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Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India

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Abstract The biodiversity of wheat associated bacteria was deciphered from the peninsular zone of India. A total of 264 isolated bacteria were analyzed through amplified ribosomal DNA restriction analysis (ARDRA, using three restriction enzymes *Alu* I, *Msp* I and *Hae* III, which led to the clustering of these isolates into 12–16 groups for the different sites at > 75% similarity index, adding up to 70 groups). 16S rRNA gene based phylogenetic analysis, revealed that all the bacteria belonged to three phyla Proteobacteria, Firmicutes, and Actinobacteria of 32 distinct species of 15 genera namely: *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Delftia*, *Enterobacter*, *Exiguobacterium*, *Klebsiella*, *Methylobacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Rhodobacter*, *Salmonella* and *Staphylococcus*. Representative strains from each cluster were screened *in vitro* for plant growth promoting traits. Among plant growth promoting activities, siderophore producers were highest (15%), when compared to indole acetic acid producers (13%), Zn-solubilizers (11%), P-solubilizers (11%), ammonia (10%), hydrogen cyanide producers (9%), biocontrol (8%), N_2-fixers (7%), 1-aminocyclopropane-1-carboxylate deaminase (6%), GA producers (6%) and K-solubilizers (5%). Among 32 representative strains, *Alcaligenes faecalis*, *Arthrobacter* sp., *Bacillus siamensis*, *Bacillus subtilis*, *Delftia acidovorans*, *Methylobacterium mesophilicum*, *Methylobacterium* sp., *Pseudomonas poae*, *Pseudomonas putida*, and *Pseudomonas stutzeri* exhibited more than six

different plant growth promoting activities at high temperature. Thermotolerant bacterial isolates may have application as inoculants for plant growth promotion and biocontrol agents for crops growing at high temperature conditions.

1. Introduction

Biodiversity is an important element of environmental conservation and is central to agriculture production. Globally wheat (*Triticum aestivum* L.) is the most important cereal crop. It is grown across a wide range of environments around the world and has the highest adaptation among all the crop species (Naresh et al., 2014). Wheat is grown in India in an area of about 30 million hectares (mha), with a production of 93 million tones. The normal national productivity is about 2.98 tones ha^{-1}. Currently, India is the second largest producer of wheat in the world after China with about 12% share in total world wheat production. Now, India is surplus and in a position to export wheat in the International Market and can earn foreign exchange (http://www.fao.org/docrep/006/y4011e/y4011e04.htm). Global warming and its associated effects are expected to impose abiotic stresses, such as extremes of temperatures, drought and flooding, that are bound to have adverse effects on food production. Climate change affects agriculture and the food production system in many ways (Godfray et al., 2011). Crop production is affected by climatic variables such as rising temperatures, changing precipitation regimes and increased atmospheric CO_2 levels. It is also affected by biological variables such as the lengths of the crop growth periods and the crop cycle.

Wheat is growing in six agro-ecological zones in India, in which peninsular zone exhibits unique characteristics in the distribution and variation in the meteorological fields compared to other parts of the country. Epiphytic, endophytic and rhizospheric bacteria have been shown to promote plant growth directly, e.g. by the fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, potassium and zinc, production of siderophores and plant growth hormones such as cytokinin, auxin and gibberellins. Several bacteria support plant growth indirectly, *via* production of antagonistic substances by inducing resistance against plant pathogens (Glick, 2015; Verma et al., 2015a; Egamberdieva et al., 2015). It has gained importance over the use of chemical fertilizers on account of deleterious effect of the latter on soil and plant health (Glick, 2015). Divergent bacterial genera are vital components of soils and plant growth. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production (Hayat et al., 2010). Indeed, the bacteria lodging around/in the plant roots, shoots and leaves are more versatile in transforming, mobilizing, and solubilizing the nutrients (Hayat et al., 2010). Therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility (Glick, 2015).

Wheat (*Triticum aestivum* L.) is a major staple food crop for more than one third of the world population and is the main staple food of Asia. The wheat associated bacterial diversity inhabiting low temperature (Mishra et al., 2011; Verma et al., 2015a), drought (Verma et al., 2014), acidic soil (Verma et al., 2013) and salinity (Egamberdieva et al., 2008; Tiwari et al., 2011) has been extensively investigated in the past few years with a focus on culture dependent techniques. However there are no reports available on thermotolerant bacterial diversity associated with wheat. The present study investigated on understanding the diversity and distribution of culturable bacteria associated with wheat growing in the peninsular zone of India. Bacteria were isolated, identified using 16S rRNA gene sequencing and characterized for plant growth promoting attributes at high temperature. The use of thermotolerant bacteria as biofertilizers and biocontrol agents would be of great use in Indian agriculture under high temperature conditions.

2. Material and methods

2.1. Sample collection and physico-chemical properties

The wheat plant with rhizospheric soil was collected from different sites in the peninsular zone of India. Forty-one samples were collected from five different sites in the peninsular zone namely: Dharwad, Nashik, Warangal, Coimbatore and Krishna Nagar, whose detail is provided in Table 1. Collected samples in sterile polythene bags were labeled and transported on ice stored at 4 °C, until samples process quickly to the laboratory (Table 1). The pH and electrical conductivity of the samples were recorded at sampling sites. Methods of soil organic carbon, total nitrogen, exchangeable cations and

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

Sampling sites	GPS coordinate	No. of samples	Temperature (°C)	pH	Cond uctivity (mS cm^{-1})
Nashik	19° 59′ 00″ N: 73° 48′ 05″ E	16	35.5–35.7	6.8–7.9	261–805
Warangal	18° 01′ 05″ N: 79° 35′ 02″ E	18	33.2–42.5	7.1–8.5	376–392
Krishna Nagar	23° 24′ 01″ N: 88° 33′ 05″ E	12	35.2–45.6	7.3–8.3	345–365
Dharwad	15° 28′ 02″ N: 75° 01′ 01″ E	17	35.8–47.5	6.9–8.1	237–255
Coimbatore	10° 59′ 33″ N: 76° 57′ 41″ E	14	34.9–47.8	6.8–8.4	266–287

available phosphorus was analyzed as followed in our earlier studies (Yadav et al., 2015e).

2.2. Isolation and enumeration of bacteria

Wheat associated bacteria were isolated from leaves, shoots, roots and rhizospheric soils using different growth media (Supplementary Table S1). The culturable bacteria were isolated from rhizospheric soil through the enrichment method, using the standard serial dilution plating technique. Epiphytic and endophytic bacteria were isolated as methods described in our earlier studies (Verma et al., 2015a). Plates were incubated at 30–50 °C for the growth of bacteria. Colonies that appeared were purified by repeated streaking on their respective medium plates. The pure cultures were maintained at 4 °C as slant and glycerol stock (25%) at −80 °C for further use.

2.3. Molecular characterization and amplified rDNA restriction analysis

Genomic DNA was extracted by the method as described earlier by Verma et al. (2016b). 16S rRNA gene sequence was amplified using universal primers pA (5′-AGAGTTT GATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATC CAGCCGCA-3′). The amplification program employed in a 100 µL composed: each primer at a concentration of 1 µL (90 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 used were as described earlier (Yadav, 2015). The PCR product was purified by PCR purification kit (Qiagen USA). 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 was undertaken using NTSYS-2.02e package (Numerical taxonomy analysis program package, Exeter software, USA). Similarity among the isolates was calculated by Jaccard's coefficient and the dendrogram was constructed using UPGMA method as described earlier (Suman et al., 2015b).

2.4. Sequencing of 16S rRNA gene and phylogenetic analysis

PCR products of partial 16S rRNA gene were sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in 3130xl Applied Biosystems ABI prism automated DNA sequencer at Xcelris Labs Ltd. Ahmedabad (India). The DNA sequence was double checked by sequencing both strands using primers pA and pH for forward and reverse reactions, respectively. The partial 16S rRNA gene sequences of the isolated strains were BLAST searched on the NCBI GenBank website to identify the closest matches. Sequence alignment and comparison was performed, using the program CLUSTAL W software. 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 software (Tamura et al., 2007).

2.5. Nucleotide sequence accession numbers

The partial 16S rRNA gene sequences of 32 bacteria were submitted to NCBI GenBank under the assigned accession numbers KF054914–KF054942, KF054945 and KF573002–KF573003.

