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Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India

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The diversity of culturable Bacilli was investigated in six wheat cultivating agro-ecological zones of India *viz*: northern hills, north western plains, north eastern plains, central, peninsular, and southern hills. These agro-ecological regions are based on the climatic conditions such as pH, salinity, drought, and temperature. A total of 395 Bacilli were isolated by heat enrichment and different growth media. Amplified ribosomal DNA restriction analysis using three restriction enzymes *Alu*I, *Msp*I, and *Hae*III led to the clustering of these isolates into 19–27 clusters in the different zones at >70% similarity index, adding up to 137 groups. Phylogenetic analysis based on 16S rRNA gene sequencing led to the identification of 55 distinct Bacilli that could be grouped in five families, Bacillaceae (68%), Paenibacillaceae (15%), Planococcaceae (8%), Staphylococcaceae (7%), and Bacillales incertae sedis (2%), which included eight genera namely *Bacillus*, *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, and*Staphylococcus*. All 395 isolated Bacilli were screened for their plant growth promoting attributes, which included direct-plant growth promoting (solubilization of phosphorus, potassium, and zinc; production of phytohormones; 1-aminocyclopropane-1-carboxylate deaminase activity and nitrogen fixation), and indirect-plant growth promotion (antagonistic, production of lytic enzymes, siderophore, hydrogen cyanide, and ammonia). To our knowledge, this is the first report for the presence of *Bacillus endophyticus*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planomicrobium okeanokoites*, *Sporosarcina* sp., and *Staphylococcus succinus* in wheat rhizosphere and exhibit multifunctional PGP attributes. These niche-specific and multifarious PGP Bacilli may serve as inoculants for crops growing in respective climatic conditions.

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Introduction

Microbial world unique in each ecosystem niche forms the basis of the diversity associated. Agriculture is highly dependent on soils and climatic conditions. The ever increasing need for food to support the growing population in the country demands a systematic appraisal of its soil and climate resources in order to

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prepare effective lands. India is gifted with heterogeneous landforms such as the lofty mountains, the riverine deltas, high altitude forests, and peninsular plateaus; different climatic conditions of temperature varying from arctic cold to equatorial hot, rainfall from extreme aridity to humid, acidic soil to alkaline soil and low saline soil to hypersaline soil. Wheat (*Triticum aestivum* L.) is one of the most important cereals worldwide and it is grown in different environments. The increasing global population creates significant concern over the agricultural production to cope with requirement of sufficient food for all. In this context, management of diseases in cereal and pulse crops is vital to alleviate food shortages and to improve efficiency in food production.

Plant growth promoting rhizobacteria (PGPR) promote plant growth directly by either facilitating resource acquisition or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogenic agents on plant growth and development, that is, by acting as biocontrol bacteria [1–3]. PGPR can have an impact on plant growth providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment [1]. Microbes stimulate plant growth in multiple ways *viz* synthesis of phytohormones (auxins, indole-3-acetic acid), production of siderophore, ammonia, hydrogen cyanide, and 1-aminocyclopropane-1-carboxylate (ACC), solubilization of phosphorus, potassium and zinc, nitrogen fixation, and suppression of pathogenic organisms [1, 2].

Global work on PGPR for different crops is brief carried out on a hypothesis that PGPR can overcome the burden caused by chemical fertilizer on environment [1, 2]. There are diverse conditions for crops growing in different abiotic stresses of pH, salinity, temperature, and drought. Green revolution has revolutionized the production of wheat in India, which requires a high input of chemical fertilizers. Excessive use of these chemical inputs N, P, K has affecting the soil health leading to plateauing yield. Diverse microbial communities working for nutrient mobilization are the backbone of healthy soil. The wheat associated bacterial diversity has been extensively investigated in the past few years with a focus on culture dependent techniques [2, 4, 5]. PGPR can enhance plant resistance towards biotic and abiotic stresses and improve plant growth along with soil conditions [6]. Tolerance to stress provided by microbial inoculants become more significant with the perspectives of crop production losses due to the severity of abiotic stresses [6].

The genus *Bacillus* is a heterogeneous collection of aerobic or facultative anaerobic endospores forming bacteria that are ubiquitous in many environments. The sequencing of 16S rDNA led to the identification phylogenetically distinct groups within the class Bacilli of phylum Firmicutes. Since 2001, several new genera like *Planomicrobium okeanokoites* [7]; *Exiguobacterium undae* and *Exiguobacterium antarcticum* [8]; *Sporosarcina macmurdoensis* [9]; *Bacillus aerius*, *Bacillus aerophilus*, *Bacillus stratosphericus* and *Bacillus altitudinis* [10]; *Lysinibacillus fusiformis* and *Lysinibacillus sphaericus* [11]; *Paenibacillus tundra* and *Paenibacillus xylanexedens* [12]; *Bacillus methylotrophicus* [13]; and *Planococcussalinarum* [14] have been sort out. Bacterial species of class Bacilli are phenotypically and genotypically heterogeneous and they are widely used in agriculture as plant growth promoting and disease suppressing agents.

In an effort to understand the diversity and distribution of culturable Bacilli associated with wheat growing in the diverse environments which included saline soil (north eastern and western plains zone), acidic soil (southern hills zone), water deficient/drought stress (central zone), high temperature (peninsular zone), and low temperature regions (northern hills zone) in India. Bacilli were isolated, enumerated, and characterized for tolerances abiotic stresses and plant growth promoting attributes. The isolated Bacilli were identified using 16S rRNA gene sequencing. The use of *Bacillus* and *Bacillus* derived genera (BBDG) and related genera as biofertilizers and biocontrol agent would be of great use in Indian agriculture under diverse climatic conditions of temperature, pH, salt, and drought.

Materials and methods

Soil samples collection

The rhizospheric soil samples were collected from wheat growing in six agro-ecological zones of India, whose details are given in Table 1. A total of 96 soil samples were collected from different zones *viz:* Northern hills zone (Hilly areas of J&K, Himachal Pradesh, Uttarakhand, and Sikkim); North western plains zone (Punjab, Haryana, western Uttar Pradesh, Rajasthan, Delhi, Tarai region of Uttarakhand, Jammu, Samba and Kathua districts of Jammu and Kashmir, and Chandigarh); North eastern plains zone (Eastern UP, Bihar, Jharkhand, West Bengal, Assam, and Odisha); Central zone (Madhya Pradesh, Gujarat, Chhattisgarh, Udaipur division of Rajasthan, and Jhansi division of Uttar Pradesh); Peninsular zone (Maharashtra, Tamil Nadu, Karnataka, and Andhra Pradesh) and Southern

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

Sampling zones	Latitude:Longitude	Habitat	No. of samples	Temperature (°C)	pH	Conductivity (mS cm^{-1})
Northern hills	31° 06′ 12″ N: 77° 10′ 20″ E	Low temperature	16	10.5–22.5	6.0–8.0	261–805
North western plains	28° 36′ 50″ N: 77° 12′ 32″ E	Salinity/alkalinity	18	20.5–33.5	7.0–9.0	376–392
North eastern plains	25° 51′ 39″ N: 85° 46′ 56″ E	Salinity/alkalinity	12	25.8–30.5	7.0–9.0	345–365
Central	22° 43′ 31″ N: 75° 51′ 55″ E	Drought	17	26.5–37.5	6.0–8.0	237–255
Peninsular	20° 04′ 59″ N: 74° 07′ 00″ E	High temperature	14	25.3–38.5	6.0–8.0	266–287
Southern hills	11° 22′ 12″ N: 06° 48′ 00″ E	Acidity	19	28.3–35.5	3.8–5.0	268–281

hills zone (Nilgiris and Palani hills of Tamil Nadu). Samples were labeled, transported on ice, and stored at 4 °C until analysis.

Physico-chemical properties of samples

The pH and conductivity of rhizospheric soil samples were recorded at sampling sites. Total nitrogen (%) was analyzed using kjeldahl's procedure by N-analyzer UDK-149 (VELP Scientifica SRL, Italy). Soil sample were analyzed for soil organic carbon, according to rapid titration method of Walkley and Black [15]. Soil organic matter was determined by the loss of ignition method. Exchangeable cations (Ca, K, Mg, and Na) were extracted with 1 M ammonium acetate (pH 7.0) and determined with an atomic absorption spectrophotometer. Available phosphorus was determined by the Bray II method [16]. Soil analysis was done at Division of Soil Sciences, Indian Agricultural Research Institute, New Delhi, India.

