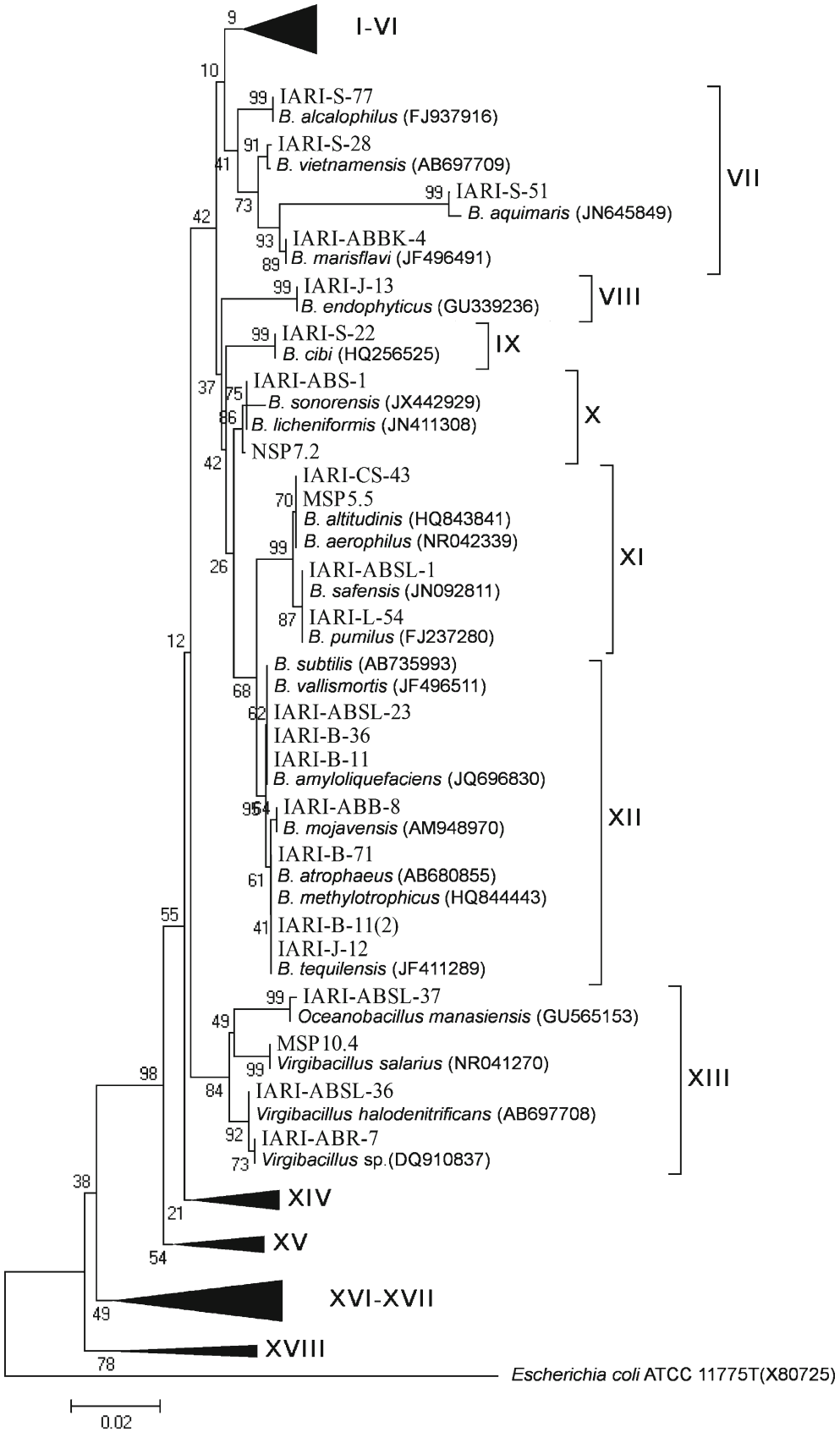


Fig. 2 (continued)

Table 4 Distribution of predominant BBDG in the different extreme environments

S. No	Species	Extreme environments[a]	No of isolates
1.	*Bacillus aryabhattai*	MB, SW, BC, BO, AN	5
2.	*Bacillus cereus*	LL, LC, RP, VH, MB, JR, KG, BK, SW, BC, BO, CL, AN, KK	14
3.	*Bacillus endophyticus*	SL, JR, KG, AN	4
4.	*Bacillus firmus*	LL, LC, SL, JR, BK, BC, CL	7
5.	*Bacillus flexus*	LL, MB, SL, BK, BC, CL	6
6.	*Bacillus licheniformis*	LL, MB, KG, BK, SW, BC	6
7.	*Bacillus megaterium*	MB, JR, KG, BK, SW, BC, BO, CL, AN, KK	10
8.	*Bacillus pumilus*	LL, LC, MB, SL, KG, BC, CL, AN, KK	9
9.	*Bacillus simplex*	LL, RP, MB, JR, BK, BO, CL	7
10.	*Bacillus* sp.	LL, LC, VH, MB, SL, JR, KG, BK, SW, BC, BO, CL, AN, KK	14
11.	*Bacillus subtilis*	LL, VH, MB, KG, SW, BO, CL	7
12.	*Bacillus thuringiensis*	RP, JR, SW, BO, KK	5
13.	*Lysinibacillus* sp.	LL, MB, BC, BO, AN	5

[a] *LC* Chumathang, Jammu and Kashmir; *VH* Vashisht, Himachal Pradesh; *MH* Manikaran, Himachal Pradesh; *BW* Bakreshwar, West Bengal; *BC* Balrampur, Chhattisgarh; *LL* Leh, Ladakh, Jammu and Kashmir; *RP* Rohtang Pass, Himachal Pradesh; *BO* Bhitarkanika, Odisha; *SW* Sunderbans, West Bengal; *SL* Sambhar Lake, Rajasthan; *KG* Rann of Kutch, Gujarat; *CL* Chilka Lake, Odisha; *JR* Jaisalmer, Rajasthan; *AN* Andaman and Nicobar Islands; *KK* Kollam, Kerala

focus of investigations for innovative applications useful to mankind.