2.6. Statistical analysis for bacterial diversity

In order to compare the bacterial diversity among five different sites in the peninsular zone, the 16S rRNA gene sequences of the isolates showing ⩾97% sequence similarity were grouped into the same OTU (phylotype). The Shannon index (H), Evenness (J) Simpson's index (D) and Chao-1 were calculated as described earlier (Yadav et al., 2015c). Using 16S rRNA gene sequences, a rarefaction curve was generated to compare the relative diversity and coverage of each sample. Principal coordinate analysis (PCA) was performed for different physico-chemical properties and distribution ratio, using the XLSTAT program (http://www.xlstat.com).

2.7. Phenotypic characterization

Representative isolates from each cluster were screened for tolerance to temperatures (30–70 °C), pH (3–11), and salinity (3–20% NaCl concentration) as describes earlier (Yadav et al., 2015e).

2.8. Functional characterization

2.8.1. Qualitative estimation

Wheat associated bacterial isolates were initially screened for direct PGP attributes which included the production of phytohormones indole-3-acetic acid, gibberellic acid and 1-aminocy clopropane-1-carboxylate (ACC) deaminase as described earlier (Verma et al., 2016b). Solubilization of phosphorus, potassium and zinc was carried according to methods described by Pikovskaya (Pikovskaya, 1948), Hu et al. (2006) and Fasim et al. (2002) using Pikovskaya agar, Aleksandrov medium and nutrient agar medium supplemented with 0.5% tricalcium phosphate, 0.2% potassium aluminosilicate minerals and 0.1% insoluble zinc compounds (ZnO, ZnS, Zn$_3$(PO$_4$)$_2$ and ZnCO$_3$) respectively. The bacterial strains were screened for their ability to utilize the 1-Aminocyclopropane-1-carboxylate (ACC) as the sole nitrogen source, a trait that is a consequence of the activity of the enzyme ACC deaminase (Jacobson et al., 1994). The bacterial strains were spotted on MDF (modified Dworkin and Foster medium) agar plate, MDF agar plate supplemented with 0.3 g L^{-1} of ACC and MDF agar plate with ammonium sulfate 0.3 g L^{-1}. After 72 h of incubation, plates were observed for growth. All the representative strains were also screened for indirect PGP attributes which included the production of siderophores (Schwyn and Neilands, 1987), ammonia (Cappucino and Sherman, 1992), and HCN (Bakker and Schippers, 1987). All assays were done in triplicate.

2.8.2. Quantitative estimation

Representative strains were further screened quantitatively for the solubilization of phosphorus, potassium and zinc; biological nitrogen fixation and indole acetic acid production as methods described in our earlier studies (Verma et al., 2016b). Gibberellic acid production was estimated by colorimetric method of Holbrook et al. (1961). One milliliter bacterial culture was inoculated in 50 mL nutrient broth in Erlenmeyer flasks (100 mL), and incubated at 30 °C in a rotary shaker at 180 rpm. The bacterial cultures were harvested and centrifuged at 10,000g for 30 min. The culture supernatant was acidified (pH 2.5 using 2.0 N HCl) and extracted by adding an equal volume of ethyl acetate in three stages to obtain 45 mL extract. The extract was evaporated to 5.0 ml and gibberellic acid was estimated by colorimetric method using 100 µg/ml gibberellic acid (Sigma, USA) as standard.

2.9. Antagonistic property

In vitro antagonistic activity of bacterial isolates was evaluated against three fungal pathogens *Fusarium graminerum* ITCC 1856, *Rhizoctonia solani* ITCC 2775 and *Macrophomina phaseolina* ITCC 3134 according to the method described by Sijam and Dikin (2005). The fungal pathogens involved in root-rot complex in crops, were obtained from the Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute, New Delhi, India. The fungal strains were inoculated on the potato dextrose agar plates and allowed to grow for 3–5 days. Bacterial strains were spot inoculated on potato dextrose agar plates already inoculated with fungal colony. Control plates with only the mycelia plug were set up and, when the pathogen had grown across these control plates, the diameter of growth in the challenge plates was measured. Triple culture assays were repeated three times per isolate and for each fungus.

3. Results

3.1. Isolation of wheat associated bacteria

The populations of bacterial isolates were enumerated in wheat plant samples collected from five diverse sites in the peninsular zones of India (Table 1). A total of 264 bacteria were isolated from Nashik (48), Warangal (43), Krishna Nagar (59), Dharwad (62) and Coimbatore (52). The significant variations were observed among the culturable bacterial populations of each sample on different media and at different sites (Supplementary Table S1). The abundance of bacteria varied from 1.4×10^3 to 7.2×10^6 CFU g^{-1} soils, 1.9×10^6 to 6.32×10^6 CFU g^{-1} leaves and 1.2×10^6 to 2.58×10^6 CFU g^{-1} roots with the lowest and highest values recorded in Nashik and Warangal, respectively. Among the different media used, tryptic soy agar supported the highest population of bacteria 7.2×10^6 CFU g^{-1} soil and Jensen's agar medium the least growth for bacteria 1.4×10^3 CFU g^{-1} soil. The highest population and number of bacteria (172) were isolated from the rhizospheric samples, whereas the least number of bacteria (26) was recovered from putative endophytic samples (Supplementary Table S1).

3.2. Molecular characterization 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 3–7 fragments ranging in size from 100 to 850 base pairs. The dendrogram was constructed for each site to determine the percent similarity among the isolates. The isolates were grouped into clusters; and the number of clusters ranged from 12–16 adding up to 70 groups (Table 2). All 70 strains were sequenced and BLAST analysis showed >97–100% similarity with the sequences available in GenBank (Table 3), that reveled the identification of 32 distinct species of fifteen genera namely: *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Delftia*, *Enterobacter*, *Exiguobacterium*, *Klebsiella*, *Methylobacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Rhodobacter*, *Salmonella* and *Staphylococcus*. The eleven isolates showing <96% similarity in partial 16S rRNA gene sequences within the GenBank database need further characterization and validation to confirm their taxonomic position. The phylogenetic tree of 32 identified species of bacteria and related genera were constructed to determine their affiliations (Fig. 1a and b).

Analysis of the 16S rRNA gene sequences revealed that 32 strains belonged to three phyla namely Proteobacteria (22 strains), Firmicutes (10 strains) and Actinobacteria (2 strains) (Fig. 1a and b). The Proteobacteria were further distributed into 3 class, as α-proteobacteria (4 strains), β-proteobacteria (7 strains) and γ-proteobacteria (11 strains). Analysis of the 16S rRNA gene sequences revealed that 65%, 29% and 6% bacteria belong to three phyla namely Proteobacteria, Firmicutes and Actinobacteria respectively, with 32 distinct species of 15 genera (Fig. 2a and b). The Proteobacteria was the most pre-dominant phylum followed by Firmicutes. *Arthrobacter* from Actinobacteria, *Bacillus* from Firmicutes and *Pseudomonas* from Proteobacteria were the most frequently recovered genera, respectively (Table 2; Fig. 1).

3.3. Statistical analysis

All 264 isolates from the five different sites of wheat growing in the peninsular zone were categorized into 32 clusters, based on a similarity index of >75% of the ARDRA pattern. Sequencing of representative isolates from each cluster led to the identification of 32 distinct strains. Of the 32 strains identified, four strains, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Methylobacterium* sp. and *Pseudomonas aeruginosa* were common to all five sites (Table 2). Shannon's diversity index (H' = 2.73) and species richness was the highest for Krishna Nagar, whereas Warangal recorded the lowest value (H' = 2.42). These observations are supported by bacterial diversity parameters, such as Simpson's index, Chao-1, Evenness, and Shannon Entropy and individual rarefaction curves (Table 2). The individual rarefaction curves for all the samples of five sites indicated that the bacterial population were the least diverse in Warangal followed by Coimbatore and most diverse in Krishna Nagar followed by Dharwad (Fig. 3a). Principal coordinate analysis was used to investigate relation-

Table 2 Distribution and diversity indices of bacteria isolates from different sites in the peninsular zone of India.