Isolation and enumeration of Bacilli

The population of BBDG and related genera in the wheat rhizosphere were isolated through enrichment using the standard serial dilution plating technique. Rhizospheric soil from respective zones were pooled and one gram of each soil sample in 9 ml of sterile distilled water was heated at 80 °C for 15 min in hot water bath to kill the vegetative cells. Selective isolation of *Bacillus thuringiensis* was done using sodium acetate (0.25 M and 0.75 M) buffer, followed by Luria broth enrichment and heat treatment at 80 °C for 15 min as described earlier by Yadav et al. [17]. Diverse growth medium (nutrient agar, tryptic soy agar, R2 agar, soil extract agar, chemically defined medium, and halophilic medium) with different pH (3–11), NaCl concentration (5–20%), temperatures (5–50 °C) and PEG-8000 (−0.5 to −1.5 MPa) were used to isolate diverse groups of microorganisms *viz*. acidophilic, alkaliphilic, psychrophilic, thermophilic, halophilic, or drought tolerant (Supporting Information Table S1). Plates were incubated at different temperatures and time as described earlier by Yadav et al. [17]. Cultures were purified and maintained at 4 °C as slant and glycerol stock (20%) at −80 °C for further use.

PCR amplification of 16S rRNA gene and amplified rDNA restriction analysis

Genomic DNA was extracted according to protocol of Pospiech and Neumann [18] with some modification. Purified pelleted bacterial cells from 1.5 ml broth were washed 2–3 times in 1.0 ml of TE Buffer (10 mM Tris–HCl and 1 mM EDTA pH 8.0). Bacterial lysis was performed using 0.5 ml SET buffer (75 mM NaCl, 25 mM EDTA, and 20 mM Tris) with 10 μl of lysozyme (10 mg ml^{-1}) for 30 min at 37 °C and 10% SDS with 20 mg ml^{-1} proteinase K for 1 h at 55 °C. DNA was extracted using phenol/chloroform/isoamyl alcohol and aqueous phase was transferred to a fresh tube. Finally, the washed DNA pellet was incubated at 37 °C for 25–30 min to completely remove ethanol and then resuspended in 50 μl of TE buffer. The amount of DNA extracted was electrophoresed on 0.8% agarose gel. The primers pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′) were used for the amplification of 16S rRNA gene. The amplification was carried out in a 100 μl composed: each primer at a concentration of 100 ng, each deoxynucleoside triphosphate at a concentration of 200 μM, 5 U of Taq DNA polymerase, 1.5 mM $MgCl_2$, 90 ng template DNA, and 10 μl 10X Taq buffer and amplification conditions were used for the PCR as 95 °C, 5 min; 39 cycles of 94 °C, 1 min; 52 °C, 1 min; 72 °C, 2 min, and final elongation at 72 °C, 8 min. After amplification the PCR products were resolved by electrophoresis in 1.2% agarose gel in 1X TAE buffer. Gels were stained with ethidium bromide (10 mg ml^{-1}), visualized on a gel documentation system (Alpha–Imager) and gel images were digitalized.

The PCR amplified 16S rDNA 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 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 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 and dendrogram was constructed using the UPGMA method.

16S rRNA gene sequencing and phylogenetic analysis

The nucleotide sequences of purified 16S rDNA were di-deoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in 3130xl Applied Biosystems ABI prism automated DNA sequencer. The DNA sequence was double checked by sequencing both strands using primers pA and pH for forward and reverse reaction respectively. The partial 16S rRNA gene 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 $\geq$97% with that of a prototype strain sequence in the GenBank. Sequence alignment and comparison was performed, using the program CLUSTAL W. One sequence from each group was selected as a representative operational taxonomic unit (OTU). The phylogenetic tree was constructed on the aligned datasets using the neighbor–joining method implemented in the program MEGA 4.0.2 [19].

Screening of isolates for tolerance to pH, temperature, salinity, and drought

Representative strains from each cluster were screened for stress tolerance to alkalinity (pH 9–11), acidity (pH 3–5), salinity (5–20% NaCl concentration), drought [low water potential on polyethylene glycol (PEG–8000)-infused plates of -0.25 to -1.25 MPa water potential] and temperatures (4–75 °C) as described earlier by Yadav et al. [17]. Selected bacterial strains from northern hills, peninsular, north western plains/north eastern plains, southern hills, and central zone were screened for low temperatures, high temperatures, alkalinity/salinity, acidity, and drought, respectively.

Assessment for plant growth promoting attributes

Qualitative estimation direct and indirect PGP attributes

All isolates were initially screened qualitatively for direct PGP attributes which included biological N_2-fixation, production of phytohormones indole-3-acetic acid [20], gibberellic acid [21], and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [22]. Solubilization of phosphorus, potassium, and zinc were carried according to methods described by Pikovskaya [23], Hu et al. [24] and Fasim, et al. [25] using Pikovskaya agar, Aleksandrov medium and nutrient agar medium supplemented with 0.5% tricalcium phosphate, 0.2% potassium aluminosilicates minerals, and 0.1% insoluble zinc compounds (ZnO, ZnS, $Zn_3(PO_4)_2$, and $ZnCO_3$), respectively. Methods of screening for indirect PGP attributes were as followed in our earlier studies, which included production of ammonia, HCN, siderophore, different lytic enzymes [β-glucanase, chitinase, lipase, and protease], and biocontrol against *Fusarium graminearum*, *Rhizoctonia solani*, and *Macrophomina phaseolina* [26, 27]. All assays were done in triplicate.

Quantitative estimation direct PGP attributes

Biological nitrogen fixation. The nitrogen-fixing attribute of bacterial strains were tested by using the acetylene reduction assay (ARA) as described by Han and New [28]. Selected Bacilli were inoculated in a 12 ml vials containing 6 ml semisolid NFb medium and incubated for 24–36 h at desired incubation temperature. The vials were sealed with rubber septa and gas phase of each vial was replaced with a gas mixture of nitrogen, air, and acetylene (90:10:10, v/v), and cultures were re-incubated at for 24 h. The amount of ethylene produced by acetylene reduction was measured by a Perkin Elmer F-11 gas chromatograph. Protein concentration of each strain was determined by standard method of Bradford [29].

Solubilization of P, K, and Zn. Quantitative estimation of P, K, and Zn -solubilization was performed by inoculating 1 ml of bacterial suspension in 50 ml Pikovskaya, Aleksandrov and nutrient broth supplemented with 0.5% tricalcium phosphate, 0.2% potassium aluminosilicates minerals and 0.1% zinc carbonate respectively. After 7 days of incubation, the culture suspension were centrifuged at 10,000*g* for 15 min and the P, K, and Zn content in the supernatant was estimated according to method of Murphy and Riley [30], Hu et al. [24], and Fasim et al. [25], respectively.

IAA production. Indole acetic acid production was estimated by inoculating the bacterial suspension of 1 ml aliquots (3×10^7 CFU ml^{-1}) in 50 ml Luria Bertani broth containing L-tryptophan (100 μg ml^{-1}) and incubated it at desired temperature for 72 h on rotary shaker at 180 rpm. The bacterial cultures were harvested and centrifuged at 10,000*g* for 30 min. Two milliliter of supernatant was mixed with two-three drops of orthophosphoric acid and 4 ml of Salkowski reagent (mixture of 50 ml, 35% of perchloric acid and 1 ml 0.5 M $FeCl_3$) and incubated for 25 min at room temperature. The development of pink-color in the culture filtrate indicated the occurrence of IAA. Intensity of the color was measured

by spectrophotometer at 530 nm and concentration of IAA produced by cultures were measured using standard graph of IAA (Sigma–Aldrich) obtained in the range of 10–100 $\mu g\,ml^{-1}$ [31].

Accession numbers

The partial 16S rRNA gene sequences of 137 Bacilli were submitted to NCBI GenBank and the assigned accession numbers were KJ875388-KJ875524. Representative bacterial strains were deposited in National Agriculturally Important Microbial Culture Collection (NAIMCC) at ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM) culture collection facility, Mau Nath Bhanjan, Uttar Pradesh, India.

