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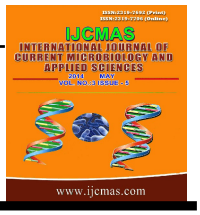

Original Research Article

Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (*Triticum aestivum*) growing in central zone of India

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A B S T R A C T

The diversity of plant growth promoting bacteria was investigated from wheat growing in different sites in central zone of India. Epiphytic, endophytic and rhizospheric bacteria were isolated using different growth medium. Bacterial diversity was analysed through amplified ribosomal DNA restriction analysis (ARDRA) using three restriction enzymes *Alu* I, *Hae* III, and *Msp* I which led to the grouping of 348 isolates into 24-29 clusters at >75% similarity index. 16S rRNA gene based phylogenetic analysis, revealed that 134 strains belonged to three phyla namely actinobacteria, firmicutes and proteobacteria with 38 distinct species of 17 genera. *Bacillus* and *Pseudomonas* were dominant in rhizosphere while *Methylobacterium* were in phyllosphere. Endophytic niche specific bacteria were identified as *Delftia* and *Micrococcus*. Sampling of different sites showed variation in diversity indices. *In vitro* plant growth promoting activities of bacteria exposed more than three beneficial traits which may act independently or concurrently. Phosphate solubilization and siderophores production are the predominant traits exhibited by these microbes. The many species of genera *Bacillus, Exiguobacterium, Micrococcus, Pseudomonas* and *Psychrobacter* showed antagonistic properties against fungal pathogens *Fusarium graminerum, Rhizoctonia solani* and *Macrophomina phaseoli.* These promising isolates showing a range of useful plant growth promoting attributes insist to be explored for agricultural applications.

K e y w o r d s

Epiphytic; Endophytic; Rhizospheric; PGPB; Drought stress; Biocontrol

Introduction

Wheat (*Triticum durum* L.) is one of the most important cereals world-wide and it is grown in different environments. Drought is one of the major constraints which hamper wheat production in India. The central zone of India (Madhaya Pradesh, Kota region of Rajasthan and Jhansi region of Uttar Pradesh) were characterised for water stress ecosystem. Plant growth promoting bacteria (PGPB)

are free-living soil, rhizospheric, epiphytic and endophytic bacteria that can either directly or indirectly have an impact on plant growth (Glick et al. 1999; Verma et al. 2013). PGPB stimulate plant growth in multiple ways *viz* N_2 fixation, synthesize phytohormones (auxin and cytokinin), production of siderophores and suppress pathogenic organisms. PGPB has been reported not only to improve plant growth but also to suppress the plant pathogens, of which *Pseudomonas* and *Bacillus* were well characterised. Pink-pigmented facultative methylotrophs synthesize a variety of metabolites useful for the plants including phytohormones (Ivanova et al. 2001; Verma et al. 2013) that promote plant growth and yield. PGPB are used as biological control agents to reduce the development of plant diseases caused by plant pathogenic fungi, bacteria, viruses and nematodes.

In the last decade, a number of PGPB associated with wheat and different cereals crops have been identified including *Acinetobacter, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Citricoccus, Kocuria, Lysinibacillus, Methylobacterium, Paenibacillus, Providencia*, *Pseudomonas* and *Serratia* (Coombs and Franco 2003; Conn and Franco 2004; Streptomyces 2005; Jha and Kumar 2009; Wellner et al. 2011; Meena et al. 2012; Verma et al. 2013). The phyllosphere is common niche for synergism between bacteria and plant. Many bacteria such as *Pseudomonas* and *Methylobacterium* have been reported in the wheat phyllosphere (Wellner et al. 2011; Meena et al. 2012; Verma et al. 2013). Rhizospheric bacteria have the ability to attach to the root surfaces (rhizoplane) allowing these to derive maximum benefit from root exudates. Endophytic bacteria live in plant tissues without causing substantive harm to the host. Bacterial endophytes such as *Achromobacter, Microbiospora, Micrococcus*, *Micromomospora, Pantoea*, *Planomonospora, Pseudomonas*, *Stenotrophomonoas*, S*treptomyces* and *Thermomonospora* have been reported from wheat (Zinniel et al. 2002; Coombs and Franco 2003; Verma et al. 2013). A number of bacterial species associated with the wheat rhizosphere were recovered belonging to genera *Azospirillum*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium, Methylobacterium, Pseudomonas*, *Rhizobium* and *Serratia* (Xie et al. 1996; Lavania et al. 2006; Chaiharn and Lumyong 2011; Meena et al. 2012; Verma et al. 2013).

The present study attempted to elucidate the bacterial diversity associated with wheat growing in central zone of India, employing different growth media. ARDRA analysis was done for phylogenetic clustering of the moderately drought tolerant isolates. Sequencing the 16S rRNA gene of representative strains was undertaken for identification. Representative strain from each cluster was screen *in vitro* for plant growth promotion in drought stress condition. PGPB inoculants are inexpensive, simple to use and have no unpleasant effects to land. The use of PGPB may prove useful in developing strategies to facilitate wheat and other cereals crops growth in drought area.

Materials and Methods

Samples collection

Wheat plants of two *var*. IWP5007 and HD2987 with rhizospheric soil were collected from five different sites in

central zone of India, which included Gwalior, Sagar and Indore region of Madhya Pradesh, Jhansi region of Uttar Pradesh and Kota region of Rajasthan (Table 1). A total forty samples, eight from each site were collected in sterile polythene bags labelled transported on ice and processes immediately.