Bacterial isolates	Nashik	Warangal	Krishna Nagar	Dharwad	Coimbatore
Achromobacter spanius	3*				
Alcaligenes faecalis		5			
Alcaligenes sp.	2			5	
Arthrobacter sp.	3	5	2	1	
Bacillus altitudinis					5
Bacillus amyloliquefaciens	5@	2	3	5	4
Bacillus flexus	3	4	3	2	
Bacillus licheniformis	5		5	4	
Bacillus mojavensis					5
Bacillus siamensis		3	5		
Bacillus subtilis	3	2	3	5	4
Delftia acidovorans					5
Delftia lacustris		5			
Delftia sp.	5		4		3
Delftia tsuruhatensis					4
Enterobacter asburiae				5	
Enterobacter hormaechei				6	
Exiguobacterium acetylicum			5		
Klebsiella oxytoca		5			
Micrococcus luteus	5		4		
Methylobacterium sp.	2	3	5	5	4
M. mesophilicum					5
Paenibacillus amylolyticus	2				
Pseudomonas aeruginosa	3	4	5	2	2
Pseudomonas japonica			3		
Pseudomonas poae			2		
Pseudomonas putida		2	4	5	2
Pseudomonas stutzeri	5			3	
Rhodobacter capsulatus				4	
Rhodobacter sphaeroides				5	
Salmonella bongori		3		5	4
Staphylococcus succinus	2		3		5
Total isolates	48	43	59	62	52
Species richness	14	12	16	15	13
Chao-1	14	12	16	15	13
Niche-specific	02	03	02	04	05
Simpson's (D)	0.91	0.90	0.93	0.92	0.91
Shannon (H)	2.57	2.42	**2.73**	2.63	2.52
Evenness (J´)	0.93	0.94	0.95	0.93	0.96

*Niche-specific bacteria; @common bacteria at all sites.

ships between bacterial diversity (Shannon's diversity index). The first two dimensions of PCA (PCA1 and PCA2) explained 83.87% of the total variation, with component 1 accounting for 69.08% and component 2 for 14.79% of the variance (Fig. 2b). Correlation analysis proved the existence of significant relationship between the different parameters such as temperature, pH, conductivity, organic carbon, available nitrogen, phosphorus, potassium, zinc, exchangeable sodium,

dehydrogenase and alkaline phosphatase of sampling sites. The first two factorial axes of biplot represented 26.15–46.29% variance in the data (Fig. 3c).

3.4. Phenotypic characterization

All 264 bacteria were screened for tolerance to different abiotic stresses and results are represented by representative strains

Table 3 Identification and plant growth promoting attributes of bacterial isolates from wheat cultivating in different sites in the peninsular zone of India.

Nearest phylogenetic relative	Strain number	Relative distribution (%)	Solubilization		
			Phosphorus	Potassium	Zinc[#]
Achromobacter spanius	IARI-NIAW2-15	1.1	61.9 ± 0.2	–	2.4 ± 0.1
Alcaligenes faecalis	IARI-NIAW1-6	3.8	55.9 ± 1.4	–	3.3 ± 0.2
Alcaligenes sp.	IARI-NIAW1-8	6.8	63.0 ± 1.0	–	1.6 ± 0.9
Arthrobacter sp.	IARI-NIAW1-4	7.2	66.0 ± 0.7	–	2.0 ± 1.1
Bacillus altitudinis	IARI-NIAW1-38	3.8	45.7 ± 1.1	23 ± 0.5	4.3 ± 0.2
Bacillus amyloliquefaciens	IARI-NIAW1-23	12.5	–	–	–
Bacillus flexus	IARI-NIAW2-25	8.0	47.8 ± 0.1	–	–
Bacillus licheniformis	IARI-NIAW2-3	8.7	45.6 ± 1.0	–	–
Bacillus mojavensis	IARI-NIAW2-23	3.8	43.9 ± 0.7	–	–
Bacillus siamensis	IARI-NIAW1-21	6.1	–	27 ± 0.9	2.6 ± 0.1
Bacillus subtilis	IARI-NIAW1-13	11.7	47.9 ± 1.4	17 ± 0.9	4.5 ± 0.2
Delftia acidovorans	IARI-NIAW1-20	3.8	47.2 ± 1.4	33 ± 0.5	5.0 ± 0.6
Delftia lacustris	IARI-NIAW1-34	3.8	43.7 ± 0.9	–	1.1 ± 0.4
Delftia sp.	IARI-NIAW1-31	7.2	–	12 ± 0.8	2.4 ± 0.8
Delftia tsuruhatensis	IARI-NIAW1-15	3.0	–	–	2.0 ± 1.3
Enterobacter asburiae	IARI-NIAW2-21	3.8	–	–	1.5 ± 0.1
Enterobacter hormaechei	IARI-NIAW2-34	4.5	–	–	–
Exiguobacterium acetylicum	IARI-NIAW2-27	3.8	–	–	–
Klebsiella oxytoca	IARI-NIAW2-11	3.8	–	–	–
Micrococcus luteus	IARI-NIAW1-1	4.9	–	–	–
Methylobacterium sp.	IARI-NIAW2-37	13.6	41.6 ± 0.1	27 ± 0.9	3.6 ± 0.1
Methylobacterium mesophilicum	IARI-NIAW1-41	3.8	43.2 ± 1.1	29 ± 0.5	3.4 ± 0.2
Paenibacillus amylolyticus	IARI-NIAW2-33	0.8	–	–	–
Pseudomonas aeruginosa	IARI-NIAW1-2	11.0	–	33 ± 1.2	1.6 ± 0.7
Pseudomonas japonica	IARI-NIAW2-24	2.3	64.6 ± 0.9	–	2.6 ± 0.5
Pseudomonas poae	IARI-NIAW2-1	1.5	34.4 ± 1.2	–	3.6 ± 1.2
Pseudomonas putida	IARI-NIAW1-16	9.8	54.6 ± 0.9	–	–
Pseudomonas stutzeri	IARI-NIAW1-3	3.0	51.6 ± 1.0	–	2.1 ± 1.1
Rhodobacter capsulatus	IARI-NIAW1-9	3.8	–	–	–
Rhodobacter sphaeroides	IARI-NIAW1-7	9.1	45.0 ± 1.2	–	–
Salmonella bongori	IARI-NIAW2-19	6.8	55.7 ± 0.5	28 ± 1.2	–
Staphylococcus succinus	IARI-NIAW2-28	1.1	40.4 ± 1.1	–	–

Strain number	Production					Other activities		
	IAA	Sidero[#]	GA	HCN	NH$_3$	ACC	N$_2$F	BC
IARI-NIAW2-15	27.8 ± 1.2	1.0 ± 0.1	–	–	+	–	11.5 ± 1.5	–
IARI-NIAW1-6	21.4 ± 1.3	4.9 ± 0.1	–	–	+	+	17.5 ± 1.0	+
IARI-NIAW1-8	20.4 ± 1.1	2.1 ± 0.1	45.5 ± 1.8	+	–	–	–	–
IARI-NIAW1-4	11.4 ± 1.5	2.6 ± 0.1	–	+	+	+	–	–
IARI-NIAW1-38	16.6 ± 1.0	3.5 ± 0.2	–	–	+	–	–	–
IARI-NIAW1-23	–	2.5 ± 0.1	–	+	+	–	–	–
IARI-NIAW2-25	–	2.6 ± 0.1	–	–	–	–	–	–
IARI-NIAW2-3	–	–	–	+	+	–	55.5 ± 1.5	–
IARI-NIAW2-23	15.6 ± 0.7	–	22.6 ± 1.5	+	–	–	45.3 ± 1.2	–
IARI-NIAW1-21	6.6 ± 1.0	–	12.2 ± 0.3	+	–	–	23.5 ± 1.3	+
IARI-NIAW1-13	8.75 ± 1.2	1.8 ± 0.2	–	+	–	+	–	+
IARI-NIAW1-20	17.8 ± 1.2	2.1 ± 0.1	15.5 ± 1.5	+	–	–	31.5 ± 1.6	+
IARI-NIAW1-34	–	1.5 ± 0.1	–	+	–	–	13.5 ± 1.7	+
IARI-NIAW1-31	–	1.2 ± 0.2	–	–	+	–	–	+
IARI-NIAW1-15	22.8 ± 0.8	3.1 ± 0.1	–	+	–	–	–	+
IARI-NIAW2-21	28.6 ± 1.0	4.1 ± 0.2	–	+	+	–	–	–
IARI-NIAW2-34	–	3.2 ± 0.2	–	–	–	–	–	+
IARI-NIAW2-27	17.2 ± 1.1	2.2 ± 0.5	11.8 ± 0.3	–	+	–	–	+
IARI-NIAW2-11	12.2 ± 1.0	3.2 ± 0.5	10.1 ± 0.5	–	+	+	–	–
IARI-NIAW1-1	18.2 ± 1.0	4.2 ± 0.5	33.3 ± 0.6	–	+	+	–	–
IARI-NIAW2-37	25.5 ± 1.2	3.6 ± 0.1	17.1 ± 0.8	+	+	–	–	+
IARI-NIAW1-41	31.6 ± 0.5	4.1 ± 0.2	43.1 ± 1.2	+	+	–	–	+
IARI-NIAW2-33	27.2 ± 1.0	2.4 ± 1.2	32.1 ± 1.6	–	+	+	–	–
IARI-NIAW1-2	–	2.2 ± 1.0	18.1 ± 0.5	–	+	+	–	–