Statistical analysis

In order to compare the bacterial diversity among six different zones, 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) was used to calculate Shannon index (H), Evenness (J), and the Simpson's index (D). Using 16S rRNA gene sequences, the rarefaction curve was generated to compare the relative diversity at different zones. Principal coordinate analysis (PCA) was used to determine the statistical correlation between population diversity of six zones surveyed. PCA was performed for different physico–chemical properties and sampling sites, using the XLSTAT program (http://www.xlstat.com).

Results

Enumeration and characterization of Bacilli

The populations of BBDG and related genera were enumerated in wheat rhizospheric soil samples collected from six diverse agro-ecological zones of India (Table 1). A total 395 Bacilli were isolated and significant variations were observed among the culturable bacterial population of each sample on different media. The abundance of bacteria in the rhizospheric soil samples varied from 1.0×10^3–2.25×10^6 CFU g^{-1} soil with the lowest and highest values recorded at southern hills and northern hills zone, respectively. Among the different media used, nutrient agar supported highest population of bacteria 2.25×10^6 CFU g^{-1} soil and chemically defined medium least growth for bacteria 1.0×10^4 CFU g^{-1} soil. The diversity of morphotypes was highest (82) at southern hills zone and lowest (51) at north eastern plains zone (Supporting Information Table S1).

16S rRNA gene sequencing and phylogenetic analysis

PCR amplification of 16S rRNA gene 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 three restriction enzymes, which generated profiles having three to seven fragments ranging in size from 100–850 base pairs. The dendrogram were constructed for each zone to determine the percent similarity among the isolates. The isolates were grouped into clusters; and the number of clusters ranged from 19–27 (Supporting Information Table S1). All 137 strains (NHZ-23, NWPZ-27, NEPZ-21, CZ-25, PZ-19, and SHZ-22) were sequenced and BLAST analysis showed >97 to 100% similarities with the sequences within the GenBank (Table 2). 16S rRNA gene sequencing of 137 strains, reveled the identification of 55 distinct species of Bacilli. Twenty five isolates showing <96% similarity in partial 16S rRNA gene sequences within the GenBank database needs further characterization and validation to confirm their taxonomic position. The phylogenetic tree of 55 identified species of BBDG was constructed to determine their affiliations (Fig. 1a and b).

Analysis of the 16S rRNA gene sequences revealed that 55 strains belonged to five families namely Bacillaceae (33 strains), Paenibacillaceae (8 strains), Planococcaceae (6 strains), Staphylococcaceae (5 strains), and Bacillales incertae sedis (3 strains) (Fig. 1a and b). The Bacillaceae members were further distributed into six major clusters, as *Bacillus cereus* group (*B. cereus* and *B. thuringiensis*), *Bacillus subtilis* group (*B. altitudinis*, *B. pumilus*, *B. amyloliquefaciens*, and *B. subtilis*), and *Bacillus megaterium* group (*B. flexus*, *B. megaterium*, *B. aryabhattai*, and *B. muralis*). The derived genera of *Bacillus* of family Bacillaceae, such as *Lysinibacillus* and *Paenibacillus* were presented as separate clusters. Members of all other families present in a separate cluster (Fig. 1a and b). *P. okeanokoites* the member of family Planococcaceae present with members of family of Staphylococcaceae (Fig. 1a). Two species of *Bacillus*, *B. fusiformis* and *B. sphaericus* are present with its relative derived genera with *Lysinibacillus* (Fig. 1b). Overall *Bacillus* from Bacillaceae and *Paenibacillus* from Paenibacillaceae were the most frequently recovered genera (Supporting Information Table S3).

Statistical analysis

The 395 isolates from the six different wheat agro-ecological zones could be categorised into 137 clusters, based on similarity index of >70% of the ARDRA pattern. Sequencing of representative isolates from each cluster led to the identification of 55

Table 2. Identification, distribution, and plant growth promoting attributes of wheat associated Bacilli, isolated from six wheat agro-ecological zones of India.

		Direct PGP attributes						
Strains	**Nearest phylogenetic relative**	**PO_4**	**K**	**Zn**	**IAA**	**N_2-fixation**	**GA**	**ACC**
BSH15	*Bacillus aerophilus*	15.6 ± 1.0	43.1 ± 1.2	4.1 ± 0.2	16.2 ± 1.0	–	–	–
BCZ14	*Bacillus alcalophilus*	24.9 ± 0.8	–	5.5 ± 0.5	–	–	–	–
BPZ4	*Bacillus altitudinis*	43.9 ± 0.7	–	–	–	10.5 ± 1.5	–	–
BNE12	*Bacillus amyloliquefaciens*	39.4 ± 2.4	–	–	14.2 ± 1.0	–	+	–
BCZ13	*Bacillus aquimaris*	426 ± 1.5	–	–	–	–	–	–
BCZ17	*Bacillus aryabhattai*	45.6 ± 1.0	–	–	15.6 ± 0.7	67.2 ± 1.2	–	–
BSH3	*Bacillus atrophaeus*	32.6 ± 1.0	38.5 ± 0.2	5.8 ± 0.5	11.2 ± 1.2	–	+	–
BSH5	*Bacillus barbaricus*	–	–	±	35.2 ± 1.6	18.2 ± 1.6	–	–
BSH9	*Bacillus cereus*	–	18.6 ± 1.5	–	–	–	–	+
BSH11	*Bacillus circulans*	22.6 ± 1.0	23.6 ± 1.0	6.1 ± 0.5	14.2 ± 1.0	45.3 ± 1.5	–	–
BNW9	*Bacillus endophyticus*	45.9 ± 1.9	–	3.9 ± 0.6	46.1 ± 0.5	68.5 ± 1.3	+	–
BPZ7	*Bacillus flexus*	36.2 ± 0.6	–	–	–	–	–	+
BNW5	*Bacillus fusiformis*	46.5 ± 1.2	–	4.5 ± 0.2	225.2 ± 1	98.3 ± 1.3	+	–
BNH7	*Bacillus horikoshii*	–	22.7 ± 0.9	3.6 ± 0.5	8.75 ± 1.2	–	–	+
BPZ5	*Bacillus licheniformis*	47.2 ± 1.4	27.9 ± 0.8	2.9 ± 0.2	–	12.5 ± 1.5	–	–
BNH1	*Bacillus megaterium*	45.7 ± 1.1	32.3 ± 0.5	6.5 ± 0.5	16.6 ± 1.0	–	–	–
BNE2	*Bacillus methylotrophicus*	3.6 ± 1.0	–	–	24.3 ± 2.6	18.5 ± 1.8	–	–
BPZ6	*Bacillus mojavensis*	35.6 ± 1.0	30.2 ± 1.5	4.1 ± 0.5	18.2 ± 1.0	22.5 ± 1.2	+	–
BNH12	*Bacillus muralis*	–	–	3.5 ± 0.6	28.6 ± 1.0	–	–	–
BSH7	*Bacillus nanhaiensis*	14.7 ± 0.6	–	6.4 ± 0.3	–	–	–	–
BSH10	*Bacillus nealsonii*	16.5 ± 0.9	–	4.9 ± 0.5	–	–	–	–
BCZ15	*Bacillus pumilus*	–	13.5 ± 1.0	7.5 ± 0.5	–	–	–	+
BSH4	*Bacillus rigui*	45.9 ± 1.9	–	3.3 ± 0.6	46.1 ± 0.5	32.5 ± 1.2	+	–
BPZ2	*Bacillus siamensis*	–	–	3.6 ± 0.5	35.1 ± 1.0	26.5 ± 1.0	–	+
BSH8	*Bacillus solisalsi*	–	–	–	28.2 ± 1.0	–	–	–
BNH9	*Bacillus* sp.	47.2 ± 1.4	23.1 ± 1.2	±	–	19.5 ± 1.3	–	–
BNW8	*Bacillus sphaericus*	31.6 ± 1.0	16.3 ± 1.2	6.4 ± 0.3	16.2 ± 1.0	80.5 ± 1.0	+	–
BPZ1	*Bacillus subtilis*	24.8 ± 0.8	–	6.7 ± 0.5	102.8 ± 05	–	+	+
BNE10	*Bacillus tequilensis*	56.2 ± 0.6	–	–	30.8 ± 1.1	–	–	–
BNH2	*Bacillus thuringiensis*	58.9 ± 0.7	–	2.8 ± 0.5	–	–	–	–
BPZ8	*Exiguobacterium acetylicum*	9.9 ± 1.0	–	3.4 ± 0.5	–	–	–	–
BNH11	*Exiguobacterium antarcticum*	–	11.9 ± 1.5	4.5 ± 0.2	22.8 ± 0.8	–	–	–
BNH10	*Exiguobacterium* sp.	43.7 ± 0.9	–	2.4 ± 0.5	–	–	–	–
BSH2	*Lysinibacillus fusiformis*	46.5 ± 1.2	–	7.5 ± 0.3	122.3 ± 2	43.5 ± 1.6	–	–
BSH6	*Lysinibacillus sphaericus*	45.6 ± 1.0	±	4.7 ± 0.4	21.2 ± 1.0	19.5 ± 1.0	–	–
BCZ8	*Lysinibacillus xylanilyticus*	–	–	6.3 ± 0.5	–	–	–	+
BNW7	*Paenibacillus alvei*	–	–	4.5 ± 0.6	–	–	–	–
BCZ16	*Paenibacillus amylolyticus*	24.4 ± 1.0	26.6 ± 1.5	–	–	–	–	–
BCZ2	*Paenibacillus dendritiformis*	73.2 ± 1.4	22.4 ± 1.1	3.5 ± 0.5	45.2 ± 1.1	–	–	–
BCZ10	*Paenibacillus durus*	32.3 ± 1.4	–	–	–	46.5 ± 1.0	–	+
BNW13	*Paenibacillus lautus*	–	–	–	31.7 ± 0.8	–	+	–
BNW6	*Paenibacillus polymyxa*	95.6 ± 1.0	18.3 ± 1.6	3.9 ± 0.5	36.7 ± 0.8	–	+	–
BCZ3	*Paenibacillus tundrae*	–	–	4.8 ± 0.8	–	–	–	–
BCZ12	*Paenibacillus xylanexedens*	66.2 ± 0.6	–	–	30.8 ± 1.1	–	+	–
BNE6	*Planococcus citreus*	65.6 ± 1.0	36.5 ± 1.2	3.8 ± 0.8	31.2 ± 1.0	–	–	–
BSH13	*Planococcus salinarum*	6.7 ± 0.7	46.9 ± 1.2	5.5 ± 0.6	–	–	–	–
BNE8	*Planomicrobium okeanokoites*	8.9 ± 1.0	–	–	72.1 ± 1.0	60.5 ± 1.2	–	–
BSH14	*Planomicrobium* sp.	8.9 ± 1.0	–	–	75.1 ± 1.5	15.5 ± 1.0	–	–
BNW4	*Sporosarcina* sp.	75.6 ± 1.0	–	6.0 ± 0.5	37.2 ± 1.0	43.5 ± 1.3	–	–
BNH13	*Staphylococcus arlettae*	–	–	3.8 ± 0.2	–	–	+	–
BCZ24	*Staphylococcus devriesei*	9.9 ± 1.0	–	7.3 ± 0.3	–	–	–	–
BSH16	*Staphylococcus epidermidis*	–	–	2.1 ± 0.5	22.8 ± 1.9	–	–	–
BNE7	*Staphylococcus equorum*	–	–	3.6 ± 0.6	–	–	–	–
BNW11	*Staphylococcus* sp.	–	–	7.5 ± 0.8	–	–	–	–
BPZ9	*Staphylococcus succinus*	15.6 ± 1.0	–	5.4± 0.5	12.2 ± 1.0	–	–	–