Physico-chemical properties of samples

The pH and conductivity of the soil samples were recorded on sampling site. Soil samples analyzed for soil organic carbon, total nitrogen (%), soil organic matter, exchangeable cations and available phosphorus was determined as described earlier Verma et al. (2013). Soil analysis was done at Division of Soil Sciences, Indian Agricultural Research Institute, New Delhi, India.

Enumeration of wheat associated bacteria

The culturable bacteria from soil and rhizosphere soil were isolated through enrichment using the standard serial dilution plating technique employing nine different growth media as described earlier (Verma et al. 2013). Endophytic and epiphytic bacteria were isolated using methods described by Conn and Franco (2004) and Holland and Polacco (1994) respectively. The plates were incubated at 30 °C and the population was counted after 3-7 days. Colonies that appeared were purified by repeated re-streaking to obtain pure colonies using respective medium plates. The pure cultures were maintained at 4 °C as slant and glycerol stock (25 %) at -80 °C for further use. All the isolates were screened in triplicates for tolerance to pH and drought [Low water potential on polyethylene glycol (PEG-8000) - infused plates] as described earlier Yadav et al. (2014).

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

Genomic DNA was extracted by the method as earlier described by Kumar et al. (2013). The amount of DNA extracted was electrophoresed on 0.8% agarose gel. Amplification of 16S rDNA was done by using the universal primers pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3'). The amplification was carried out in a 100 µl volume and amplification conditions were used as described earlier (Pandey et al. 2013). The PCR amplified 16S rDNA were purified by QIA quick PCR product purification kit (Qiagen). 100 ng purified PCR products were digested separately with three restriction endonucleases *Alu* I, *Hae* III and *Msp* I (GeNei) in 25 µl reaction volumes, using the manufacturer's recommended buffer and temperature. The clustering analysis was undertaken using the software, NTSYS-2.02e package (Numerical taxonomy analysis program package, Exeter software, USA). Similarity among the isolates was calculated by Jaccard's and dendrogram was constructed using the UPGMA method (Nei and Li 1979).

16S rDNA Sequencing and phylogenetic analysis

PCR amplified 16S rRNA genes were purified and sequenced using both pA and pH primers for forward and reverse reactions respectively. Sequencing employed a dideoxy cycle with fluorescent terminators and was run in a 3130xl Applied Biosystems ABI prism automated DNA sequencer (Applied Biosystems, Foster City, CA) at SCI Genome Chennai, India. 16S rRNA gene sequences were analysed using codon code aligner v.4.0.4.

The 16S rRNA gene sequences were aligned to those of closely related bacterial species available at GenBank database using BLASTn program. Bacterial isolates were identified based on percentage of sequence similarity (≥97%) with that of a prototype strain sequence in the GenBank. The phylogenetic tree was constructed on the aligned datasets using the neighbour-joining method (Saitou and Nei 1987) implemented in the program MEGA 4.0.2 (Tamura et al. 2007). Bootstrap analysis was performed as described by Felsenstein (1981) on 1000 random samples taken from the multiple alignments. The partial 16S rRNA gene sequences were submitted to NCBI GenBank and accession numbers were assigned from KF054878- KF054913 and KF572999-KF573001.

In vitro screening of isolates for PGP traits

Representative isolates from each cluster were screened for PGP attributes initially as qualitative estimation for *in vitro* production of ammonia (Cappucino 1992), siderophore (Schwyn and Neilands 1987), HCN (Bakker and Schippers 1987), gibberllic acid (Brown and Burlingham 1968) and indole-3-acetic acid (Bric et al. 1991). The strains were screened for solubilization of phosphorus (Pikovskaya 1948), potassium (Hu et al. 2006) and zinc (Saravanan et al. 2004). The ability to fix nitrogen was evaluated using semi-solid nitrogen-free NFb medium (Dobereiner et al. 1996). The bacterial strains were screened for their ability to utilize the 1-aminocyclopropane-1-carboxylate (ACC) as sole nitrogen source, a trait that is consequence of the activity of the enzyme ACC deaminase (Jacobson et al. 1994). *In vitro* antagonistic activity of bacterial isolates was evaluated against three fungal pathogens *Fusarium graminerum*, *Rhizoctonia solani* and *Macrophomina phaseoli* according to the method described by Verma et al. (2013).

Statistical Analysis

In order to compare the bacterial diversity among five different sites, the 16S rRNA gene sequences of the isolates showing ≥ 97 % sequence similarity were grouped into the same OTU (phylotype). The software Shannon–Wiener Diversity Index/Shannon Entropy Calculator (http://www.changbioscience.com/genetics/shannon.html) and Rarefaction Calculator (http://www2.biology.ualberta.ca/ jbrzusto/rarefact.php) were used to calculate Shannon index (H), Evenness (J) and the Simpson's index (D). Principal coordinate analysis (PCA) was used to determine the statistical correlation between population diversity of five sites survey (Rico et al. 2004).

Results and Discussion

Enumeration and characterization of wheat associated bacteria

A total of 348 bacteria were isolated from five different sites in central zone of India (Table 2). Significant variations were observed among the culturable bacterial population (CFU) of each sample on different growth media. The Population of bacteria varied from 2.1×10^6 to 7.8×10^6, 1.2×10^6 to 8.8×10^6, 1.0×10^6 to 1.8×10^6 for isolation sources of phyllosphere, rhizosphere and endophytic respectively (Table 2). The pure colonies obtained from each sample on different media were isolated based on colony morphology and cultural characteristics. The representative strains were screened for tolerance to range of pH and PEG mediated water deficit (drought). Among the 38 isolates, 22 were able to grow on PEG-8000 infused plates with water potential of -0.25

Mpa and 11 isolates tolerates upto -0.5 Mpa (Fig. 5).