With the progress of exploration of extreme environments, the richness of microbial diversity is becoming increasingly evident. Among bacteria, aerobic, endospore-forming BBDG are pervasive in the environment and play a significant role in agriculture, medicine and industry. Extreme environments can be a source for novel species of *Bacillus*, as these bacteria can tolerate extremes of pH, temperature, salinity and moisture stress. In recent years, several studies have been conducted to look for the diversity of BBDG in different environments, such as geothermal areas of Deception island, in the South Shetland Archipelago (Llarch et al. 1997); the Gulf of Mexico (Siefert et al. 2000); board and paper products (Suihko and Stackebrandt 2003); the biosphere reserve in Egypt (Mohamed et al. 2006), steel plant wastes (Freitas et al. 2008); deep sea hyper-saline anoxic basins within the Eastern Mediterranean Sea (Sass et al. 2008); Avord Basin, Oregon (Smith et al. 2009); marine sediments (Ettoumi et al. 2009); acidic soils (Yadav et al. 2011) Lonar Lake (Tambekar and Dhundale 2012); geothermal regions of Turkey (Cihan et al. 2012) and the hyper-arid Atacama Desert, Chile (Paulino-Lima et al. 2013). Such studies have illustrated that a large number of aerobic endospore-forming Gram positive *Bacillus* species are present in diverse environments, some of which possess unique capabilities for adapting to extreme environments.

Preliminary screening revealed the presence of many BBDG that can grow in a wide range of environments at pH 5.0–12.0, temperatures between 4 and 90 °C, in salinity from

Table 5 Diversity indices for the isolates from 15 locations in India exhibiting extreme environmental conditions

	High temperature					Low temperature		Salinity			Mangroves		Drought	Acidic	
	LC	VH	MB	BK	BC	LL	RP	SL	KG	CL	BO	SW	JR	AN	KK
Number of isolates	5	5	31	17	13	59	11	35	61	35	33	32	24	22	9
Species richness	4	4	19	9	10	20	11	16	22	12	15	24	9	9	6
Evenness (J′)	0.96	0.96	0.92	0.89	0.95	0.85	1.0	0.92	0.75	0.85	0.89	0.96	0.89	0.84	0.97
Shannon (H)	1.33	1.33	2.73	1.97	2.20	2.53	2.40	2.57	2.30	2.13	2.41	3.04	1.97	1.85	1.73
Simpson's (D)	0.28	0.28	0.08	0.17	0.12	0.12	0.09	0.09	0.19	0.15	0.11	0.05	0.17	0.19	0.18

LC Chumathang, Jammu and Kashmir; *VH* Vashisht, Himachal Pradesh; *MH* Manikaran, Himachal Pradesh; *BW* Bakreshwar, West Bengal; *BC* Balrampur, Chhattisgarh; *LL* Leh, Ladakh, Jammu and Kashmir; *RP* Rohtang Pass, Himachal Pradesh; *BO* Bhitarkanika, Odisha; *SW* Sunderbans, West Bengal; *SL* Sambhar Lake, Rajasthan; *KG* Rann of Kutch, Gujarat; *CL* Chilka Lake, Odisha; *JR* Jaisalmer, Rajasthan; *AN* Andaman and Nicobar Islands; *KK* Kollam, Kerala

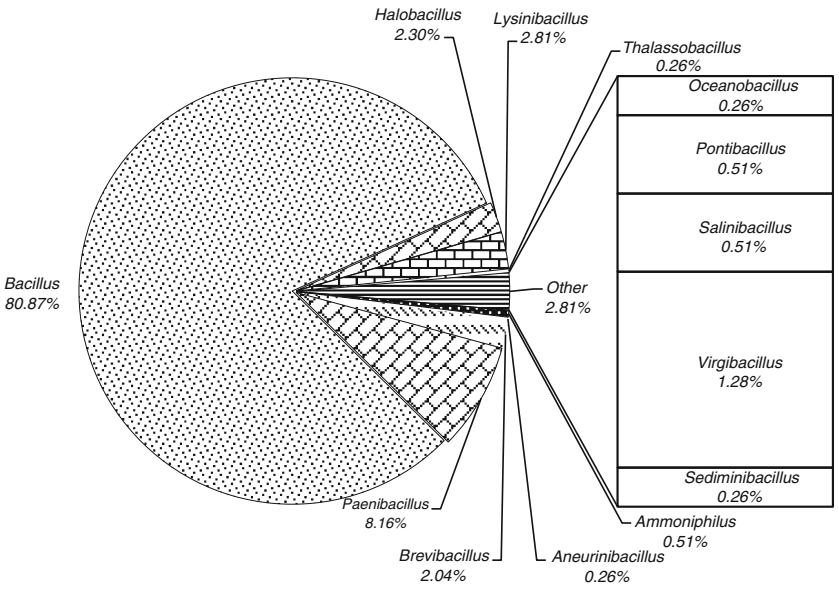


Fig. 3 Abundance of different *Bacillus* and *Bacillus* derived genera in the samples surveyed

0 to 30 % NaCl and at moisture stress ranging from 0 to –0.75 Mpa. Many such isolates were also found to tolerate multiple stresses. The population count of BBDG showed variations among the sites and the media employed; and the counts varied from 4.5×10^3 to 4.0×10^6 CFU g^{-1} sediment or CFU ml^{-1} water. In general, for most of the sites, the population was in the range of 1.1×10^4 to 8.8×10^4, indicating that BBDG predominate most of the extreme environments. In earlier studies, the populations of *Bacillus* in acidic soils of Kollam, Kerala were reported to range from 5×10^4 to 300×10^4 cells g^{-1} soil (Yadav et al. 2011), while samples from Sambhar Salt Lake recorded a total bacterial count in the sediment samples ranging from 32 to 59×10^5 CFU g^{-1}, whereas in water samples it ranged from 14 to 38×10^5 CFU ml^{-1} (Sahay et al. 2012). In deep sea hyper-saline anoxic sediments in the Mediterranean, a high number of endospores, ranging from 3.8×10^5 to 1.2×10^6 g^{-1} dw sediment, were reported (Ventosa et al.