		Indirect PGP attributes							
Strains	Distribution ratio (%)	Siderophore	BC	NH_3	HCN	Chitinase	Protease	Lipase	β-glucanase
BSH15	1.3	−	−	+	−	−	−	−	−
BCZ14	0.5	−	+	+	−	+	+	−	−
BPZ4	2.3	+	+	+	+	−	+	+	+
BNE12	4.8	+	−	−	−	−	−	−	−
BCZ13	0.8	−	+	+	−	−	+	−	−
BCZ17	2.8	−	−	+	+	−	−	−	−
BSH3	2.5	−	−	+	−	−	−	−	−
BSH5	2.5	−	+	−	−	+	+	−	+
BSH9	4.6	−	+	+	+	−	+	−	−
BSH11	1.0	−	−	+	−	−	−	−	−
BNW9	0.8	+	+	+	+	−	+	−	−
BPZ7	5.3	−	−	+	−	−	−	−	−
BNW5	0.8	−	+	+	−	−	+	−	−
BNH7	0.8	+	+	−	+	−	+	−	−
BPZ5	4.3	−	+	+	+	−	+	−	+
BNH1	3.8	+	−	+	−	−	−	−	−
BNE2	0.8	+	−	+	+	−	−	−	−
BPZ6	0.5	−	−	+	−	−	−	−	−
BNH12	0.8	+	−	+	+	+	+	+	−
BSH7	0.8	+	+	−	+	−	+	−	−
BSH10	0.8	+	−	−	+	−	−	−	+
BCZ15	4.1	+	−	+	−	−	−	+	−
BSH4	1.0	+	+	+	+	−	+	−	−
BPZ2	0.8	−	−	+	−	−	−	−	−
BSH8	1.3	−	+	−	+	−	+	−	−
BNH9	1.8	+	+	+	+	−	+	−	−
BNW8	0.8	−	−	+	−	−	−	−	−
BPZ1	4.3	+	−	+	−	+	+	−	−
BNE10	1.0	+	+	−	−	−	+	−	−
BNH2	4.3	+	−	+	+	−	−	−	−
BPZ8	0.5	−	+	+	−	−	+	−	−
BNH11	0.5	+	+	+	+	−	−	+	+
BNH10	1.0	+	+	+	−	−	+	+	+
BSH2	3.3	−	+	−	−	−	+	−	−
BSH6	2.3	−	−	−	−	+	+	−	−
BCZ8	0.5	−	−	−	−	−	−	−	−
BNW7	1.3	−	−	+	−	−	+	−	+
BCZ16	2.3	−	−	+	−	−	−	−	−
BCZ2	0.8	+	−	+	−	−	+	−	+
BCZ10	0.5	−	+	+	−	−	−	−	−
BNW13	0.5	−	−	+	−	−	−	−	−
BNW6	5.1	−	+	+	−	+	+	−	+
BCZ3	1.8	−	−	+	−	−	+	−	−
BCZ12	2.0	+	+	+	−	−	+	−	−
BNE6	0.8	−	−	+	−	+	−	+	+
BSH13	2.3	+	−	+	−	−	+	+	−
BNE8	2.3	+	+	+	−	−	+	−	−
BSH14	1.8	+	+	−	−	−	+	−	−
BNW4	1.3	−	−	−	−	−	−	+	+
BNH13	1.8	+	+	+	−	+	+	−	−
BCZ24	1.3	−	+	+	−	+	+	−	−
BSH16	1.8	+	+	+	−	+	−	−	−
BNE7	0.5	+	+	+	−	−	+	−	−
BNW11	1.8	+	+	+	−	−	−	−	−
BPZ9	0.8	+	+	+	−	−	+	−	−

P, K and Zn solubilization (mg L^{-1}); IAA, indole acetic acid (μg mg^{-1} protein day^{-1}); N_2-fixation (nmol ethylene h^{-1} mg^{-1} protein); BC, biocontrol; GA, gibberellic acid; ACC, 1-aminocyclopropane-1-carboxylate; HCN, hydrogen cyanide; −, negative for the attributes; +, positive for the attributes.

strains. Of the 137 strains identified, nine Bacilli were common to all six zones (Supporting Information Table S3). Shannon's diversity index ($H' = 3.253$) and species richness was highest for north western plains zone, whereas peninsular zone recorded the lowest value ($H' = 2.84$). These observations are supported by bacterial diversity parameters, such as Simpson's index, Chao-1, Evenness, Shannon Entropy, and