Physico-chemical properties of samples

Physical and chemical characteristics of the soil varied considerably amongst the different soil samples (Table1). Available nitrogen content was highest in Gwalior sample and it ranges from 149-177 kg ha^{-1}. Organic carbon content was significantly higher, achieving of 4.9 % organic carbon in Indore followed by 4.3 % in Gwalior samples (Table 1).

PCR amplification of 16S rDNA and ARDRA

PCR amplification of 16S rDNA followed by ARDRA with three restriction endonucleases was carried out to look for the species variation among the morphotypes selected. The 16S rDNA amplicons were digested with restriction enzymes, which generated profiles having 3 to 7 fragments ranging in size from 100 to 860 base pairs. ARDRA results revealed that among the restriction endonucleases, *Alu* I was more discriminatory as compared to *Msp* I and *Hae* III. A combined dendrogram was constructed for each sampling site to determine the percent similarity among the isolates. At a level of 75 % similarity, the isolates were grouped into clusters; and the number of clusters ranged from 24 (for JCZ) to 29 (for ICZ). The total number of clusters was 134, summed up for all the sites (Table 2).

16S rRNA gene sequencing and phylogenetic analysis

16S rRNA gene sequencing and phylogenetic analysis of a representative isolate from each cluster revealed that all the isolates showed > 97 to 100 % similarity with the sequences within the GenBank (Table 3). One sequence from each group was selected as a representative operational taxonomic unit (OTU) and all the isolates were classified into 38 OTUs using a $\geq$ 97 % sequence similarity cut-off value. The phylogenetic tree of 38 identified bacteria was constructed to determine their affiliations (Fig.1). Analysis of the 16S rRNA gene sequences revealed that 134 strains belonged to 3 phyla namely actinobacteria (18 %), firmicutes (38 %) and proteobacteria (43%) with 38 distinct species of 17 genera (Table 3; Fig. 2a, b).

Three major clusters were formed in which proteobacteria were most pre-dominant phylum followed by firmicutes. Out of the 38 OTUs, 17 strains belonged to phylum firmicutes were grouped into three families of bacilli namely Bacillaceae (11 strains *Bacillus subtilis, Bacillus alcalophilus, Bacillus aquimaris, Bacillus aryabhattai, Bacillus barbaricus, Bacillus cereus, Bacillus megaterium, Bacillus pumilus, Bacillus tequilensis, Bacillus thuringiensis* and *Lysinibacillus xylanilyticus*); Bacillales incertae sedis (1 strain *Exiguobacterium acetylicum)* and Paenibacillaceae (5 strains *Paenibacillus amylolyticus, Paenibacillus dendritiformis, Paenibacillus durus, Paenibacillus* sp. and *Paenibacillus tundrae*). Second cluster of phylum actinobacteria consist of five strains, *Arthrobacter humicola, Corynebacterium callunae, Kocuria* sp., *Micrococcus luteus* and *Micrococcus* sp. (Fig. 1). Phylum proteobacteria consist three grouped of alpha proteobacteria (3 strain *Methylobacterium extorquens, Methylobacterium mesophilicum* and *Methylobacterium radiotolerans*), beta proteobacteria (2 strains *Duganella*

violaceusniger and *Delftia* sp.) and gamma proteobacteria (11 strains *Acinetobacter* sp., *Pantoea ananatis, Pseudomonas fuscovaginae, Pseudomonas lini, Pseudomonas monteilii, Pseudomonas stutzeri, Pseudomonas thivervalensis, Psychrobacter fozii, Serratia marcescens, Stenotrophomonas maltophilia* and *Stenotrophomonas* sp.) (Fig. 1). Overall *Micrococcus* from actinobacteria, *Bacillus* from firmicutes, and *Pseudomonas* from proteobacteria were the most frequently recovered genera (Table 3).

Statistical analysis

The 134 isolates from the five sampling sites based on similarity index of > 97 % at the 16S rRNA gene sequences could be categorised into 24-29 clusters (Table 4). Shannon's diversity index was recorded highest (H´=3.3) for Kota and lowest (H´=3.12) value for Jhansi using 16S rRNA sequences and ARDRA data. The highest species richness was recorded in Indore (Table 4). The individual rarefaction curves for all the five sites indicated that the bacterial populations were the least diverse in Jhansi and most diverse in Indore (Fig. 3). 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 67.29 % of the total variation, with component 1 accounting for 48.42 % and component 2 for 18.87 % of the variance (Fig. 4).

Plant growth promoting (PGP) attributes

Out of 38 representatives, 29, 10 and 21 strains were positive for solubilisation of phosphorus, zinc and potassium respectively (Table 3). Production of siderophores, IAA, gibberellic acid and ammonia were positive for 21, 17, 12 and 21 strains respectively (Table 3). Nine strains showed nitrogen fixation confirmed by acetylene reduction assay. ACC deaminase activity was shown by 9 strains. Isolate IARI-IIWP-20 solubilised highest amount of phosphorus (326±1.5 µg $mg^{-1}day^{-1}$) followed by IARI-IHD-5 (126±1.2 µg $mg^{-1}day^{-1}$). Isolate IARI-IHD-3 showed highest IAA production (280.4±0.5 µg mg^{-1} protein day^{-1}) followed IARI-IIWP-2 (102.8±05 µg mg^{-1} protein day^{-1}). Highest solubilization of potassium by isolates IARI-IIWP-12 (3.8±0.8 mm). Isolate IARI-IHD-4 show highest zinc solubilization (9.8±1.5 mm). Of 38 stains, 11 strains were anatagonastic against *Fusarium graminerum, Rhizoctonia solani* and *Aspergillus fumigatus* (Table 3).