Table 3 (continued)

Strain number	Production					Other activities		
	IAA	Sidero#	GA	HCN	NH$_3$	ACC	N$_2$F	BC
IARI-NIAW2-24	–	3.1 ± 0.1	–	+	+	–	–	+
IARI-NIAW2-1	15.2 ± 1.1	1.6 ± 0.2	–	+	–	–	67.5 ± 1.6	+
IARI-NIAW1-16	70.8 ± 1.5	4.1 ± 0.1	9.8 ± 0.5	+	+	+	45.5 ± 1.2	–
IARI-NIAW2-29	20.8 ± 1.5	1.8 ± 0.1	–	–	+	+	87.5 ± 2.5	+
IARI-NIAW1-3	25.4 ± 1.2	–	–	+	+	–	14.5 ± 1.0	+
IARI-NIAW1-9	69.1 ± 0.5	4.6 ± 0.1	–	–	–	–	11.5 ± 1.1	–
IARI-NIAW1-7	18.2 ± 1.1	4.4 ± 0.1	–	–	–	+	–	+
IARI-NIAW2-19	66.7 ± 0.5	2.5 ± 0.2	–	+	–	–	18.5 ± 1.8	–
IARI-NIAW2-28	66.1 ± 0.7	2.4 ± 0.1	–	–	–	+	–	–

IAA, Indole 3-acetic acid-(μg mg^{-1} protein day^{-1}); phosphorus (mg L^{-1}); potassium (mg mL^{-1})' *GA*, gibberellic acid (μg ml^{-1}); *HCN*, Hydrogen cyanide, *ACC*, 1-aminocyclopropane-1-carboxylate; *N$_2$F*, N$_2$-fixation; *Numerical values are mean ± SD of three independent observations; # Radius of halo zone in mm; (−), negative for the attributes; (+), positive for the attributes

from each cluster at different sites. Among the representative 32 strains, tolerant to a temperature of 50 °C or more 15 strains could grew at up to 65 °C. Among 32 strains, majority of strains could tolerate 5–8 pH. Three strains *B. amyloliquefaciens* IARI-NIAW1-23, *Enterobacter asburiae* IARI-NIAW2-21, and *P. aeruginosa* IARI-NIAW1-2 could be grouped as acido-thermotolerant bacteria. Five strains *Bacillus siamensis* IARI-NIAW1-21, *P. aeruginosa* IARI-NIAW1-2, *Pseudomonas poae* IARI-NIAW2-1, *Staphylococcus succinus* IARI-NIAW2-28 and *Delftia acidovorans* IARI-NIAW1-20 could tolerate 15% NaCl said to be halo-thermotolerant bacteria (Supplementary Table S2).

3.5. Plant growth promoting attributes

All 264 isolates were screened for direct and indirect PGP traits and results were represented by 32 representative isolates. Of 32 representatives, 20, 9 and 18 strains exhibited solubilization of phosphorus, potassium and zinc, respectively (Table 3). Out of 32 representatives, 25 strains produced indole-3-acetic acid, while only 12 strains produced gibberellic acid (Table 2). Nitrogen fixation and ACC deaminase activity were exhibited by 13 and 11 strains, respectively. Of 32 representatives 29, 19, 18, and 16 strains exhibited activity for siderophore, ammonia, HCN and biocontrol respectively (Table 3).

The strain *Arthrobacter* sp. IARI-NIAW1-4 solubilized the highest amount of phosphorus (66.0 ± 0.7 mg L^{-1}) followed by *Pseudomonas japonica* IARI-NIAW2-24 (64.6 ± 0.9 mg L^{-1}). *Methylobacterium mesophilicum* IARI-NIAW1-41 exhibited the highest solubilization of potassium (Table 3). *Pseudomonas putida* IARI-NIAW1-16 showed the highest IAA production (70.8 ± 1.5 μg mg^{-1} protein day^{-1}) followed by *Rhodobacter capsulatus* IARI-NIAW1-9 (69.1 ± 0.5 μg mg^{-1} protein day^{-1}). *Alcaligenes faecalis* IARI-NIAW1-6 showed the highest production of siderophore (4.9 ± 0.1 mm). Sixteen strains showed antagonistic activity against *Fusarium graminerum*, *Rhizoctonia solani* and *Macrophomina phaseolina* (Table 3). Among thirty-two strains, ten strains were identified as *A. faecalis*, *Arthrobacter* sp., *B. siamensis*, *B. subtilis*, *D. acidovorans*, *M. mesophilicum*, *Methylobacterium* sp., *P. poae*, *P. putida*, and *Pseudomonas stutzeri* exhibiting more than six different plant growth promoting activities at high temperatures (Fig. 3).

4 Discussion

Over the past decades, climate change has directly affected the plant growth with different abiotic stresses and change ecosystems. For instance, thermotolerant microbes used as plant growth promoting to protect the diverse stresses have become more production and yield in many crops. The extreme environment of high temperature harbors microbial diversity, which may be fundamental for the maintenance and conservation of global genetic resources. Our present study deciphers the diversity of microbial communities coupled with the epiphytic, endophytic and rhizosphere of wheat crop and their PGP attributes. A total of 264, wheat allied bacterial isolates were obtained from different sites in the peninsular zone of India. To the best of our knowledge, this is the first report, which elucidates the bacterial diversity associated with rhizosphere, endophytic and epiphytic of wheat crops and their PGP attributes at high temperature condition.