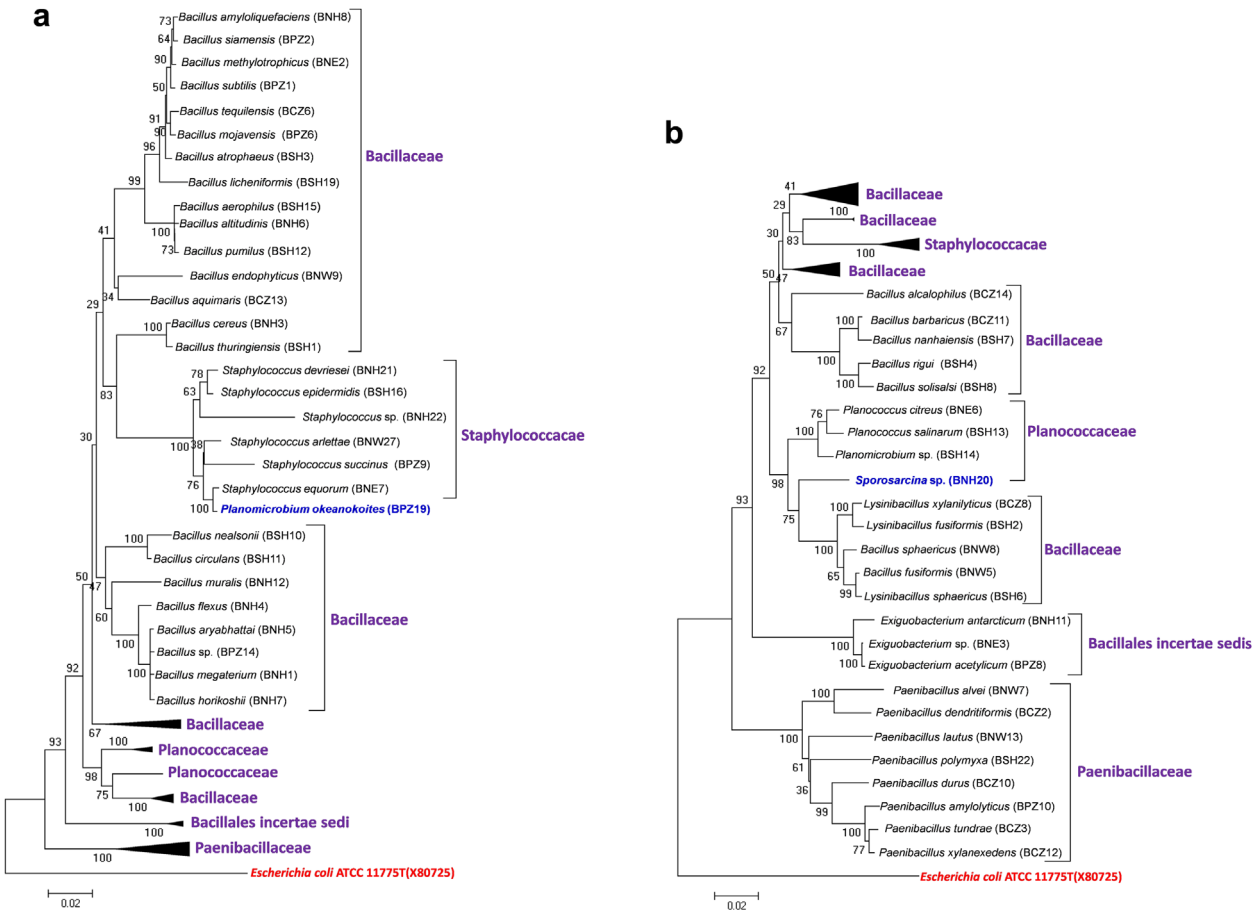

Figure 1. Phylogenetic tree showing the relationship among 55 Bacilli, 16S rRNA gene sequences with reference sequences obtained through BLAST analysis. The sequence alignment was performed using the CLUSTAL W program and tree was constructed using Neighbor Joining with algorithm using MEGA4 software [19].

individual rarefaction curves (Table 3, Fig. 2a). Principal coordinate analysis was used to investigate relationships between bacterial diversity (Shannon's diversity index). The first two dimensions of PCA (PCA1 and PCA2) explained 55.0% of the total variation, with component 1 accounting for 36.49% and component 2 for 18.51% of the variance (Fig. 2b). Correlation analysis proved existence of significant relationship between the different parameters and sampling sites. The first two factorial axes of biplot represent 22.70–56.45% variance in the data (Fig. 2c).

Tolerance of isolates for pH, salt, drought, and temperatures

All 395 Bacilli were screened for tolerance different abiotic stresses and results are represented by representative strains from each cluster at different zones. Among representative 23 strains from northern hills zone, 11 and 12 strains could be grouped as psychrotolerant and psychrotrophic Bacilli as they could grow between 4–30 °C and 10–37 °C, respectively. Representative 48 strains from north western plains/north eastern plains zone could grow up to 15% NaCl concentrations (27 strains) and 11 pH (19 strains). Among the representative 25 strains from central zone, 13 were able to grow

Table 3. Diversity indices for the Bacilli associated with wheat from six agro-ecological zones of India.

Isolates	NHZ	NWPZ	NEPZ	CZ	PZ	SHZ
No of isolates	60	78	51	72	52	82
Species richness	23	27	21	25	19	22
Simpson (D)	0.95	0.96	0.95	0.96	0.94	0.95
Shannon (H)	3.08	3.253	3.02	3.16	2.84	3.05
Evenness (J′)	0.94	0.96	0.98	0.95	0.89	0.96
Brillouin	2.59	2.79	2.51	2.70	2.38	2.67
Menhinick	2.97	3.06	2.94	2.95	2.64	2.43
Margalef	5.37	5.97	5.09	5.61	4.56	4.77
Chao-1	23	27	21	25	19	22

NHZ, northern hills zone; NWPZ, north western plains zone; NEPZ, north eastern plains zone; CZ, central zone; PZ, peninsular zone; SHZ, southern hills zone.

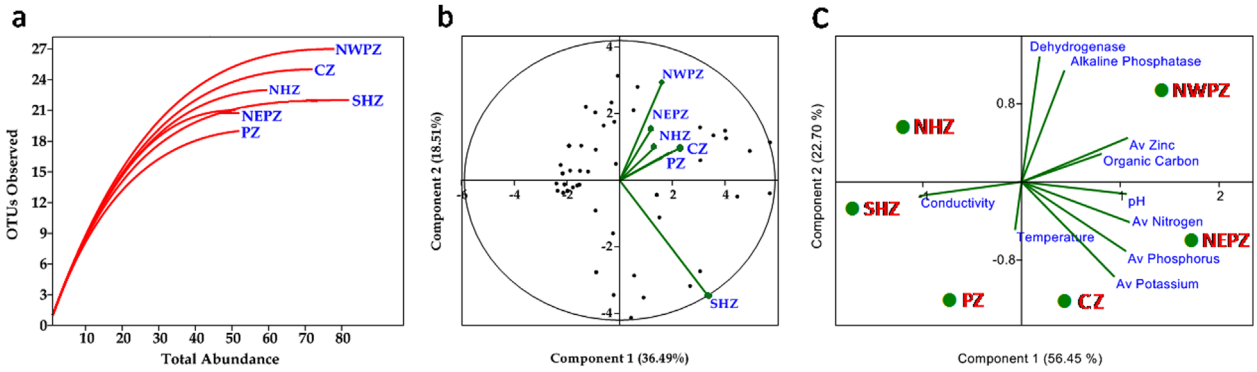

Figure 2. (a) Rarefaction curves of observed OTUs in the samples from six agro-ecological zones of India; (b) Principal Coordinate Analysis of the diversity indices (H) of the 16S rRNA gene PCR-ARDRA profiles of the six agro-ecological zones in relation to 16S rRNA gene sequences, component 1 and component 2 accounted for 36.49% and 18.51% of the total variation, respectively; (c) Biplot showing relationship between different sampling sites and temperature, pH, conductivity, organic carbon, available NPK and Zn, exchangeable sodium, dehydrogenase, and alkaline phosphatase.