Bacteria associated with wheat have been frequently isolated and identified as endophytic and rhizobacteria but this paper provides the diversity of bacteria present in endophytic, epiphytic as well as rhizospheric. Representative strains were screened for eleven different plant growth promoting attributes including solubilization of phosphorus, potassium and zinc; production of ammonia, gibberellic acid, HCN, IAA, siderophores; Nitrogen fixation and ACC deaminase activity. *Invitro* antagonistic activity was done against three pathogenic fungus *Fusarium graminerum, Rhizoctonia solani* and *Aspergillus fumigatus*.

In present study we have isolated wheat associated bacteria (Epiphytic, endophytic and rhizospheric) form five locations in central zone (One of the wheat agro-ecological zones) in India. From the phyllosphere a total of 89 bacteria isolated, belong to different genera of *Arthrobacter, Bacillus, Corynebacterium, Methylobacterium, Paenibacillus, Pseudomonas* and *Psychrobacter.* Pink-

pigmented facultative methylotrophs (PPFMs) *Methylobacterium extorquens, Methylobacterium mesophilicum and Methylobacterium radiotolerans* are a physiologically and taxonomically diverse group of bacteria with prominent plant growth promoting attributes (Verma et al. 2013). The genus *Methylobacterium* is among the commonly recorded leaf epiphytes and represents abundant and stable members of the phyllosphere community of a wide range of crop plants (Holland and Polacco 1994; Meena et al. 2012; Verma et al. 2013).

A total of 222 rhizospheric bacteria were isolated, belonged to twelve genera namely *Acinetobacter, Bacillus, Duganella, Exiguobacterium, Kocuria, Lysinibacillus, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Serratia* and *Stenotrophomonas* (Table 3). Thirty seven endophytic bacteria were isolated and identified belonging to genera of *Delftia, Micrococcus, Pseudomonas* and *Stenotrophomonas* (Table 3).

Among plant growth promoting activities, P-solubilization were highest (17 %) when compared to zinc solubilization (13 %), ammonia, IAA and siderophore production (12 %), Biocontrol and GA (7%), K-solubilization (6%), Nitrogen fixation, ACC deaminase (5 %) and HCN production (4%). Among PGPR, members of *Bacillus* and *Bacillus* derived genera (BBDG) are ubiquitous in nature that included both free-living PGPR and pathogenic species. PGPR belonging to BBDG have been reported to enhance the growth of several plants such as wheat, tomato, sugar beet, sorghum and peanut. A next to BBDG, another group of PGPR belonged to the genus *Pseudomonas* (Yadav et al. 2013; Verma et al. 2013). Previously it is reported that *Pseudomonas* PGPR are highly resistant to various environmental stresses (Paul and Nair 2008). Production of ACC deaminase by *Pseudomonas fluorescence* increases the resistance of plants to salt stress (Sandhya et al. 2010).

Table.1 Sampling sites and physico-chemical properties of soil

Sampling sites	pH	EC (mS/cm)	%OC	Avail. N (kg ha^{-1})	Avail. P (kg ha^{-1})	Avail. K (kg ha^{-1})	Zinc (mg kg^{-1})	Exch. Na (mg kg^{-1})
Gwalior GCZ)	7.8-8.3	46.8	4.3	177	13.7	1286	1.50	51.83
Sagar (SCZ)	7.2-8.2	49.2	4.0	167	11.2	1119	1.36	45.33
Indore (ICZ)	7.2-8.5	47.9	4.9	163	12.5	1245	1.39	49.23
Jhansi (JCZ)	7.1-7.9	41.3	3.8	156	11.1	1086	1.12	39.85
Kota (KCZ)	7.4-8.9	40.2	3.4	149	10.3	1036	1.02	39.28

Table.2 Total viable count of bacteria associated with wheat growing in different site from central zone of India

Sampling sites	Total viable count (CFU g^{-1} soil × 10^6) on different media*											M#
	Epiphytic			Rhizospheric						Endophytic		
	NA	AMS	TSA	NA	T_3A	KB	JA	SEA	TSA	LB	MDM	
GCZ	5.3	3.4	7.8	8.8	2.9	4.7	2.2	4.9	4.5	1.2	1.3	60
SCZ	5.2	3.4	7.2	8.4	2.6	4.2	2.7	4.1	4.0	1.5	1.4	59
ICZ	5.8	3.9	7.6	7.9	2.8	4.4	2.5	4.4	4.7	1.8	1.6	80
JCZ	4.2	2.3	6.5	6.4	2.1	3.7	1.6	3.2	3.6	1.0	1.2	53
KCZ	4.8	2.1	6.2	6.3	1.7	3.2	1.2	3.7	3.1	1.0	1.1	66

*Ammonium minerals salt (AMS); Jensen's agar (JA); King's B agar (KB); Luria bertani agar (LB); Modified Dobereiner medium (MDM); Nutrient agar (NA); Soil extract agar (SEA); T_3 agar (T3A); Tryptic soy agar (TSA) M#- Total morphotypes.