Sequencing of 16S rRNA gene of the representative strain from each cluster of all five different sites was identified and selected 32 distinct species were taken up for phylogenetic analysis. Partial sequencing of the smaller subunit of 16S rRNA gene assigned and all the representative bacteria could be grouped into 3 phyla, Actinobacteria (6%), Firmicutes (29%) and Proteobacteria (64%) (Fig. 4a). Among the five sites analyzed, at four sites all three phyla were present whereas in Coimbatore only members of phyla Proteobacteria and Firmicutes are present (Fig. 4b). Many species of *Bacillus*, *Pseudomonas* and *Methylobacterium* were found to be common to all five different sites. Apart from dominant and common species of bacteria at different sites, niche specific bacterial species were also identified at all the sites. These niche specific bacteria were represented by *Bacillus altitudinis*, *Bacillus mojavensis*, *D. acidovorans*, *Delftia tsuruhatensis*, and *M. mesophilicum* in Coimbatore; *E. asburiae*, *Enterobacter hormaechei*, *R. capsulatus* and *Rhodobacter sphaeroides* in Dharwad; *Exiguobacterium acetylicum*, *P. japonica* and *P. poae* in Krishna Nagar; *A. faecalis*, *Delftia lacustris* and *Micrococcus luteus* in Warangal; *Achromobacter spanius* and *Paenibacillus amylolyticus* in Nashik (Table 2; Fig. 4c). Other reports are also available on niche-specific bacterial diversity from different habitats (Pandey et al., 2013; Kumar et al., 2014a,b; Verma et al., 2016b; Yadav et al., 2015a–c, 2016).
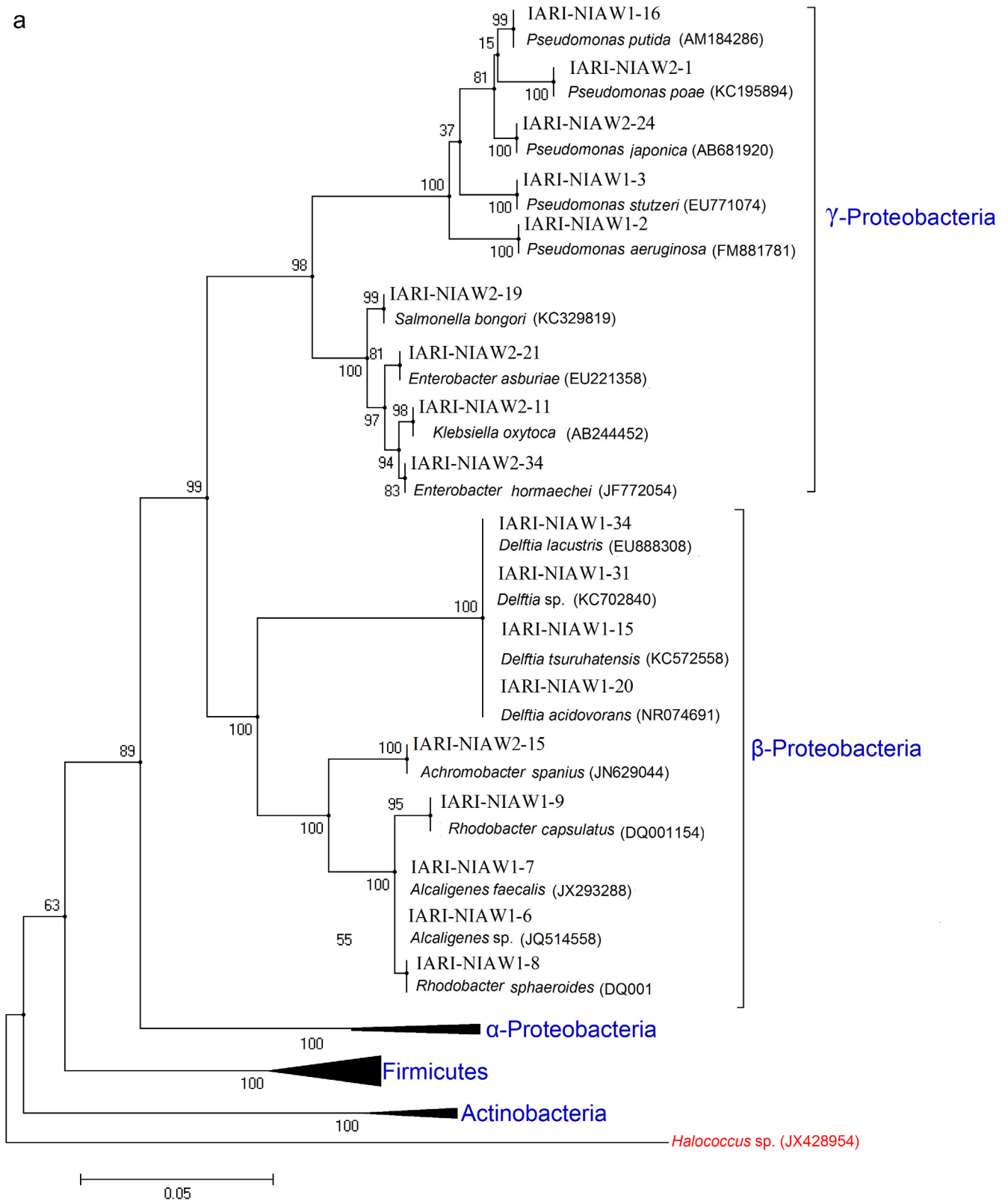
a

Figure 1 Phylogenetic tree showing the relationship among 32 bacteria isolates, 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).
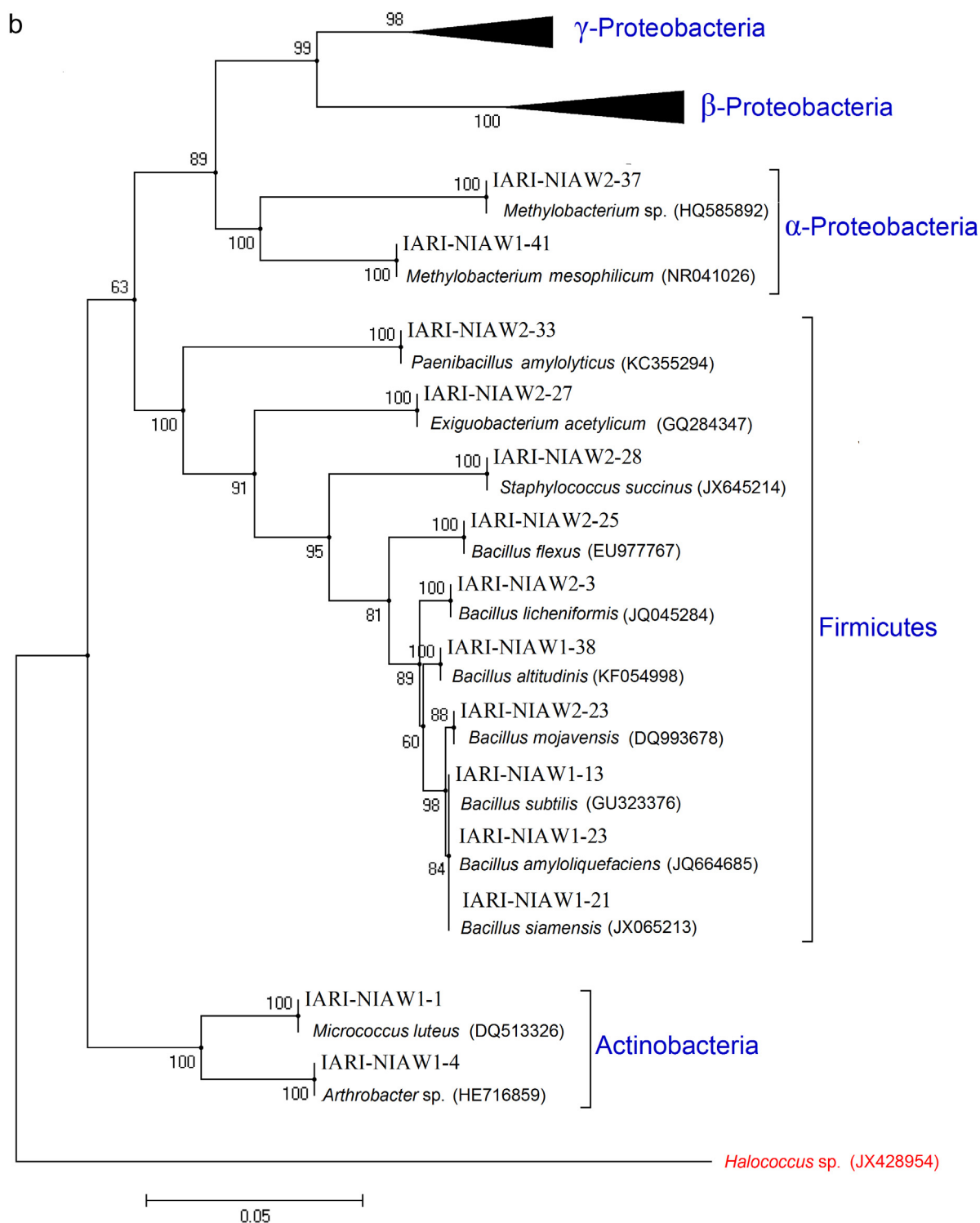
Fig. 1 (*continued*)

Distribution ratio of each phylum showed a clear difference between each sites. The wheat growing in Nashik and Krishna Nagar showed genera and species that are more diverse than other three sites in the peninsular zone of India (Table 4). Among 32 distinct species genera *Bacillus* have been reported as the most dominant in Nashik (33.3% distribution ratio) followed by Krishna Nagar (32.2% distribution ratio) (Table 4).