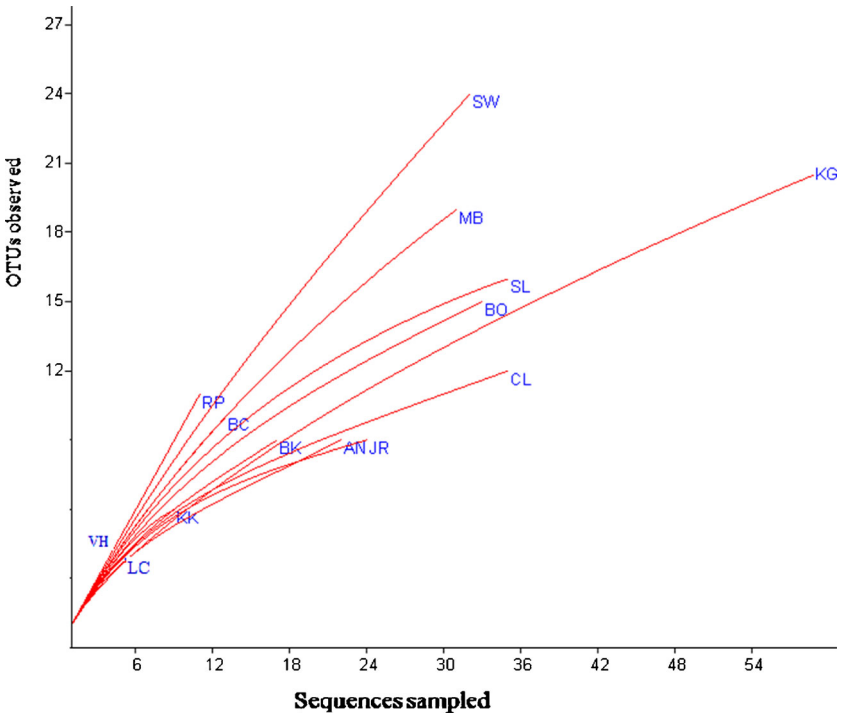


Fig. 4 Rarefaction curves of observed OTUs in the soil/sediment samples from 15 sites in India

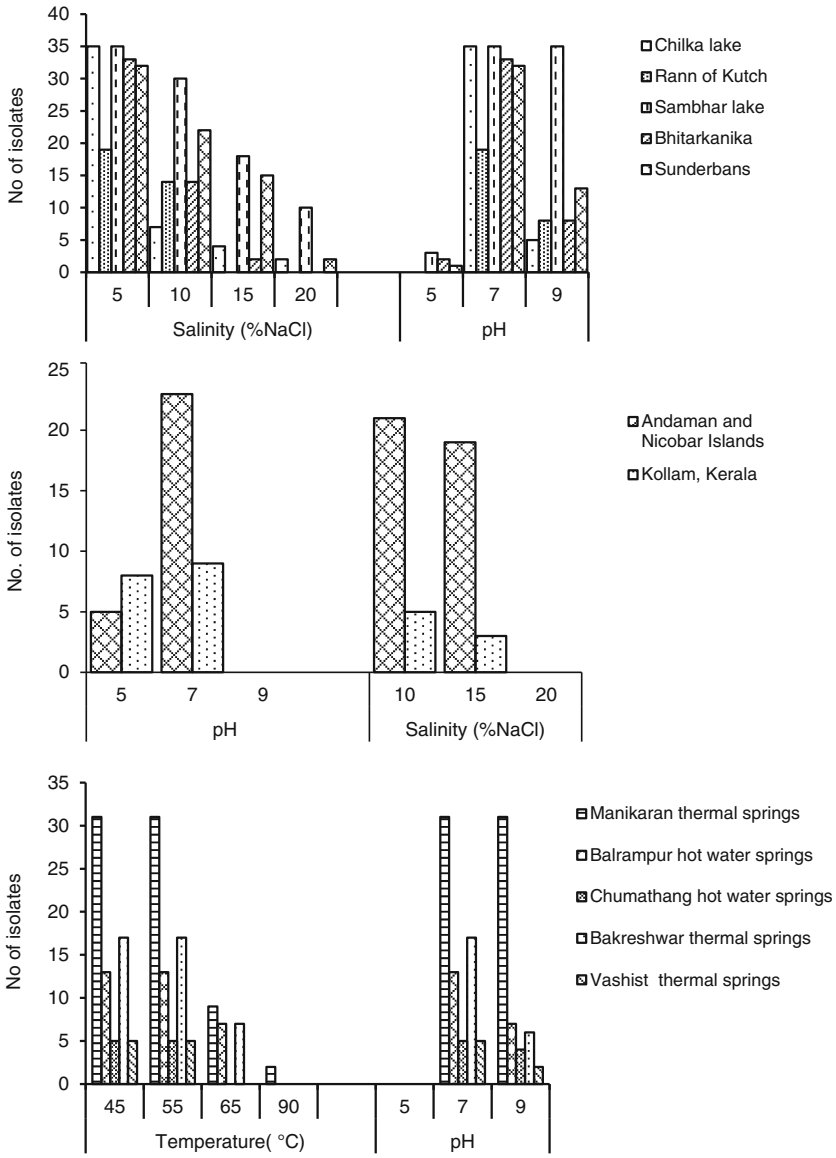


Fig. 5 Enumeration of bacterial isolates from **a** Chilka Lake, Sambhar Lake, Rann of Kutch, Bhitarkanika and Sunderbans on the basis of their salt and pH tolerance; **b** from Andaman and Nicobar Islands and Kollam, based on their pH and salt tolerance; **c** from thermal springs of Manikaran, Bakreshwar, Vashisht, Balrampur and Chumathang on the basis of temperature and pH tolerance

1998). Microbial abundance in snow cover has been reported to range from 10^3 to 10^5 per ml in melted snow and varies with altitude and latitude; though it was less abundant in Antarctic than in mountainous and Arctic snow and increased with altitude.

A total of 893 putative *Bacillus* isolates were obtained from 15 different extreme environments, employing different media. Morphological variations among the isolates obtained on different media further confirmed that members of the genus *Bacillus* exhibit a wide range of nutritional requirements. On the basis of restriction fragment analysis with restriction enzymes *Alu*I, *Msp*I and *Hae*III, all the isolates grouped into 559 clusters. Among the restriction enzymes, *Alu*1 was found to be more discriminatory and yielded several fragments. In earlier studies (Yadav et al. 2011; Vardhan et al. 2011; Cihan et al. 2012), *Alu*I was found to be ideal for restriction analysis and clustering of *Bacillus* isolates. Sequencing of 16S rRNA gene of the representative isolates from 559 clusters identified 392 as BBDG; these were taken up for phylogenetic analysis. Partial sequencing of the smaller subunit of 16S rRNA gene assigned all the 392 isolates to 75 different species, grouped in thirteen genera of Phylum Firmicutes and belonging to two families (Bacillaceae 89.03 %, and 10.97 %, Paenibacillaceae). Priest (1993) also reported a global distribution of species of *Bacillus* and *Paenibacillus*. Multiple species of *Bacillus* and *Paenibacillus* were reported from both bulk and rhizosphere soils, and their counts ranged from 10^3 to 10^6 cells ml^{-1} (Vargas-Ayala et al. 2000).