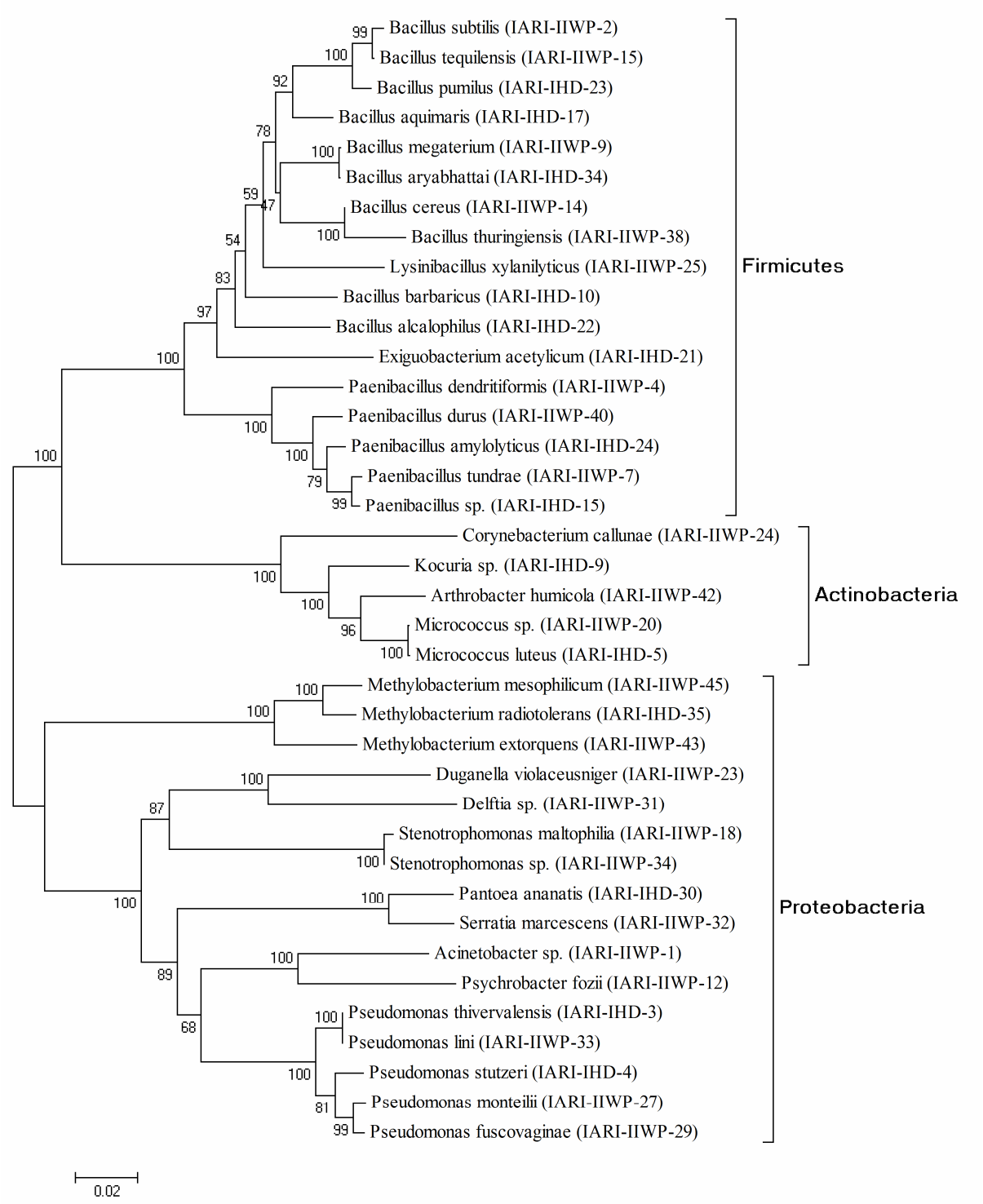

Fig.1 Unrooted phylogenetic tree based on the 16S ribosomal DNA sequences of bacteria isolated from wheat growing in central zone of India. The trees were constructed using Neighbor joining with algorithm using MEGA4 software (Tamura et al. 2007). One thousand bootstrap replicates were performed. Bootstrap values are indicated on the branches.

Table.3 Identification and functional attributes of the bacterial isolates associated with wheat growing in central zone of India