Among three phyla, Proteobacteria were most dominant followed by Firmicutes. Of 32 representative strains, 5 distinct species with two genera *Rhodobacter* and *Methylobacterium* belonged to α-proteobacteria while three strains *Alcaligenes*, *Achromobacter* and *Delftia* belonged to β-proteobacteria. The most predominant class of Proteobacteria is γ-proteobacteria consisting of 10 distinct species distributed into
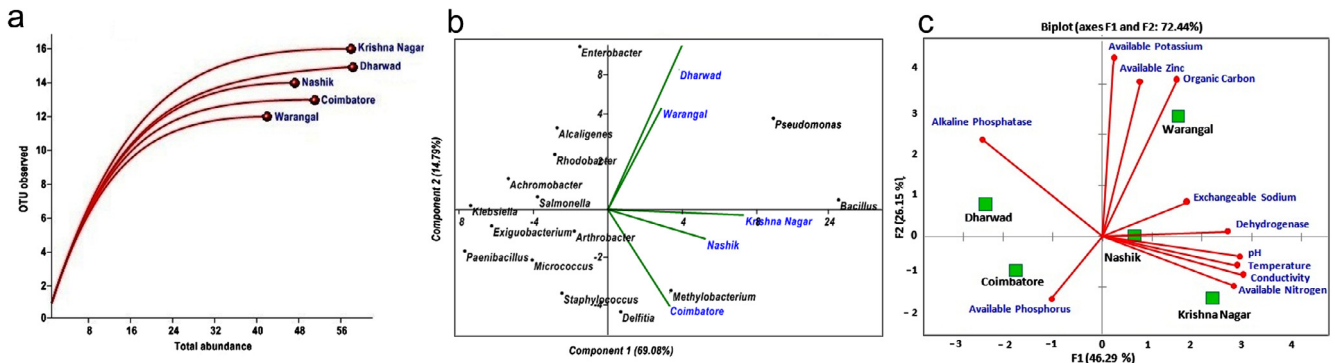
Figure 2 (a) Rarefaction curves of observed OTUs in the five samples from the peninsular zone of India; (b) principal coordinate analysis of the diversity indices (H) of the 16S rDNA PCR-ARDRA profiles of the five sites in relation to 16S rRNA gene sequences, Component 1 and Component 2 accounted for 14.79% and for 69.08% of the total variation, respectively. (c) Biplot showing relationship between different sampling sites and temperature, pH, conductivity, organic carbon, available NPK and zinc, exchangeable sodium, dehydrogenase and alkaline phosphatase.
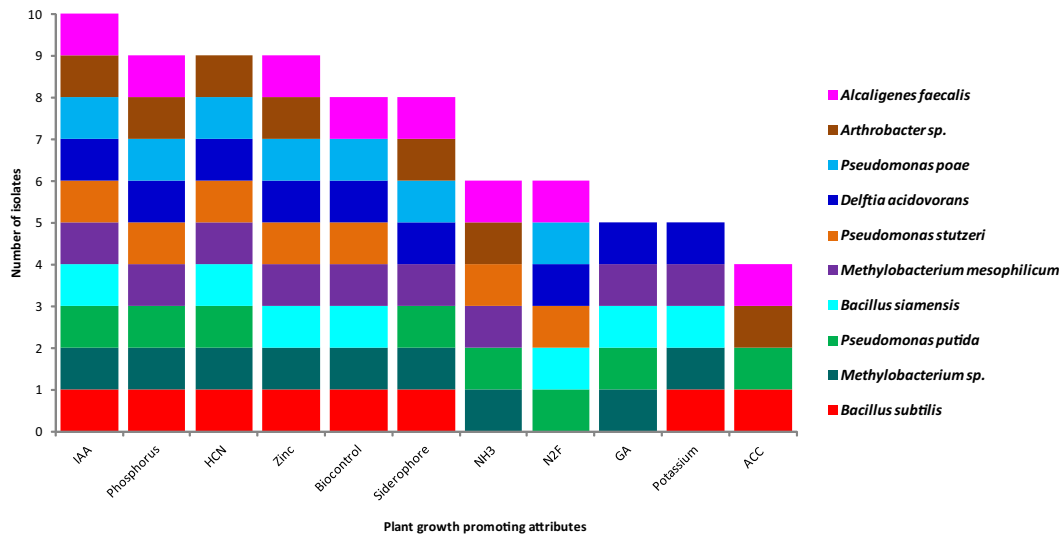

Figure 3 Characterization of ten bacteria endowed with different plant growth promoting attributes.

three orders: Pseudomonadales with five strains *P. aeruginosa*, *P. stutzeri*, *P. putida*, *P. poae* and *P. japonica*; Enterobacteriales with three strains *E. asburiae*, *E. hormaechei* and *Klebsiella oxytoca*; Xanthomonadales with *Stenotrophomonas maltophilia* (Table 4; Fig. 1). Second dominant phylum is Firmicutes, included four families; Bacillaceae with three strains *B. altitudinis*, *B. amyloliquefaciens* and *Bacillus flexus*; Bacillales Incertae Sedis, Paenibacillaceae and Staphylococcaceae with one strain *Exiguobacterium antarcticum*; *P. amylolyticus* and *S. succinus*. Two strains belonged to the phylum Actinobacteria, represented by *Arthrobacter* sp. and *M. luteus* (Table 4; Fig 1).

Different groups of bacterial isolates were investigated from wheat such as *Arthrobacter*, *Bacillus* and *Alcaligenes*. The epiphytic bacterial strains identified as *B. amyloliquefaciens*, *A. faecalis* and *P. poae* were most dominant in phyllosphere. *B. amyloliquefaciens* are contributed to its plant-growth-promoting effect of many crop plants (Idriss et al. 2002; Verma et al. 2015a; Verma et al. 2016a; Verma et al. 2015b). *A. faecalis* and *P. poae* were identified as epiphytic PGPB for

the first time herein, from wheat growing at arid land and high temperature environments (Joo et al. 2005). *A. faecalis* first time reported from ammonium removal by heterotrophic nitrification-aerobic denitrification. It is a niche specific bacteria isolated from wheat growing in Warangal, Telangana. It is a peach colored thermotolerant bacterium that could tolerate 5% NaCl and produced IAA, siderophore and ammonia; solubilized phosphorous and zinc; fixed nitrogen; exhibited ACC deaminase activity and showed antagonistic against *Fusarium graminerum*, *Rhizoctonia solani* and *Macrophomina phaseolina* at high temperatures. *P. poae* was earlier reported from phyllosphere of grasses (Behrendt et al., 2003) while first time reported here from wheat phyllosphere. It is a light yellow colored thermotolerant and alkalitolerant bacterium that could tolerate 10% NaCl and produced IAA and siderophore; and solubilized phosphorus and zinc at high temperatures.

In the present investigated endophytes, *A. spanius*, *B. flexus*, *D. acidovorans*, *Delftia* sp., *D. lacustris*, *M. luteus* and *P. aeruginosa* were isolated and identified from wheat. *A.*
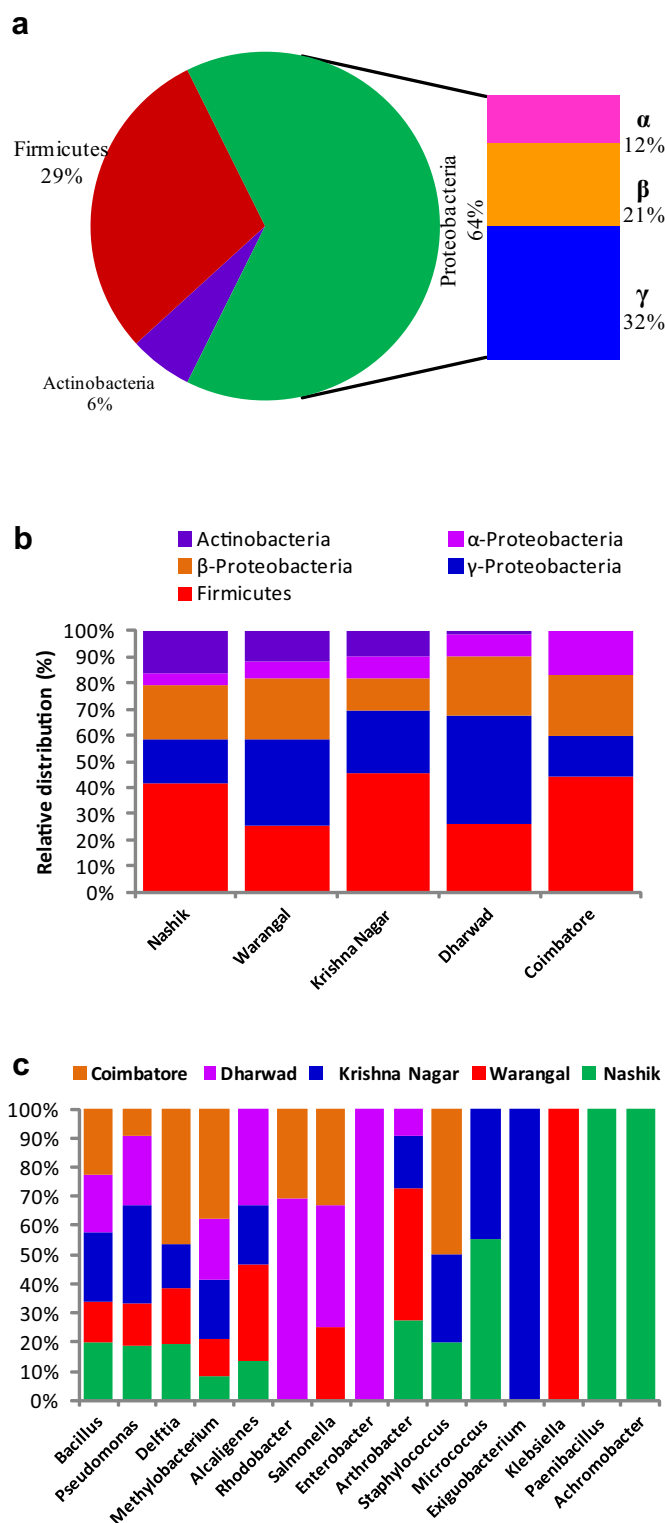
a

b

c

Figure 4 Abundance of different bacteria; (a) distribution of phylum and group in the samples surveyed; (b) distribution of total bacteria in five sampling sites; (c) distribution of different genera in five sampling sites in the peninsular zone of India.