It is well known that 16S rDNA sequences are reliable tools to establish phylogenetic relationships among bacteria, and in the present study they proved valuable in the identification of BDDG. However, a large number of isolates were classified as

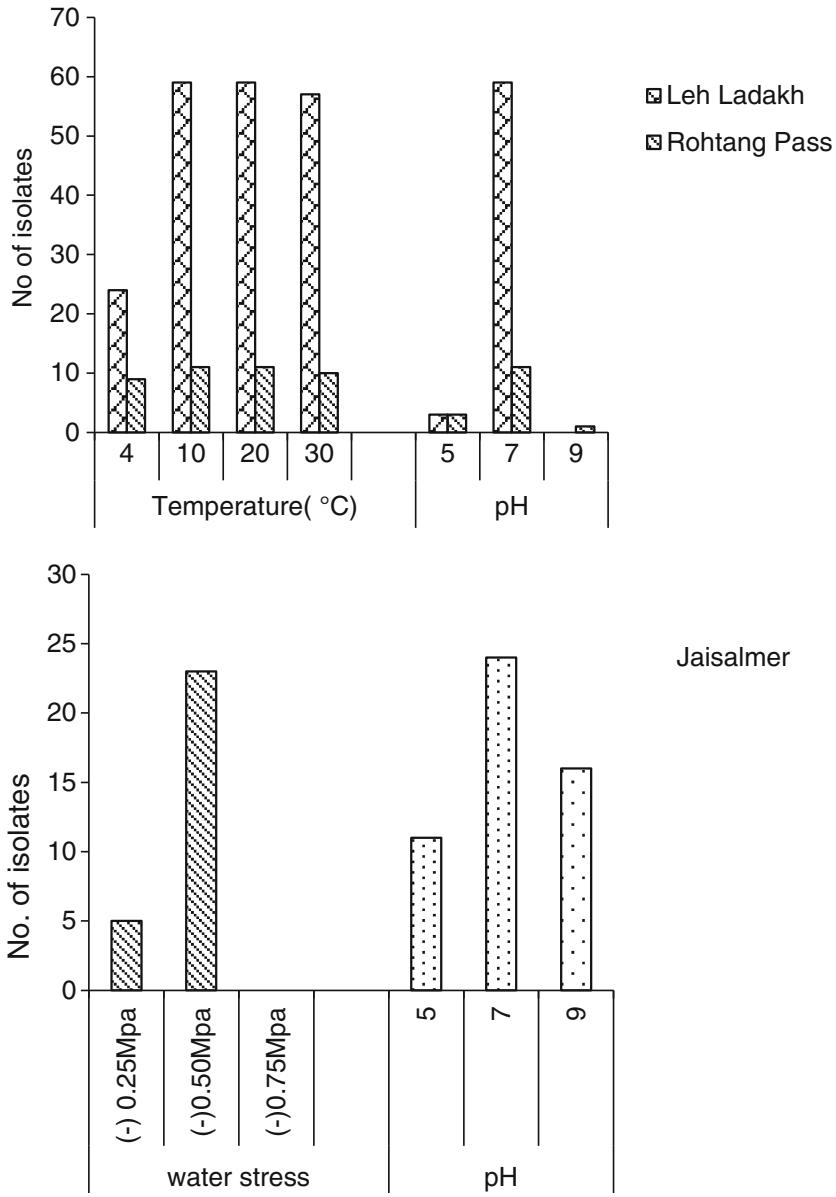


Fig. 6 Distribution of bacterial isolates based on their degree of tolerance and belonging to **a** Cold deserts of Leh and Rohtang Pass, with respect to temperature and pH; **b** Hot/arid desert of Jaisalmer, with respect to water stress and pH

Bacillus sp. with no species assigned to them. Among the Bacillaceae, species of *Bacillus cereus* appeared to be the most predominant species recovered from all extreme environments, except from Sambhar Salt Lake. The other predominant species were *Bacillus megaterium*, *B. subtilis*, *B. firmus* and *B. pumilus* (Table 3). The wide occurrence of *B. subtilis* and *B. cereus* has been reported in different studies (Ettoumi et al. 2009). Among 96 bacilli isolated from marine sediments, 68 % were identified as species of *B. subtilis*, *B. licheniformis*, *B. pumilus* and *B. cereus* (Ettoumi et al. 2009). In another study, 23 marine isolates were clustered, based on 16S rRNA gene sequencing, into four species belonging to *Bacillus licheniformis*, *B. cereus*, *B. subtilis* and *B. pumilus* (Miranda et al. 2008). The diversity of *Bacillus* genotypes in soil samples from El-Omayed Biosphere reserve in Egypt also revealed that a majority of the isolates were closely related to the members of the *B. cereus*/*B. thuringiensis* group (Mohamed et al. 2006). *Bacillus megaterium* has also been reported to be among the most abundant in some soils (Liu and Sinclair 1992). Diversity indices further illustrated that these diverse environments posses a high degree of genetic diversity.

The cold deserts represent hot spots of biodiversity, and several novel cold-tolerant bacterial species have been isolated from these regions (Shivaji et al. 2005). Significant prokaryotic diversity has been detected, including heterotrophic bacteria, cyanobacteria and eukaryotes, with many related to known psychrophilic and psychrotolerant species (Amato et al. 2007). Among the 145 isolates obtained, BBDG exhibited great variations. Some niche-specific bacilli as *Bacillus*

muralis (L-74), *Lysinibacillus fusiformis* (L-2) *and Paenibacillus terrae* (L-57) from Leh, and *Bacillus psychrosaccharolyticus* (R-2), *Paenibacillus lautus* (R-27) and *Virgibacillus halodenitrificans* (ABR-18) from Rohtang Pass, were isolated. It is well documented that the most frequently encountered bacterial genera in cold environments belong to Proteobacteria, *Cytophaga—Flavobacterium-Bacteroidetes* (CFB) group and low and high G/C Gram-positive genera (Amato et al. 2007). The presence of these genera in these cold environments suggests their adaptability to low temperature, which may be attributed to the presence of pigments or polyunsaturated fatty acids that have been implicated in cold adaptation (Shivaji et al. 2007). In our study, all the isolates were capable of growing between 4 and 30°C, indicating that psychrotolerant bacteria are predominant in the sediments of Leh and Rohtang Pass. In the present study, many of the isolates obtained from cold deserts showed pigmentation and produced copious amount of exopolysaccharides. Bacteria living in cold aquatic environments (e.g., Antarctic and Arctic sea ice) are known to produce high amounts of exopolymeric substances (Nichols et al. 2005). It is suggested that these exopolysaccharides help in adhesion to wet surfaces and the formation of the biofilm matrix, which traps nutrients, protects the cell against unfavorable environmental conditions, and mediates biochemical interactions (Nichols et al. 2005). *Virgibacillus halodenitrificans* (ABR-18) from Sarkund Lake in Rohtang Pass was found to grow best under cold and saline conditions, consistent with the properties of its habitat (Doran et al. 2003, 2008), and its phylogeny is predictive of a cold active and halotolerant phenotype.