Strain number	Nearest phylogenetic relative	Similarity (%)	Bacteria type	Solubilization		
				Phosphorus *	Potassium $	Zinc$
IARI-IIWP-20	*Micrococcus* sp.	99	E	326±1.5	-	-
IARI-IIWP-24	*Corynebacterium callunae*	100	P	-	-	3.5±0.5
IARI-IIWP-42	*Arthrobacter humicola*	100	P	61.9±0.2	-	2.4±0.1
IARI-IHD-5	*Micrococcus luteus*	99	R	126±1.2	-	-
IARI-IHD-9	*Kocuria* sp.	100	R	-	-	5.3±1.2
IARI-IIWP-2	*Bacillus subtilis*	99	R	24.8±0.8	-	7.0±1.0
IARI-IIWP-4	*Paenibacillus dendritiformis*	100	R	58.9±0.7	3.3±1.2	9.8±2.1
IARI-IIWP-7	*Paenibacillus tundrae*	99	R	-	-	2.2±0.5
IARI-IIWP-9	*Bacillus megaterium*	99	R	45.7±1.1	2.2±0.5	4.3±0.2
IARI-IIWP-14	*Bacillus cereus*	99	R	-	-	-
IARI-IIWP-15	*Bacillus tequilensis*	99	R	-	-	-
IARI-IIWP-25	*Lysinibacillus xylanilyticus*	100	R	-	-	2.8±0.8
IARI-IIWP-38	*Bacillus thuringiensis*	99	R	63.2±1.4	-	3.1±0.1
IARI-IIWP-40	*Paenibacillus durus*	100	R	32.3±1.4	-	-
IARI-IHD-10	*Bacillus barbaricus*	100	R	-	-	2.3±0.8
IARI-IHD-15	*Paenibacillus* sp.	99	R	56.2±0.6	-	-
IARI-IHD-17	*Bacillus aquimaris*	99	R	326±1.5	-	-
IARI-IHD-21	*Exiguobacterium acetylicum*	100	R	9.9±1.0	-	6.1±1.2
IARI-IHD-22	*Bacillus alcalophilus*	100	R	24.9±0.8	-	2.0±0.5
IARI-IHD-23	*Bacillus pumilus*	100	R	21.5±1.0	-	-
IARI-IHD-24	*Paenibacillus amylolyticus*	100	P	24.4±1.0	2.8±1.2	
IARI-IHD-34	*Bacillus aryabhattai*	100	P	45.6±1.0	-	-
IARI-IIWP-43	*Methylobacterium extorquens*	99	P	23.6 ± 1.0	-	-
IARI-IIWP-45	*Methylobacterium mesophilicum*	100	P	12.6 ± 1.5	-	-
IARI-IHD-35	*Methylobacterium radiotolerans*	98	P	14.6 ± 1.2	-	-
IARI-IIWP-23	*Duganella violaceusniger*	99	R	58.9±0.7	3.3±1.2	5.3±2.1
IARI-IIWP-31	*Delftia* sp.	100	E	32.2±1.4	-	6.3±0.6
IARI-IHD-3	*Pseudomonas thivervalensis*	100	R	73.5±0.6	2.0±0.5	3.8±0.5
IARI-IHD-4	*Pseudomonas stutzeri*	99	R	-	-	7.8±1.5
IARI-IHD-30	*Pantoea ananatis*	100	R	-	-	2.1±0.1
IARI-IIWP-1	*Acinetobacter* sp.	99	R	21.6±1.0	-	-
IARI-IIWP-12	*Psychrobacter fozii*	99	P	20.83±1	3.8±0.8	5.8±1.5
IARI-IIWP-18	*Stenotrophomonas maltophilia*	99	E	55.7±0.5	2.8±1.2	-
IARI-IIWP-27	*Pseudomonas monteilii*	100	E	40.5±0.4	3.3±0.5	6.3±1.5
IARI-IIWP-29	*Pseudomonas fuscovaginae*	99	P	29.2±0.8	-	7.7±0.6
IARI-IIWP-32	*Serratia marcescens*	99	R	46.6±0.9	-	-
IARI-IIWP-33	*Pseudomonas lini*	99	R	55.9±0.6	3.3±0.5	8.3±0.6
IARI-IIWP-34	*Stenotrophomonas* sp.	99	R	23.7±0.5	2.2±0.5	-

Table 3 (Continued)

Strain number	Production					Other activities		
	IAA*	Siderophore[$]	GA	HCN	NH_3	ACC	N_2 Fixation	Biocontrol
IARI-IIWP-20	-	-	+	-	-	-	-	+
IARI-IIWP-24	-	2.40±0.5	-	-	-	-	-	-
IARI-IIWP-42	27.8±1.2	1.0±0.1	-	-	+	-	+	-
IARI-IHD-5	-	-	+	-	-	-	-	+
IARI-IHD-9	32.6±2.6	-	-	-	+	+		-
IARI-IIWP-2	102.8±0.5	5.3±0.5	+	-	-	+	-	-
IARI-IIWP-4	45.2±1.1	4.7±0.5	+	-	+	-	-	-
IARI-IIWP-7	-	-	-	-	+	-	-	-
IARI-IIWP-9	16.6±1.0	3.5±0.2	-	-	+	-	-	-
IARI-IIWP-14	-	2.6±0.1	-	-	-	-	-	-
IARI-IIWP-15	-	22.5±0.5	+	-	+			+
IARI-IIWP-25	-	-	-	-	+	+	-	-
IARI-IIWP-38	-	2.5±0.1	-	+	+	-	-	-
IARI-IIWP-40	-	-	-	-	+	+	+	+
IARI-IHD-10	35.2±1.6	-	-	-	-	-	-	+
IARI-IHD-15	30.8±1.1	4.8±1.2	-	-	+	-	-	+
IARI-IHD-17	-	-	+	-	-	-	-	+
IARI-IHD-21	-	-	-	-	-	-	-	+
IARI-IHD-22	-	-	-	-		-	-	+
IARI-IHD-23	-	-	-	+	-	-	-	-
IARI-IHD-24	-	-	-	-	-	-	-	-
IARI-IIWP-43	16.2±1.1	3.5±0.2	-	-	+	-	-	-
IARI-IIWP-45	12.1±1.2	4.5±0.1	-	+	+	-	-	-
IARI-IHD-35	11.6±1.3	2.5±1.2	-	-	+	-	-	-
IARI-IHD-34	15.6±0.7	2.5±0.1	+	+	-	-	+	-
IARI-IIWP-23	45.17±1.1	4.7±0.5	+	-	+	-	-	-
IARI-IIWP-31	21.5±1.1	9.7±0.9	-	+	-	-	+	-
IARI-IHD-3	280.4±0.5	2.2±0.5	+	-	+	+	+	-
IARI-IHD-4	-	6.8±0.8	+	-	+	-	-	-
IARI-IHD-30	-	4.6±0.1	-	-	-	-	-	-
IARI-IIWP-1	15.2 ±0.4	-	-	+	-	-	+	-
IARI-IIWP-12	65.9±1.0	2.8±1.5	+	-	+	+		+
IARI-IIWP-18	66.1±0.7	2.4±0.1	-	-		+	-	-
IARI-IIWP-27	35.7±0.7	6.0±0.8	-	-	+	+	+	-
IARI-IIWP-29	28.5±1.1	7.0±0.8	-	-	+	-	+	+
IARI-IIWP-32	-	-	+	-	-	-	-	-
IARI-IIWP-33	-	4.7±1.2	-	-	-	+	-	-
IARI-IIWP-34	36.1±0.7	-	-	-	+	-	+	-