on PEG infused plates with water potential of −0.5 to −0.75 MPa, in which six strains (*Bacillus aryabhattai* BCZ17, *Bacillus licheniformis* BCZ20, *Lysinibacillus xylanilyticus* BCZ8, *Paenibacillus dendritiformis* BCZ2, *Planococcus salinarum* BCZ23, and *Staphylococcus devriesei* BCZ24) were said to be thermo-drought tolerant bacteria, as they could grow at high temperature and moisture stress condition. All representative strains obtained from the peninsular zone were tolerant to temperature of 50 °C or more. Among 19 strains, 8 strains *Bacillus altitudinis* BPZ4, *Bacillus amyloliquefaciens* BPZ3, *Bacillus cereus* BPZ11, *Bacillus subtilis* BPZ1, *Bacillus thuringiensis* BPZ15, *Exiguobacterium acetylicum* BPZ8, *Paenibacillus tundrae* BPZ18, and *Staphylococcus succinus* BPZ9 could to thermotolerant as they can grow upto 65 °C. The representative strains from southern hills zone could grow at <5 pH, in which 10 strains *Bacillus aerophilus* BSH15, *Bacillus amyloliquefaciens* BSH17, *Bacillus atrophaeus* BSH3, *Bacillus cereus* BSH9, *Bacillus circulans* BSH11, *Bacillus licheniformis* BSH19, *Bacillus pumilus* BSH12, *Lysinibacillus fusiformis* BSH2, *Paenibacillus polymyxa* BSH22, and *Planomicrobium* sp. BSH14 could grow at 3 pH (acidophilic Bacilli) (Supporting Information Table S2).

Plant growth promoting attributes

All 395 Bacilli were screened for direct and indirect PGP traits and result were represented by 55 representative Bacilli. Of 55 representatives, 39, 18, and 40 strains exhibited solubilization of phosphorus, potassium, and zinc respectively (Table 2). Out of 55 representatives, 36 strains produced indole-3-acetic acid, while only 12 strains produced gibberellic acid (Table 2). Nitrogen fixation and ACC deaminase activity exhibited by 19 and 8 strains respectively. Among indirect plant growth promoting attributes ammonia production was exhibited by 39 strains whereas only 8 strains showed lipase production (Table 2). Of 55 representatives, 27, 15, 11, 10, and 29 strains were found positive for production of siderophore, HCN, β-glucanase, chitinase, and biocontrol respectively (Table 2).

Among P, K, and Zn solubilizers, *Paenibacillus polymyxa* BNW6 solublized highest amount of phosphorus $95.6 \pm 1.0\ mg\ L^{-1}$ followed by *Sporosarcina* sp. BNW4 $75.6 \pm 1.0\ mg\ L^{-1}$. *Planococcus salinarum* BSH13 ($46.9 \pm 1.2\ mg\ L^{-1}$) and *Bacillus pumilus* BCZ15 ($7.5 \pm 0.5\ mg\ L^{-1}$) solubilized highest amount of potassium and zinc respectively. Among 55 representatives, *Bacillus fusiformis* BNW5 produced highest amount of IAA ($225.2 \pm 1\ \mu g\ mL^{-1}$ protein day^{-1}) and 16 strains produced >30 $\mu g\ ml^{-1}$ protein day^{-1} (Table 2). N_2-fixation activity varied from 10.5 ± 1.5–98.3 ± 1.3 nmol ethylene h^{-1} mg^{-1} protein (Table 2). Among plant growth promoting activities, ammonia producing Bacilli were highest (79.0%), when compared to P-solubilizer (73.9%), Zn-solubilizers (67.1%), protease producers (56.7%), IAA producers (55.2%), siderophore producers (49.1%), biocontrol activity (47.8%), K-solubilizers (39.2%), N_2-fixers (31.4%), HCN producers (27.3%), gibberellic acid producers (24.8%), chitinase activity (21.0%), ACC deaminase (20.8%), β-glucanase producers (20.5%), and lipase producers (12.9%) (Supporting Information Table S4).

Plant growth promoting attributes of Bacilli varied in all different agro-ecological zones of India. Isolates from southern hills zone showed maximum number of Bacilli possess solubilization of P, K, and Zn; production of IAA, siderophore, chitinase and protease, and biocontrol activity, whereas N_2-fixation, gibberellic acid, and ammonia production by Bacilli of north western plains zone; production of HCN and lipase by northern hills zone isolates and production of β-glucanase and ACC deaminase

by peninsular zone Bacilli (Supporting Information Table S4). Isolates from all zones were also tested for all PGP attributes at different stress condition such as low temperature, high temperature, salinity, drought, and acidity for isolates from different zones northern hills, peninsular, north western plains/north eastern plains, central and southern hills, respectively (data not published). Among 55 strains, 22 strains were identified as *Bacillus altitudinis*, *Bacillus barbaricus*, *Bacillus endophyticus*, *Bacillus fusiformis*, *Bacillus horikoshii*, *Bacillus licheniformis*, *Bacillus mojavensis*, *Bacillus muralis*, *Bacillus rigui*, *Bacillus* sp., *Bacillus sphaericus*, *Bacillus subtilis*, *Exiguobacterium antarcticum*, *Exiguobacterium* sp., *Lysinibacillus sphaericus*, *Paenibacillus dendritiformis*, *Paenibacillus polymyxa*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planococcus salinarum*, *Planomicrobium okeanokoites*, and *Staphylococcus succinus* exhibited more than seven different plant growth promoting activities (Fig. 3a).

Discussion

The extreme habitats harbor microbial diversity, which may be fundamental for the maintenance and conservation of global genetic resources. Our present study deciphers the diversity of BBDG and related genera coupled with the diverse zones of wheat crop and their PGP attributes. A total of 395, wheat allied bacterial isolates were obtained from different agro-ecological zones of India. To the best of our knowledge, this is the first report, which elucidate the BBDG and related genera associated with wheat crops and their PGP attributes at different extreme habitats.

Sequencing of 16S rRNA gene of the representative strain from each cluster of all six different zones was identified and selected 55 distinct species were taken up for phylogenetic analysis. Partial sequencing of the smaller subunit of 16S rRNA gene assigned and all the representative Bacilli could be grouped into five families: Bacillaceae (68%), Paenibacillaceae (15%), Planococcaceae (8%), Staphylococcaceae (7%), and Bacillales incertae sedis (2%), which included 55 distinct species of eight genera namely *Bacillus*, *Exiguobacterium*, *Lysinibacillus*, *Paenibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, and *Staphylococcus*. Among the six zones analyzed, *Bacillus* diversity was found more diverse in three zones namely, north western plains, north eastern plains, and peninsular (Fig. 4b). The distribution of 55 distinct Bacilli from six zones was represented in Fig. 4c.