spanius, *D. acidovorans* and *D lacustris* were isolated first time from internal tissue of wheat. *A. spanius* have been earlier isolated from clinical, fresh water and phenol (Coenye et al., 2003; Juárez-Jiménez et al., 2010). Firstly reported from wheat internal tissues, it is a light yellow colored thermotolerant that could tolerate 7% NaCl and produced IAA, siderophore and ammonia; solubilized phosphorus and zinc and also exhibited nitrogen fixation. The potential of endophytic bacteria exert several beneficial effects on host plants, such as stimulation of plant growth, nitrogen fixation and induction of resistance

Table 4 Distribution of wheat associated bacteria isolated from five sites in the peninsular zone of India.

Sampling sites	Phylum	Genera	Distribution ratio (%)
Dharwad (8 Genus)	Actinobacteria (22%)	*Arthrobacter*	1.6
	Firmicutes (11%)	*Bacillus*	25.8
	α-proteobacteria (22%)	*Rhodobacter*	14.5
		Methylobacterium	8.06
	β-proteobacteria (11%)	*Alcaligenes*	8.1
	γ-proteobacteria (34%)	*Pseudomonas*	16.1
		Salmonella	8.1
		Enterobacter	17.7
Krishna Nagar (10 Genus)	Actinobacteria (22%)	*Arthrobacter*	3.4
		Micrococcus	6.8
	Firmicutes (34%)	*Bacillus*	32.2
		Exiguobacterium	8.5
		Staphylococcus	5.1
	α-proteobacteria (11%)	*Methylobacterium*	8.5
	β-proteobacteria (22%)	*Alcaligenes*	5.1
		Delftia	6.8
	γ-proteobacteria (11%)	*Pseudomonas*	23.7
Coimbatore (10 Genus)	Firmicutes (28%)	*Bacillus*	32.1
		Staphylococcus	8.9
	α-proteobacteria (29%)	*Rhodobacter*	7.1
		Methylobacterium	16.1
	β-proteobacteria (14%)	*Delftia*	21.4
	γ-proteobacteria (29%)	*Pseudomonas*	7.1
		Salmonella	7.1
Nashik (10 Genus)	Actinobacteria (20%)	*Arthrobacter*	6.3
		Micrococcus	10.4
	Firmicutes (30%)	*Bacillus*	33.3
		Paenibacillus	4.2
		Staphylococcus	4.2
	α-proteobacteria (10%)	*Methylobacterium*	4.2
	β-proteobacteria (30%)	*Alcaligenes*	4.2
		Delftia	10.4
		Achromobacter	6.25
	γ-proteobacteria (10%)	*Pseudomonas*	16.7
Warangal (9 Genus)	Actinobacteria (12%)	*Arthrobacter*	11.6
	Firmicutes (12%)	*Bacillus*	25.6
	α-proteobacteria (13%)	*Methylobacterium*	7.0
	β-proteobacteria (25%)	*Alcaligenes*	11.6
		Delftia	11.6
	γ-proteobacteria (38%)	*Pseudomonas*	14.0
		Salmonella	7.0
		Klebsiella	11.6

to plant pathogens (Suman et al., 2015a). There were obvious differences among the rhizosphere, endophytic and epiphytic bacterial communities in terms of CFU count and phylotype. Lower bacterial diversity was observed in the phyllospheric compared to rhizospheric and endophytic. Pink-pigmented facultative methylotrophs (PPFMs) *M. mesophilicum*, and *Methylobacterium* sp. were identified as epiphytic. In our present study, *A. faecalis*, *B. amyloliquefaciens* and *P. poae* were isolated as epiphytic whereas *A. spanius*, *B. flexus*, *D. acidovorans*, *Delftia* sp., *D. lacustris*, *M. luteus* and *P. aeruginosa* as endophytic only. *Alcaligenes* genera was recovered from both epiphytic and rhizospheric.

Wheat associated PGPB have a high potential for agriculture because they can improve plant growth, under limiting or stress conditions of temperatures. PGPB can directly facilitate the proliferation of their plant host through the production of the stimulatory phytohormones. IAA is a phytohormone, a type of best characterized auxin, which is essential for the growth and development of plants. Along with phytohormone production, plant growth promotion is known to be mediated by a variety of mechanisms including the solubilization of phosphorus, potassium and zinc; production of ammonia, siderophores and HCN (Verma et al., 2015a; Tilak et al., 2005; Verma et al., 2016b). There are considerable populations of P- or K-solubilizing bacteria in soil and in plants rhizosphere. P-solubilizing bacteria (PSB) have the ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate (Vyas et al., 2009; Yadav et al., 2015d). In the present study P-solubilization activity was exhibited by many genera such as *Alcaligenes*, *Arthrobacter*, *Achromobacter*, *Bacillus*, *Delftia*, *Methylobacterium*, *Pseudomonas*, *Rhodobacter*, *Staphylococcus* and *Salmonella*. K-solubilizing bacteria (KSB)

were found to resolve potassium, silicon and aluminum from insoluble minerals. To the our best of knowledge, *Delftia* and *Salmonella* are first time reported as potassium at high temperatures. The K-solubilizing bacteria may have use in the amelioration of K-deficient soil in agriculture at high temperatures. In the present investigation diverse groups of bacteria were characterized for nitrogen fixation, such as *Achromobacter*, *Alcaligenes*, *Bacillus*, *Delftia*, *Providencia*, *Pseudomonas*, *Rhodobacter* and *Salmonella*. *A. faecalis*, *B. subtilis*, *D. acidovorans* and *M. mesophilicum* that showed more than six plant growth promoting activities. Selected nitrogen fixing, P and K-solubilizing bacteria could be effectively used as biofertilizers in the place of chemical fertilizers. NPK could increase soil productivity to improve sustainability of agriculture production.

In conclusion, utility of such thermotolerant bacterial strains in the context of temperature, considering the unique crops growing in the climatic conditions of the high-temperature agricultural eco-systems. Such systems require situation-specific microbial inoculants that withstand extremities of temperature tolerance and retain their functional traits for PGP. The PGP potential of the bacterial strain dealt in this study requires further evaluation and validation before its use as bio-inoculants. The selection of native functional plant growth promoting microorganisms is a mandatory step for reducing the use of energy intensive chemical fertilizers. The strain reported in this study seems to be an ideal candidate for promotion as a bio-inoculant, due to its high temperature tolerance and multiple traits for plant growth promotion. PGP thermotolerant bacteria are another important isolates that could be developed as suitable inoculants for summer season crops grown in the arid, sub-arid, high plateau and high temperature regions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sjbs.2016.01.042.

References

Bakker, A.W., Schippers, B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. Soil Biol. Biochem. 19, 451–457.

Behrendt, U., Ulrich, A., Schumann, P., 2003. Fluorescent pseudomonads associated with the phyllosphere of grasses; *Pseudomonas trivialis* sp. nov., *Pseudomonas poae* sp. nov. and *Pseudomonas congelans* sp. nov. Int. J. Syst. Evol. Microbiol. 53, 1461–1469.

Cappucino, J.C., Sherman, N., 1992. Nitrogen cycle. In: Microbiology: A Laboratory Manual, 4th ed. Benjamin/Cumming Pub. Co., New York, pp. 311–312.

Coenye, T., Vancanneyt, M., Falsen, E., Swings, J., Vandamme, P., 2003. *Achromobacter insolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. Int. J. Syst. Evol. Microbiol. 53, 1819–1824.