P- Phyllospheric; E-Endophytic; R-Rhizospheric; K-Potassium; IAA-Indole 3-acetic acid; GA-Gibberellic acid ACC-1-aminocyclopropane-1-carboxylate; *Numerical values are mean ± SD of three independent observations; Phosphate (µg mg-1day-1); IAA (µg mg-1 protein day-1); # Radius of halo zone in mm; -, negative for the attributes; +, positive for the attributes

Table.4 Diversity indices for the isolates associated with wheat from five sites in central zone of India

	GCZ	SCZ	ICZ	JCZ	KCZ
No of isolates	68	64	87	59	70
Species richness	27	26	29	24	28
Evenness (J´)	0.95	0.94	0.94	0.94	0.97
Shannon (H)	3.25	3.20	3.31	3.12	3.30
Simpson's (D)	0.96	0.95	0.96	0.95	0.96
Chao-1	27	26.27	29	24	28

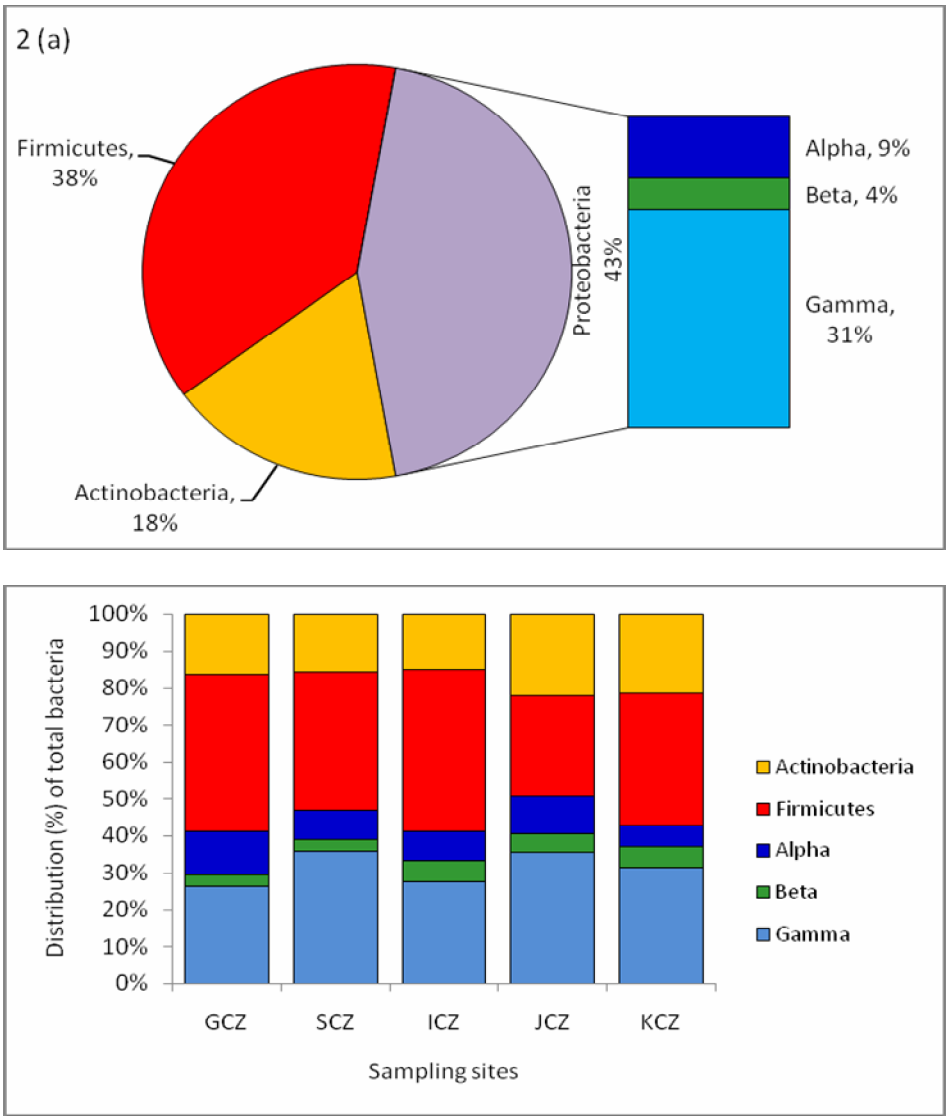

Fig. 2 Abundance of different bacteria; **a** Distribution of phylum and group in the samples surveyed; **b** Distribution of total bacteria isolated from five different site of central zone of India