Thermal springs represent extreme niches that have been a source of biotechnologically important microorganisms best illustrated by *Thermus aquaticus*. In the last decade, several attempts have been made for phylogenetic characterization of microflora from thermal springs in different parts of the world (Reysenbach et al. 2000; Derekova et al. 2008; Singh et al. 2010; Kumar et al. 2013). Recently, the draft genome sequence of *Thermus* sp. isolated from Manikaran thermal springs has been published (Dwivedi et al. 2012). In the present study, 195 isolates from Indian hot water springs (Manikaran, Balarampur, Vashisht, Chumathang and Bakreshwar) were isolated and identified. In general, isolates obtained from Manikaran showed tolerance to higher temperatures. Two isolates, *Paenibacillus pabuli* (M-10) and *Paenibacillus tylopili* (M-18), could grow at temperatures > 80°C. There were many species and derived genera that were niche-specific and typically isolated from Manikaran. Some BBDG from the present study include *Aneurinibacillus danicus* (M-35), *Bacillus beijingensis* (M-74), *B. vireti* (M30), *Brevibacillus agri* (M-73), *Paenibacillus pabuli* (M-10), *P. tylopili* (M-18) *and Lysinibacillus xylanilyticus* (M-52). *Bacillus fusiformis* (B-10) from Bakreshwar and *Brevibacillus* from Vashisht and Balrampur were identified as niche-specific bacilli. *Aneurinibacillus danicus and Brevibacillus* sp. have been earlier reported as thermophilic bacteria (Goto et al. 2004). *Bacillus licheniformis and B. megaterium* were previously reported to be the most dominant bacilli in thermal springs (Llarch et al. 1997). Most of the thermophilic bacteria isolated from Indian springs were alkalitolerant. Earlier reports also suggest the occurrence of thermo-alkali tolerant xylanase-producing bacteria from Manikaran thermal springs (Singh et al. 2010).

In the present study, a large number of halophilic or halotolerant species, such as *Bacillus halodurans* (ABSL-8), *Bacillus methanolicus* (ABSL-11), *Ammoniphilus* sp. (ABSL-2), *Halobacillus trueperi* (ABSL-21), *Bacillus vallismortis* (ABSL-23) and *Halobacillus dabanensis* (ABSL-29) from Sambhar Lake, *Marinococcus halophilus* (ABK-3) from Rann of Kutch, and *Pontibacillus* sp. (AB-2) from Chilka Lake, were identified. Sambhar Lake has been previously investigated (Sahay et al. 2012). Similarly, many other BBDG have been reported from saline alkaline habitats, such as *Bacillus vallismortis* from Death Valley, California (Spring et al. 1996), *Ammoniphilus*, halo-alkalitolerant bacilli from the rhizosphere of sorrel (*Rumex acetosa*) and from decaying wood, Finland (Zaitsev et al. 1998), *Bacillus halodurans* from a Kenyan soda lake (Hashim et al. 2004), *Halobacillus dabanensis* and *Oceanobacillus manasiensis* sp. nov. from salt lakes in Xinjiang, China (Wang et al. 2010), and *Pontibacillus* from a sea urchin (Chen et al. 2009). Three species of the genus *Halobacillus (H. halophilus, H. litoralis* and *H. trueperi*) were reported to require NaCl ions for growth (Spring et al. 1996).

Sunderbans, the world's largest coastal wetland comprising mangrove forests, and Bhitarkanika, the second largest mangrove ecosystem of India, are hot spots of biodiversity. A total of 184 bacteria were isolated from these Indian mangroves and characterized for their salt tolerance. Three isolates, *Bacillus vietnamensis* (S-28), *B. barbaricus* (S-31), *B. marisflavi* (S-14) from Sunderbans, and *B. atrophaeus* (B-71) from Bhitarkanika mangroves were able to tolerate 15 % NaCl concentration, while two isolates *Halobacillus litoralis* (S-91) and *Bacillus hwajinpoensis* (S-93) from Sunderbans and one isolate *Bacillus mojavensis* (AB-10) from Bhitarkanika could tolerate 20 % NaCl concentration. Some niche-specific bacilli from Sunderbans were identified as *Bacillus alcalophilus* (S-77), *B. aquimaris* (S-51), *B. cibi* (S-22), *B. horikoshii* (S-45), *B. hwajinpoensis* (S-93), *Halobacillus litoralis* (S-91), *Paenibacillus amylolyticus* (S-78), and *Bacillus vietnamensis* (S-28). Bhitarkanika mangrove was also represented by specific BBDG, such as *Bacillus atrophaeus* (B-71), *Bacillus methylotrophicus* (B-11), and *Bacillus bombysepticus* (B-80). *Bacillus horikoshii*, *B. atrophaeus*, and *B. methylotrophicus* have earlier been reported to tolerate 8–12 % NaCl concentration.

Bacillus horikoshii was isolated from coastal saline soils, Gujarat, India (Yousuf et al. 2012). *Bacillus hwajinpoensis* represent novel members of *Bacillus* rRNA group, which were earlier reported from sea water of the East Sea and the Yellow Sea in Korea (Yoon et al. 2004). *Bacillus vietnamensis* sp. nov., a moderately halotolerant, aerobic, endospore-forming bacterium, was initially isolated from Vietnamese fish sauce (Noguchi et al. 2004), whereas *Halobacillus litoralis* and *Halobacillus trueperi*, moderately halophilic strains, were reported from hyper-saline sediments of the Great Salt Lake in Utah (Spring et al. 1996). However, the culture-independent molecular analysis of bacterial communities in the mangrove sediment of Sunderbans, India revealed only the presence of one sequence type that showed similarity with *Bacillus subtilis* (Ghosh et al. 2010). An earlier diversity study carried out at another location—Bhitarkanika, in India—revealed *Bacillus* as one of the most predominant genera (Mishra et al. 2012).