Egamberdieva, D., Kamilova, F., Validov, S., Gafurova, L., Kucharova, Z., Lugtenberg, B., 2008. High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ. Microbiol. 10, 1–9.

Egamberdieva, D., Wirth, S., Alqarawi, A.A., Abd Allah, E., 2015. Salt tolerant *Methylobacterium mesophilicum* showed viable colonization abilities in the plant rhizosphere. Saudi J. Biol. Sci. 22, 585–590.

Fasim, F., Ahmed, N., Parsons, R., Gadd, G.M., 2002. Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiol. Lett. 213, 1–6.

Glick, B.R., 2015. Introduction to plant growth-promoting bacteria. In: Beneficial Plant-Bacterial Interactions. Springer, pp. 1–28.

Godfray, H., Pretty, J., Thomas, S., Warham, E., Beddington, J., 2011. Linking policy on climate and food. Science 331, 1013–1014.

Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann. Microbiol. 60, 579–598.

Holbrook, A.A., Edge, W., Bailey, F., 1961. Spectrophotometric method for determination of gibberellic acid. Adv. Chem. Ser. 28, 159–167.

Hu, X., Chen, J., Guo, J., 2006. Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. World J. Microbiol. Biotechnol. 22, 983–990.

Idriss, E.E., Makarewicz, O., Farouk, A., Rosner, K., Greiner, R., Bochow, H., Richter, T., Borriss, R., 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. Microbiology 148, 2097–2109.

Jacobson, C.B., Pasternak, J., Glick, B.R., 1994. Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Can. J. Microbiol. 40, 1019–1025.

Joo, H.S., Hirai, M., Shoda, M., 2005. Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4. J. Biosci. Bioeng. 100, 184–191.

Juárez-Jiménez, B., Manzanera, M., Rodelas, B., Martínez-Toledo, M.V., Gonzalez-López, J., Crognale, S., Pesciaroli, C., Fenice, M., 2010. Metabolic characterization of a strain (BM90) of *Delftia tsuruhatensis* showing highly diversified capacity to degrade low molecular weight phenols. Biodegradation 21, 475–489.

Kumar, M., Yadav, A.N., Tiwari, R., Prasanna, R., Saxena, A.K., 2014a. Deciphering the diversity of culturable thermotolerant bacteria from Manikaran hot springs. Ann. Microbiol. 64, 741–751.

Kumar, M., Yadav, A.N., Tiwari, R., Prasanna, R., Saxena, A.K., 2014b. Evaluating the diversity of culturable thermotolerant bacteria from four hot springs of India. J. Biodivers. Biopros. Dev. http://dx.doi.org/10.4172/ijbbd.1000127.

Mishra, P.K., Bisht, S.C., Ruwari, P., Selvakumar, G., Joshi, G.K., Bisht, J.K., Bhatt, J.C., Gupta, H.S., 2011. Alleviation of cold stress in inoculated wheat (*Triticum aestivum* L.) seedlings with psychrotolerant Pseudomonads from NW Himalayas. Arch. Microbiol. 193, 497–513.

Naresh, R., Tomar, S., Purushottam, S., Kumar, D., Pratap, B., Kumar, V., Nanher, A., 2014. Testing and evaluation of planting methods on wheat grain yield and yield contributing parameters in irrigated agro-ecosystem of western Uttar Pradesh, India. Afr. J. Agric. Res. 9, 176–182.

Pandey, S., Singh, S., Yadav, A.N., Nain, L., Saxena, A.K., 2013. Phylogenetic diversity and characterization of novel and efficient cellulase producing bacterial isolates from various extreme environments. Biosci. Biotechnol. Biochem. 77, 1474–1480.

Pikovskaya, R., 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya 17, 362–370.

Schwyn, B., Neilands, J., 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56.

Sijam, K., Dikin, A., 2005. Biochemical and physiological characterization of *Burkholderia cepacia* as biological control agent. Int. J. Agric. Biol. 7, 385–388.

Suman, A., Yadav, A.N., Verma, P., 2015a. Endophytic microbes in crops: Diversity and Beneficial impact for Sustainable Agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity. Research Perspectives, Vol. 1. Springer-Verlag, Germany.

Suman, A., Verma, P., Yadav, A.N., Saxena, A.K., 2015b. Bioprospecting for extracellular hydrolytic enzymes from culturable thermotolerant bacteria isolated from Manikaran thermal springs. Res. J. Biotechnol. 10, 33–42.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tilak, K., Ranganayaki, N., Pal, K., De, R., Saxena, A., Nautiyal, C. S., Mittal, S., Tripathi, A., Johri, B., 2005. Diversity of plant growth and soil health supporting bacteria. Curr. Sci. 89, 136–150.

Tiwari, S., Singh, P., Tiwari, R., Meena, K., Yandigeri, M., Singh, D., Arora, D., 2011. Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. Biol. Fertil. Soils 47, 907–916.

Verma, P., Yadav, A.N., Kazy, S.K., Saxena, A.K., 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.

Verma, P., Yadav, A.N., Kazy, S.K., Saxena, A.K., Suman, A., 2014. Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India. Int. J. Curr. Microbiol. Appl. Sci. 3, 432–447.

Verma, P., Yadav, A.N., Khannam, K.S., Panjiar, N., Kumar, S., Saxena, A.K., Suman, A., 2015a. Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with wheat (*Triticum aestivum*) from the northern hills zone of India. Ann. Microbiol. 65, 1885–1899.

Verma, P., Yadav, A.N., Shukla, L., Saxena, A.K., Suman, A., 2015b. Hydrolytic enzymes production by thermotolerant *Bacillus altitudinis* IARI-MB-9 and *Gulbenkiania mobilis* IARI-MB-18 isolated from Manikaran hot springs. Int. J. Adv. Res. 3, 1241–1250.

Verma, P., Yadav, A.N., Khannam, K.S., Kumar, S., Saxena, A.K., Suman, A., 2016a. Alleviation of cold stress in wheat seedlings by *Bacillus amyloliquefaciens* IARI-HHS2-30, an endophytic psychrotolerant K-solubilizing bacterium from NW Indian Himalayas. Natl. J. Life Sci. 12, 105–110.

Verma, P., Yadav, A.N., Khannam, K.S., Kumar, S., Saxena, A.K., Suman, A., 2016b. 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. J. Basic Microbiol. 56, 44–58.

Vyas, P., Rahi, P., Gulati, A., 2009. Stress tolerance and genetic variability of phosphate-solubilizing fluorescent *Pseudomonas* from the cold deserts of the trans-Himalayas. Microb. Ecol. 58, 425–434.

Yadav, A.N., 2015. Bacterial diversity of cold deserts and mining of genes for low temperature tolerance (PhD thesis) IARI, New Delhi/Birla Institute of Technology, Mesra, Ranchi, p. 234. <http://dx.doi.org/10.13140/2.1.2948.1283>.

Yadav, A.N., Sachan, S.G., Verma, P., Kaushik, R., Saxena, A.K., 2015a. Cold active hydrolytic enzymes production by psychrotrophic Bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J. Basic Microbiol. http://dx.doi.org/10.1002/jobm.201500230.

Yadav, A.N., Sachan, S.G., Verma, P., Saxena, A.K., 2015b. Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J. Biosci. Bioeng. 119, 683–693

Yadav, A.N., Sachan, S.G., Verma, P., Tyagi, S.P., Kaushik, R.K., Saxena, A.K., 2015c. Culturable diversity and functional annotation of psychrotrophic bacteria from cold desert of Leh Ladakh (India). World J. Microbiol. Biotechnol. 31, 95–108.

Yadav, A.N., Sharma, D., Gulati, S., Singh, S., Dey, R., Pal, K.K., Kaushik, R., Saxena, A.K., 2015d. Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. Sci. Rep. 5, 12293.

Yadav, A.N., Verma, P., Kumar, M., Pal, K.K., Dey, R., Gupta, A., Padaria, J.C., Gujar, G.T., Kumar, S., Suman, A., Prasanna, R., Saxena, A.K., 2015e. Diversity and phylogenetic profiling of niche-specific Bacilli from extreme environments of India. Ann. Microbiol. 65, 611–629.

Yadav, A.N., Sachan, S.G., Verma, P., Saxena, A.K., 2016. Bioprospecting of plant growth promoting psychrotrophic Bacilli from cold desert of north western Indian Himalayas. Indian J. Exp. Biol. 54, 142–150.