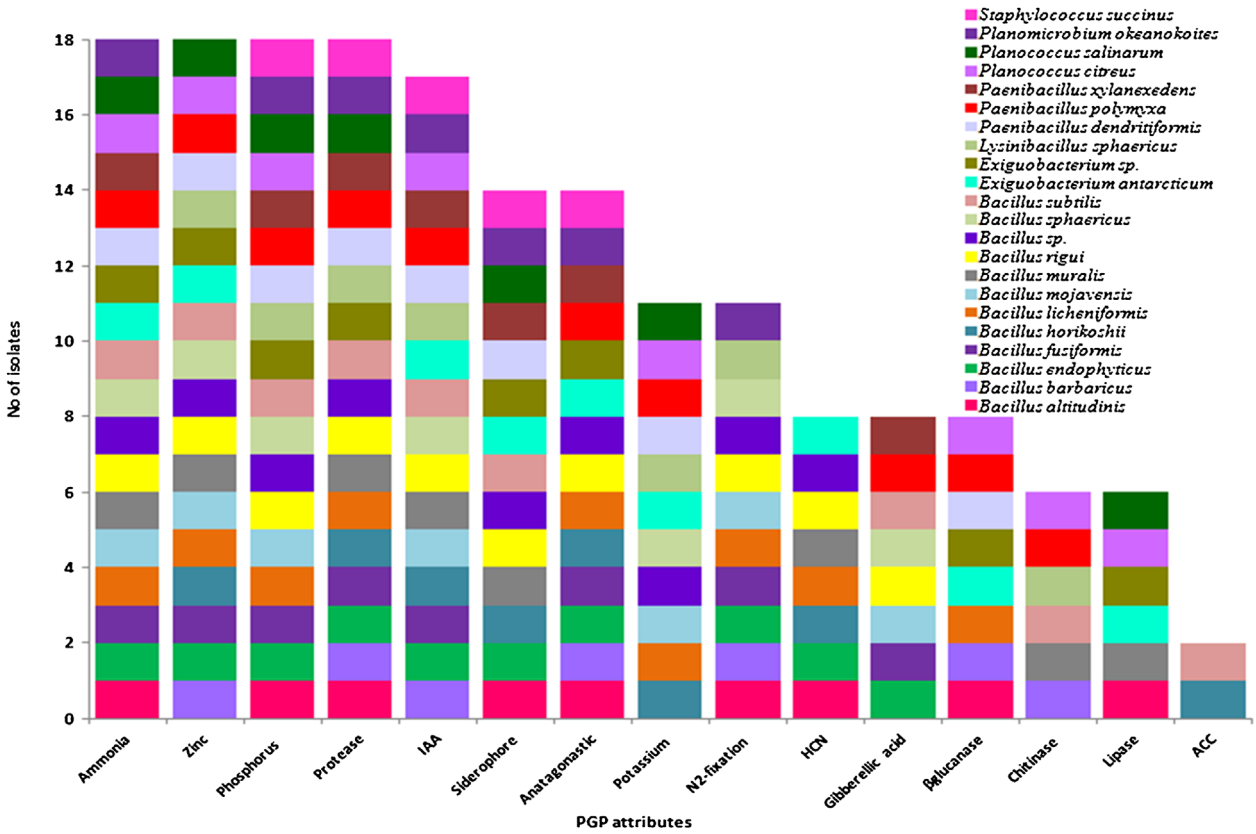

Figure 3. Characterization of 22 Bacilli endowed with different plant growth promoting attributes.

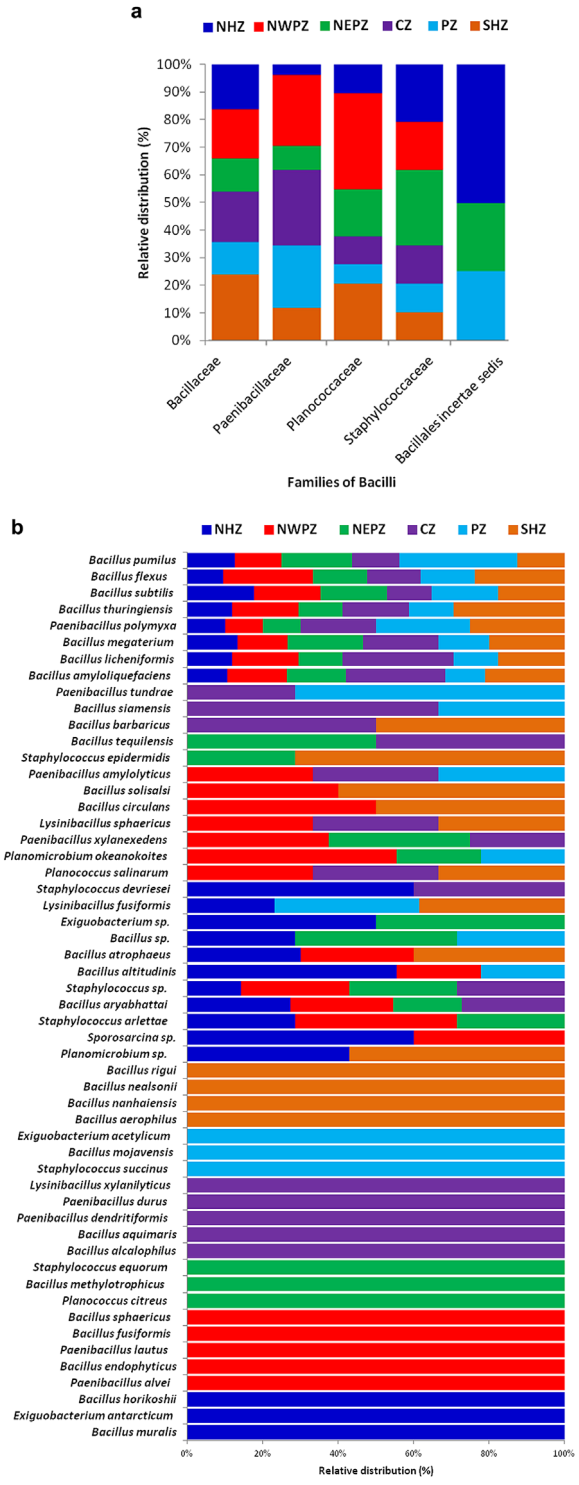

Figure 4. Abundance of different Bacilli. (a) Distribution of total Bacilli in six sampling zones; (b) distribution of different genera in six agro-ecological zones of India. NHZ, northern hills zone; NWPZ, north western plains zone; NEPZ, north eastern plains zone; CZ, central zone; PZ, peninsular zone; SHZ, southern hills zone.

In our study, *Bacillus* was most dominant genera followed by *Paenibacillus*. Among Bacilli, *B. flexus* (5.3%), *Paenibacillus polymyxa* (5.1%), *B. amyloliquefaciens* (4.8%), *B. cereus* (4.6%), *B. licheniformis* (4.6%), and *B. subtilis* (4.3%) were identified as most dominant bacteria associated with wheat rhizosphere whereas percentage ratio is 0.5% for *B. alcalophilus*, *B. mojavensis*, *Exiguobacterium acetylicum*, *E. antarcticum*, *Lysinibacillus xylanilyticus*, *Paenibacillus durus*, *P. lautus*, and *Staphylococcus equorum* (Table 2). Among 55 distinct species, *Bacillus amyloliquefaciens*, *B. cereus*, *B. flexus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *B. thuringiensis*, and *Paenibacillus polymyxa* were found to be common at all six zones surveyed. Apart from dominant and common species of Bacilli at diverse zones, niche-specific Bacilli were also identified at all the six zones. These niche-specific Bacilli were represented by *Bacillus horikoshii*, *Bacillus muralis*, and *Exiguobacterium antarcticum* at northern hills zone; *Bacillus endophyticus*, *Bacillus fusiformis*, *Bacillus sphaericus Paenibacillus alvei*, and *Paenibacillus lautus* at north western plains zone; *Bacillus methylotrophicus*, *Planococcus citreus*, and *Staphylococcus equorum* at north eastern plains zone; *Bacillus alcalophilus*, *Bacillus aquimaris*, *Bacillus siamensis*, *Paenibacillus dendritiformis*, and *Lysinibacillus xylanilyticus* at central zone; *Bacillus mojavensis*, *Exiguobacterium acetylicum*, and *Staphylococcus succinus* at peninsular zone and *Bacillus aerophilus*, *Bacillus atrophaeus*, *Bacillus nanhaiensis*, *Bacillus nealsonii*, and *Bacillus rigui* at southern hills zone (Fig. 5). Niche-specificity of these Bacilli may be due to their adaptation in respective extreme habitats. Other reports are also available on niche–specific bacterial diversity from different habitat [2, 17, 26, 32, 33].

In our study, psychrotolerant, salt tolerant, thermotolerant, acidotolerant, and drought tolerant bacteria were dominant at northern hills zone, north western plains/north eastern plains zone, peninsular zone, southern hills zone, and central zone respectively. Three strains, *Bacillus altitudinis* BNW15, *Paenibacillus amylolyticus* BNW23, and *Exiguobacterium* sp. BNE3 were found to grow best under high pH and saline conditions, consistent with the properties of its habitat and its phylogeny is predictive of a halo–alkaliphilic phenotype. The comprehensive analyses of diversity of BBDG and related genera by prospecting extreme habitats helped in the development of a huge database including baseline information on the distribution of BBDG and other genera in different niches and identifying niche-specific microbes.