Andaman and Nicobar Islands and Kollam, Kerala region were selected for isolation of BBDG tolerant to acid pH. A total of 74 BBDG were isolated from the two regions. Isolates, three each from Kollam, *Bacillus amyloliquefaciens* (A-2), *B. pumilus* (A-5) and *B. megaterium* (A-6), and Andaman and Nicobar Islands, *Lysinibacillus* sp. (AN-4), *Bacillus pseudomycoides* (AN-13) and *Bacillus aryabhattai* (AN-21), could grow at <pH 5.0. In an earlier study, many different species of BBDG, *Bacillus humi*, *B. megaterium*, *B. drentensis*, *B. pocheonensis*, *B. aestuarii*, *B. arbutinivorans*, *B. niacini*, were isolated from the rhizosphere of rice grown in Kollam, Kerala (Yadav et al. 2011). Niche-specific bacilli identified from Andaman and Nicobar Islands and Kollam were *Bacillus pseudomycoides* and *B. amyloliquefaciens*, respectively.

There is very little knowledge on rhizosphere microbiology of the desert plants. A total of 56 bacteria were isolated from rhizosphere samples of Jaisalmer desert. Species of *Bacillus* found to tolerate low water potential were identified as *Bacillus tequilensis* (J-12) and *Bacillus mycoides* (J-4). Some BBDG specific to Jaisalmer desert identified were *Bacillus mycoides* (J-4) and *Bacillus endophyticus* (J-13).

The comprehensive analyses of diversity of *Bacillus* and *Bacillus* derived genera by prospecting extreme environments helped in the development of a huge database including baseline information on the distribution of BBDG in different niches and identifying niche-specific microbes. This database also helped in identifying novel bacteria capable of solubilizing K and Zn under different abiotic stresses (unpublished data), besides those efficient in the production of hydrolytic enzymes of industrial significance such as cellulases and xylanases. The cultures tolerant to low and high temperature, salinity, acidic pH and water stress represent a rich bioresource for useful genes and alleles, which can aid in the generation of abiotic stress-tolerant transgenics.

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Declaration The experiments undertaken comply with the current laws of India, the country where the investigation was undertaken.

References

Amato P, Hennebelle R, Magand O, Sancelme M, Delort AM, Barbnate C, Boutron C, Ferrari C (2007) Bacterial characterization of the snow cover at Sptizberg, Svalbard. FEMS Microbiol Ecol 59:255–264

Ash C, Farrow AE, Wallbanks S, Collins MD (1991) Phylogenetic heterogeneity of the genus *Bacillus* revealed by comparative analysis of small-subunit-ribosomal RNA sequences. Lett Appl Microbiol 13:202–206

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2010) GenBank. Nucleic Acids Res 38:46–51

Chen YG, Zhang YQ, Xiao HD, Liu ZX, Yi LB, Shi JX, Zhi XY, Cui XL, Li WJ (2009) *Pontibacillus halophilus* sp. nov., a moderately halophilic bacterium isolated from a sea urchin. Int J Syst Bacteriol 59: 1635–1639

Chowdhury SP, Schmid M, Hartmann A, Tripathi AK (2009) Diversity of 16S-rRNA and *nif*H genes derived from rhizosphere soil and roots of an endemic drought tolerant grass, *Lasiurus sindicus*. Eur J Soil Biol 45:114–122

Cihan AC, Tekin N, Ozcan B, Cokmus C (2012) The genetic diversity of genus *Bacillus* and the related genera revealed by 16S rRNA Gene sequences and ARDRA analyses isolated from geothermal regions of turkey. Braz J Microbiol 309–324

Claus D, Berkeley RCW (1986) Genus *Bacillus*. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 2. The Williams and Wilkins, Baltimore, pp 1105–1140

Derekova A, Mandeva R, Kambourova M (2008) Phylogenetic diversity of thermophilic carbohydrate degrading bacilli from Bulgarian hot springs. World J Microbiol Biotechnol 24:1697–1702

Doran PT, Fritsen CH, McKay CP, Priscu JC, Adams EE (2003) Formation and character of an ancient 19-m ice cover and underlying trapped brine in an "ice-sealed" east Antarctic lake. Proc Natl Acad Sci U S A 100:26–31

Doran PT, Fritsen CH, Murray AE, Kenig F, McKay C, Kyne JD (2008) Entry approach into pristine ice-sealed lakes-Lake Vida, East Antarctica, a model ecosystem. Limnol Oceanog Methods 6: 542–547

Dwivedi V, Sangwan N, Nigam A, Garg N, Niharika N, Khurana P, Khurana JP, Lal R (2012) Draft genome sequence of *Thermus* sp. Strain RL, isolated from a hot water spring located atop the Himalayan ranges at Manikaran, India. J Bacteriol 194:3534

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853

Ettoumi B, Raddadi N, Borin S, Daffonchio D, Boudabous A, Cherif A (2009) Diversity and phylogeny of culturable spore forming bacilli isolated from marine sediments. J Basic Microbiol 49:S13–S23

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Fortina MG, Pukall R, Schumann P, Mora D, Parini C, Manachini PL, Stackebrandt E (2001) *Ureibacillus* gen. nov., a new genus to accommodate *Bacillus thermosphaericus* (Anderson et al. 1995), amendation of *Ureibacillus thermosphaericus* and description of *Ureibacillus terrenus* sp. nov. Int J Syst Evol Microbiol 51:447–455

Freitas D, Reis M, Lima-Bittencourt C, Costa P, Assis P, Chartone-Souza E, Nascimento A (2008) Genotypic and phenotypic diversity of *Bacillus* spp. isolated from steel plant waste. BMC Res Notes 1:92

Ghosh A, Dey N, BeraA TA, Sathyaniranjan KB, Chakrabarti KB, Chattopadhyay D (2010) Culture independent molecular analysis of bacterial communities in the mangrove sediment of Sundarban, India. Saline Systems 6:1–11. doi:10.1186/1746-1448-6-1

Goto K, Fujita R, Kato Y, Asahara M, Yokota A (2004) Reclassification of *Brevibacillus brevis* strains NCIMB 13288 and DSM 6472 (=NRRL NRS-887) as *Aneurinibacillus danicus* sp. nov. and *Brevibacillus limnophilus* sp. nov. Int J Syst Evol Microbiol 54: 419–27