To our knowledge, this is the first report for the presence of *Bacillus endophyticus*, *Paenibacillus xylanexedens*, *Planococcus citreus*, *Planomicrobium okeanokoites*, *Sporosarcina*

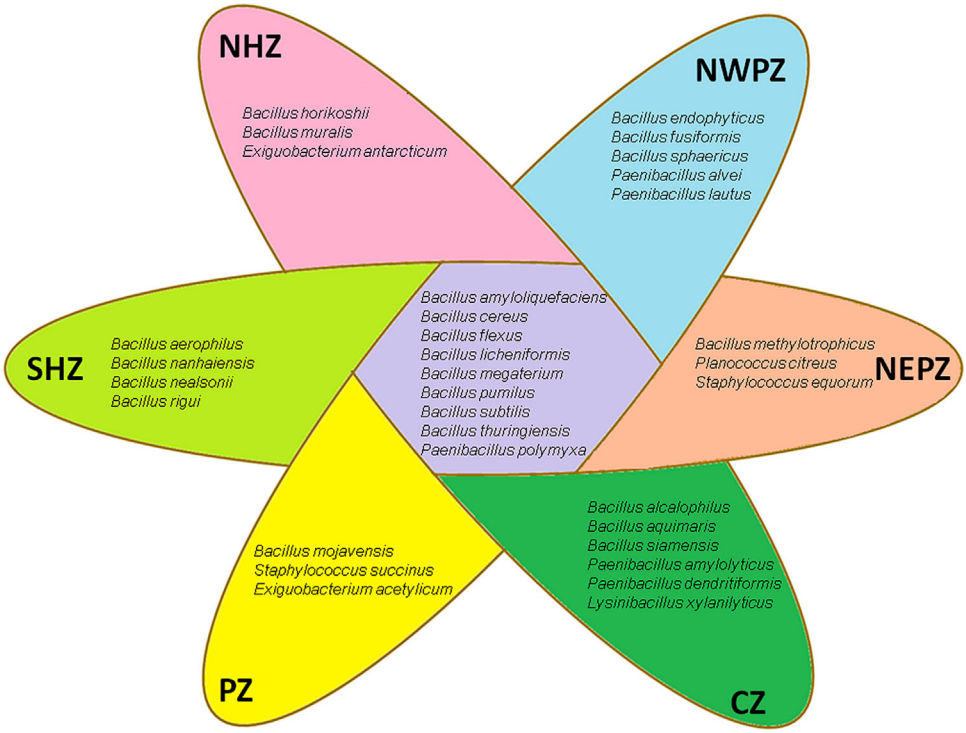

Figure 5. Distribution of niche-specific Bacilli in six agro-ecological zones of India. NHZ, northern hills zone; NWPZ, north western plains zone; NEPZ, north eastern plains zone; CZ, central zone; PZ, peninsular zone; SHZ, southern hills zone.

sp., and *Staphylococcus succinus* in wheat rhizosphere and exhibit multifunctional PGP attributes, leading significance to our study. These species have been earlier isolated from different crops. For example, *Bacillus endophyticus* was earlier isolated from the inner tissues of cotton plants (*Gossypium* sp.) [34]. It is niche-specific, isolated from wheat growing in north western plains zone of India, which distribution ratio is 0.8%. It is white colored and alkalitolerant bacterium that could tolerate 5% NaCl and produced ammonia, siderophore, HCN, protease, and IAA ($46.1 \pm 0.5\ \mu g\ mg^{-1}$ protein day^{-1}); solubilized phosphorus ($45.9 \pm 1.9\ mg\ L^{-1}$) and zinc ($3.9 \pm 0.6\ mg\ L^{-1}$), exhibited ACC deaminase and fixed 68.5 ± 1.3 nmol ethylene $h^{-1}\ mg^{-1}$ protein of nitrogen. It showed biocontrol activity against *Fusarium graminearum*, *Rhizoctonia solani*, and *Macrophomina phaseolina*. *Paenibacillus xylanexedens* was first time isolated from Alaskan tundra [12], later from cold desert of Chumathang, Indian Himalayas [35]. In present investigation, *Paenibacillus xylanexedens* is isolated from three different wheat agro–ecological zones namely, north eastern plains, north western plains and central with distribution ratio of 2.0%. It is cream/white coloured and alkalitolerant bacterium that could tolerate 10% NaCl and water stress of −0.5 MPa. It produced ammonia, siderophore, protease, gibberellic acid, and IAA ($30.8 \pm 1.1\ \mu g\ mg^{-1}$ protein day^{-1}); solubilized phosphorus ($66.2 \pm 0.6\ mg\ L^{-1}$), exhibited antagonistic against three fungal pathogen tested.

PGPR were found in several genera, such as *Arthrobacter*, *Bacillus*, *Brevundimonas*, *Burkholderia*, *Pseudomonas*, *Citricoccus*, *Exiguobacterium*, *Flavobacterium*, *Janthinobacterium*, *Kocuria*, *Lysinibacillus*, *Methylobacterium*, *Microbacterium*, *Paenibacillus*, *Providencia*, and *Serratia* [2, 35, 36]. Among PGPR, members of BBDG are ubiquitous bacteria that included both free living and pathogenic species. BBDG have been reported to enhance the growth of several plants such as wheat [2, 4, 5, 37] and rice [38]. Apart from BBDG, related genera such as *Exiguobacterium*, *Lysinibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, and *Staphylococcus* were also reported as PGPR. Wheat associated PGP Bacilli have a high potential for agriculture because they can improve plant growth, under limiting or stress

conditions [6]. Phosphorus (P) and potassium (K) are the major essential macronutrients for biological growth and development. However, the concentrations of soluble P and K in soil are usually very low, as the biggest proportions of P and K in soil are insoluble rocks, minerals, and other deposits. Different mechanisms have been proposed for solubilization of P from insoluble sources by the action of microorganisms mainly archaea, eubacteria, and fungi. The major mechanism of mineral phosphate solubilization by archaea, eubacteria, and fungi is the production of organic acids [39]. Among the organic acids produced, gluconic, 2-ketogluconic, citric, oxalic, lactic, isovaleric, succinic, glycolic, and acetic acids have been most frequently reported from P-solubilizing archaea/bacteria [39]. Production of organic acids results in the lowering of pH in the surroundings and many reports suggests a positive correlation between lowering of pH and mineral phosphate solubilization.

In the present study, P-solubilization activity was exihibited by different genera such as *Bacillus*, *Paenibacillus*, *Exiguobacterium*, *Lysinibacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, and *Staphylococcus*. K-solubilizing bacteria (KSB) were found to resolve potassium, silicon, and aluminium from insoluble minerals. To the best of our knowledge, *Paenibacillus dendritiformis*, *Planococcus citreus*, and *Planococcus salinarum* are reported as K-solubilizers, for the first time. The K-solubilizing bacteria may have use in the amelioration of K-deficient soil in agriculture. Zinc is a nutrient at low concentration but toxic at higher concentration. In present study, 11 Bacilli namely *Bacillus aerophilus* BSH15, *Bacillus atrophaeus* BSH3, *Bacillus circulans* BSH11, *Bacillus licheniformis* BPZ5, *Bacillus megaterium* BNH1, *Bacillus mojavensis* BPZ6, *Bacillus sphaericus* BNW8, *Paenibacillus dendritiformis* BCZ2, *Paenibacillus polymyxa* BNW6, *Planococcus citreus* BNE6, and *Planococcus salinarum* BSH13 exhibited all three types of solubilization of phosphorus, potassium, and zinc. Indirect plant growth promotion included antagonistic, production of lytic enzymes (protease, lipase, chitinase, and β-glucanase), siderophore, ammonia, and hydrogen cyanide. The production of siderophore by microbes influence plant growth by binding to the available iron form (Fe^{3+}) in the rhizosphere and in this process, iron is made unavailable to the phytopathogens and thus siderophore protects the plant health. HCN production by bacteria has been reported as a major means of control of diseases crop plants. This may be attributed to presence of a cyanide-resistant respiratory pathway in plants. The most interesting point is that many BBDG can play a key role in biocontrol by producing peptide antibiotics, such as polymyxins and mattacin, which are antagonistic factors.

In conclusion, the selection of native functional wheat associated plant growth promoting microbes is a mandatory step for reducing the use of energy intensive chemical fertilizers. PGP Bacilli have an advantage over other group of microbes due to their spore formation capability for survival in extreme habitats for years and thus can increase the shelf life of the bioinoculants. Niche–specific and multifarious PGP Bacilli could be used as potential inoculants in extreme habitats as they have multiple plant growth promotion attributes and adaptation to diverse abiotic stresses.

Acknowledgments

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Conflict of interest

The experiments undertaken comply with the current laws of India, the country where the investigation was undertaken. There are no conflicts of interest.

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