Hashim SO, Delgado O, Hatti-Kaul R, Mulaa FJ, Mattiasson B (2004) Starch hydrolysing *Bacillus halodurans* isolates from a Kenyan soda lake. Biotechnol Lett 26:823–828

Hengstmann U, Chin K, Janssen PH, Liesack W (1999) Comparative phylogenetic assignment of environmental sequences of genes encoding 16S rRNA and numerically abundant culturable bacteria from anoxic rice paddy soil. Appl Environ Microbiol 511: 5050–5058

Heyndrickx M, Lebbe L, Kersters K, De Vos P, Forsyth G, Logan NA (1998) *Virgibacillus:* a new genus to accommodate *Bacillus pantothenticus* (Proom and Knight 1950). amended description of *Virgibacillus pantothenticus*. Int J Syst Bacteriol 48:99–106

Jaccard P (1912) The distribution of the flora in the alpine zone. New Phytol 11:37–50

Khan M, Patel CB (2007) Plant growth promoting effect of *Bacillus firmus* strain NARS1 isolated from central Himalayan region of India on *Cicer arientnum* at low temperature. Egypt Afr Crop Sci Soc 8:1179–1181

Kumar M, Yadav AN, Tiwari R, Prasanna R, Saxena AK (2013) Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. Ann Microbiol. doi:10.1007/s13213-013-0709-7.

Larkin JM, Stokes JL (1966) Isolation of psychrophilic species of *Bacillus*. J Bacteriol 915:1667–1671

Liu ZL, Sinclair JB (1992) Population dynamics of *Bacillus megaterium* strain B153-2-2 in the rhizosphere of soybean. Phytopathology 82: 1297–1301

Llarch A, Logan NA, Castellv J, Prieto MJ, Guinea J (1997) Isolation and characterization of thermophilic *Bacillus* spp. from geothermal environments on Deception Island, South Shetland archipelago. Microb Ecol 34:58–65

Miranda CA, Martins OB, Clementino MM (2008) Species-level identification of *Bacillus* strains isolates from marine sediments by conventional biochemical, 16S rRNA gene sequencing and inter-tRNA gene sequence lengths analysis. Antonie Van Leeuwenhoek 93:297–304

Mishra RR, Swain MR, Dangar TK, Thatoi H (2012) Diversity and seasonal fluctuation of predominant microbial communities in Bhitarkanika, a tropical mangrove ecosystem in India. Rev Biol Trop 60:909–924

Mohamed EAH, Abe M, Ghanem KM, Abdel-Fattah YR, Nakagawa Y, El-Helow ER (2006) Diversity of *Bacillus* genotypes in soil samples from El-Omayed biosphere reserve in Egypt. J Cult Collect 5:78–84

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858

Nazina TN, Tourova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA, Belyaev SS (2001) Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus, Bacillus thermoleovorans, Bacillus kaustophilus, Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus, G. thermocatenulatus, G. thermoleovorans, G. kaustophilus, G. thermoglucosidasius* and *G. thermodenitrificans*. Int J Syst Evol Microbiol 51:433–446

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci U S A 76: 5269–5273

Nichols CA, Guezennec J, Bowman JP (2005) Bacterial exopolysaccharides from extreme environments with special consideration of the Southern Ocean, sea ice, and deep-sea hydrothermal vents: a review. Mar Biotechnol 7:253–271

Niimura Y, Koh E, Yanagida F, Suzuki K, Komagata K, Kozaki M (1990) *Amphibacillus xylanus* gen. nov., sp. nov., a facultatively anaerobic spore forming xylan-digesting bacterium which lacks cytochrome, quinone, and catalase. Int J Syst Bacteriol 40:297–301

Noguchi H, Uchino M, Shida O, Takano K, Nakamura LK, Komagata K (2004) *Bacillus vietnamensis* sp. nov., amoderately halo tolerant, aerobic, endo spore-forming bacterium isolated from Vietnamese fish sauce. Int J Syst Evol Microbiol 54:2117–2120

Oguntoyinbo FA, Huch M, Cho GS, Schillinger U, Holzapfel WH, Sanni AI, Franz CM (2010) Diversity of *Bacillus* species isolated from okpehe, a traditional fermented soup condiment from Nigeria. J Food Prot 73:870–878

Pandey S, Singh S, Yadav AN, Nain L, Saxena AK (2013) Phylogenetic diversity and characterization of novel and efficient cellulase producing bacterial isolates from different extreme environments. Biosci Biotechnol Biochem 77:1474–1480

Paulino-Lima IG, Azua-Bustos A, Vicuna R, Gonzalez-Silva C, Salas L, Teixeira L, Rosado A, Leitao AC, Lage C (2013) Isolation of UVC-tolerant bacteria from the hyperarid Atacama desert, Chile. Microb Ecol 65:325–335

Perez C, Munoz-Garay C, Portugal LC, Sanchez J, Gill SS, Soberon M, Bravo A (2007) *Bacillus thuringiensis* ssp. *israelensis* Cyt1Aa enhances activity of Cry11Aa toxin by facilitating the formation of a pre-pore oligomeric structure. Cell Microbiol 9:2931–2937

Pospiech A, Neumann B (1995) A versatile quick-prep of genomic DNA from gram positive bacteria. Trends Genet 11:217–218

Priest FG (1993) Systematics and ecology of *Bacillus*. In: Sonenshein AL, Hoch JA, Losick R (eds) *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society of Microbiology, Washington, pp 3–16

Reysenbach AL, Ehringer M, Hershberger K (2000) Microbial diversity at 83 C in Calcite Springs, yellowstone national park: another environment where the Aquificales and" Korarchaeota" coexist. Extremophiles 4:61–67

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Sahay H, Singh S, Kaushik R, Saxena AK, Arora DK (2011) Characterization of halophilic bacteria from environmental samples from the brackish water of Pulicat Lake, India. Biologia 66:741–747

Sahay H, Mahfooz S, Singh AK, Singh S, Kaushik R, Saxena AK, Arora DK (2012) Exploration and characterization of agriculturally and industrially important haloalkaliphilic bacteria from environmental samples of hyper saline Sambhar lake, India. World J Microbiol Biotechnol 28:3207–3217

Sahay H, Babu BK, Singh S, Kaushik R, Saxena AK, Arora DK (2013) Cold-active hydrolases producing bacteria from two different subglacial Himalayan lakes. J Basic Microbiol 53:703–714

Saitou N, Nei M (1987) The neighbor-joining method a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sass AM, McKew BA, Sass H, Fichtel J, Timmis KN, McGenity TJ (2008) Diversity of *Bacillus*-like organisms isolated from deep-sea hyper saline anoxic sediments. Saline Systems 4:1–11. doi:10.1186/1746-1448-4-8

Schlesner H, lawson PA, collins MD, Weiss N, Wehmeyer U, Volker H, Thomm M (2001) *Filobacillus milensis* gen. nov., sp. nov., a new halophilic spore-forming bacterium with Orn-D-Glu-type peptidoglycan. Int J Syst Evol Microbiol 51:425–431

Shida O, Takagi H, Kadowaki K, Komagata K (1996) Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. Int J Syst Bacteriol 46:939–946

Shivaji S, Chaturvedi P, Reddy GSN, Suresh K (2005) *Pedobacter himalayensis* sp. nov., from Hamta glacier located in the Himalayan mountain range in India. Int J Syst Evol Microbiol 55:1083–1088

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, pp 194–207

Siefert JL, Larios-Sanz M, Nakamura LK, Slepecky RA, Paul JH, Moore ER, Jurtshuk P Jr (2000) Phylogeny of marine *Bacillus* isolates from the Gulf of Mexico. Curr Microbiol 41:84–88

Singh AK, Tripathi BM, Sahay H, Singh RN, Kaushik R, Saxena AK, Arora DK (2010) Biochemical and molecular characterization of thermo-alkali tolerant xylanase producing bacteria from thermal springs of Manikaran. Indian J Microbiol 50:2–9. doi:10.1007/s12088-010-0071-4

Smith SA, Benardini JN, Strap JL, Crawford RL (2009) Diversity of aerobic and facultative alkalitolerant and halo tolerant endo spore formers in soil from the Alvord Basin, Oregon. Syst Appl Microbiol 32:233–244

Spring S, Ludwig W, Marquez MC, Ventosa A, Schleifer KH (1996) *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and *Halobacillus trueperi* sp. nov., and transfer of *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. Int J Syst Bacteriol 46:492–496

Srinivas TNR, Rao SN, Reddy PVV, Pratibha MS, Sailaja B, Kavya B, Kishore KH, Begum Z, Singh SM, Shivaji S (2009) Bacterial diversity and bio prospecting for cold-active lipases, amylases and proteases, from culturable bacteria of Kongsfjorden and Ny-Ålesund, Svalbard, Arctic. Curr Microbiol 59:537–54

Suihko ML, Stackebrandt E (2003) Identification of aerobic mesophilic Bacilli isolated from board and paper products containing recycled fibres. J Appl Microbiol 94:25–34

Tambekar DH, Dhundale VR (2012) Studies on the physiological and cultural diversity of bacilli characterized from Lonar lake (MS) India. Biosci Discov 3:34–39

Tamura K, Dudley J, Nei M, Kumar S (2007) *MEGA4*: molecular evolutionary genetics analysis (MEGA) software version 4.0.2. Mol Biol Evol 24:1596–1599

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving sensitivity of progressive multiple sequence alignments through sequence weighing, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–7680

Travers RS, Martin PAW, Reichelderfer CF (1987) Selective process for efficient isolation of soil *Bacillus* sp. Appl Environ Microbiol 53:1263–1266

Van den Burg B (2003) Extremophiles as a source for novel enzymes. Curr Opin Microbiol 6:213–218

Vardhan S, Kaushik R, Saxena AK, Arora DK (2011) Restriction analysis and partial sequencing of the 16S rRNA gene as index for rapid identification of *Bacillus* species. Antonie Van Leeuwenhoek 99:283–296

Vargas-Ayala R, Rodriguez-Kaban R, Morgan-Jones G, McInroy JA, Kloepper JW (2000) Shifts in soil microflora induced by velvet bean (*Mucuna deeringiana*) in cropping systems to control root-knot nematodes. Biol Control 17:11–22

Ventosa A, Marquez MC, Garabito MJ, Arahal DR (1998) Moderately halophilic gram-positive bacterial diversity in hyper saline environments. Extremophiles 2:297–304

Verma P, Yadav AN, Kazy SK, Saxena AK, Suman A (2013) Elucidating the diversity and plant growth promoting attributes of wheat (*Triticum aestivum*) associated acidotolerant bacteria from southern hills zone of India. Natl J Life Sci 10:219–226

Verslues PE, Bray EA (2006) Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential-induced ABA and proline accumulation. J Exp Bot 57:201–212

Waino M, Tindall BJ, Schumann P, Ingvorsen K (1999) *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. Int J Syst Bacteriol 49:821–831

Wang L, Liu WY, Gu ZJ, Chen SF, Yang SS (2010) *Oceanobacillus manasiensis* sp. nov., a moderately halophilic bacterium isolated from the salt lakes of Xinjiang, China. J Microbiol 48:312–317

Wisotzkey JD, Jurtshuk JRP, Fox GE, Deinhard G, Poralla K (1992) Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. Int J Syst Bacteriol 42:263–269

Yadav S, Kaushik R, Saxena AK, Arora DK (2011) Diversity and phylogeny of plant growth-promoting bacilli from moderately acidic soil. J Basic Microbiol 51:98–106

Yoon JH, Kim IG, Kang KH, Oh TK, Park YH (2004) *Bacillus hwajinpoensis* sp. nov. and an unnamed *Bacillus* genomospecies, novel members of *Bacillus* rRNA group 6 isolated from sea water of the East Sea and the yellow sea in Korea. Int J Syst Evol Microbiol 54:803–808

Yoon J-H, Kang S-J, Schumann P, Oh T-K (2010) *Jeotgalibacillus salarius* sp. nov., isolated from a marine saltern, and reclassification of *Marinibacillus marinus* and *Marinibacillus campisalis* as *Jeotgalibacillus marinus* comb. nov. and *Jeotgalibacillus campisalis* comb. nov., respectively. Int J Syst Evol Microbiol 60:15–20

Yousuf B, Sanadhya P, Keshri J, Jha B (2012) Comparative molecular analysis of chemolithoautotrophic bacterial diversity and community structure from coastal saline soils, Gujarat, India. BMC Microbiol 12:150–164

Zaitsev GM, Tsitko IV, Rainey FA, Trotsenko YA, Uotila JS, Stackebrandt E, Salkinoja-Salonen MS (1998) New aerobic ammonium-dependent obligately oxalotrophic bacteria: description of *Ammoniphilus oxalaticus* gen. nov., sp. nov. and *Ammoniphilus oxalivorans* gen. nov., sp. nov. Int J Syst Bacteriol 48:151–163