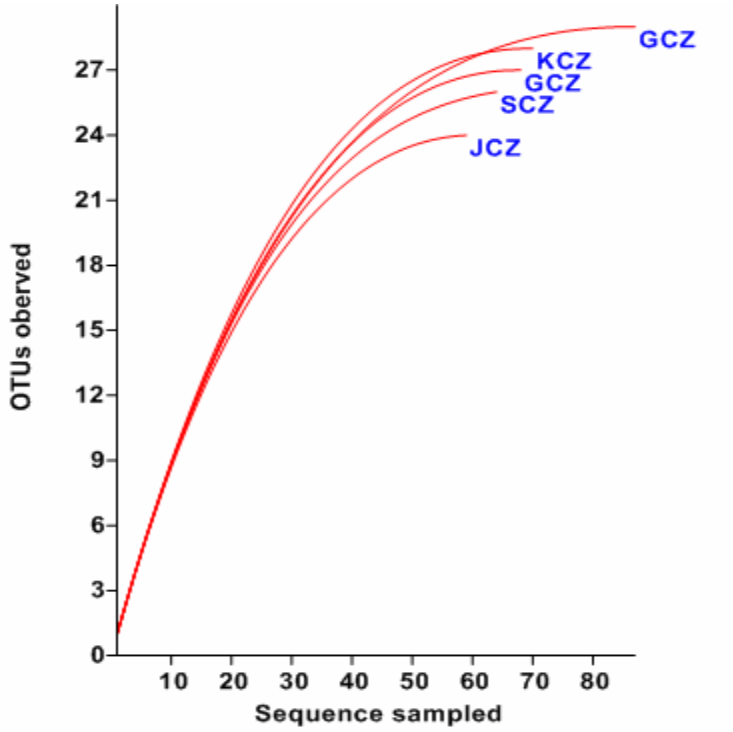

Fig. 3 Rarefaction curves of observed OTUs in wheat associated bacterial isolates from five sites of central zone of India using 16S rRNA sequencing analysis

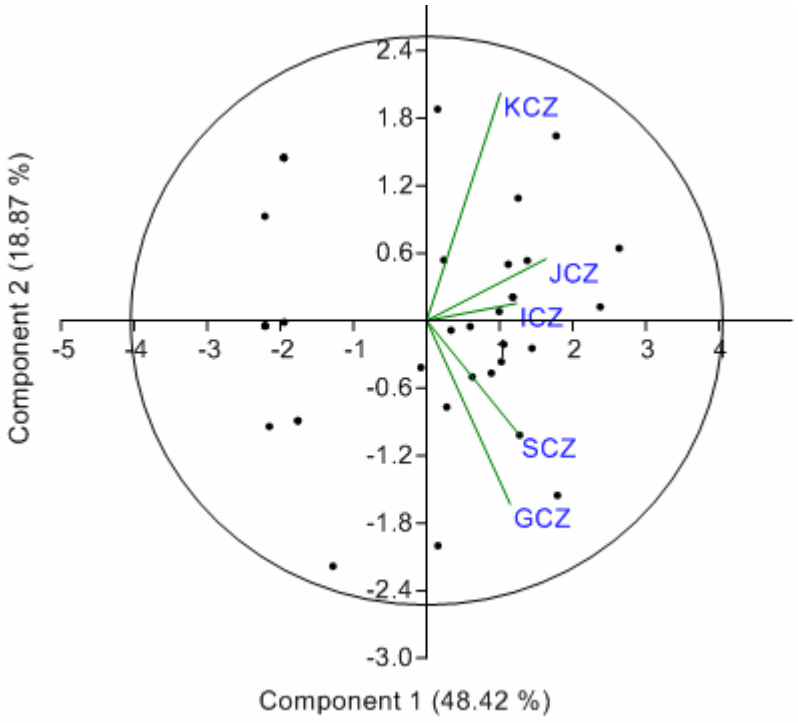

Fig. 4 Principal coordinate analysis (PCA) of the diversity indices (H) of the16S rRNA PCR-ARDRA profiles of the five sites in relation to 16S rRNA gene sequences, Component 1 and component 2 accounted for 48.42 % and for 18.87 % of the total variation, respectively.

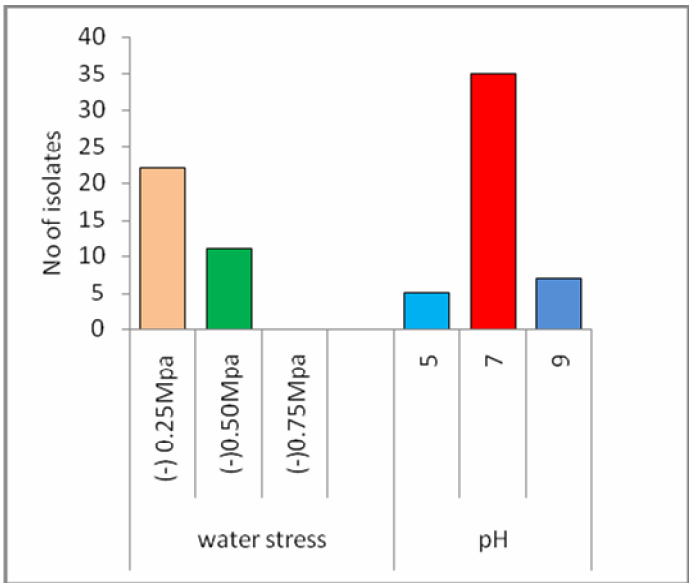

Fig. 5 Distribution of bacterial isolates based on their degree of tolerance and belonging to five different sits in central zone of India, with respect to water stress and pH.

Microbes associated with plants can be harmful and beneficial. PGPB promote growth directly by nitrogen fixation, solubilization phosphorus and potassium, production of siderophores, ammonia, HCN and production of plant growth hormones (cytokinin, auxin and gibberellic acid) (Tilak et al. 2005). Many bacteria support plant growth indirectly by improving growth restricting conditions via production of antagonistic substances. A number of bacterial species associated with the plant belonging to genera *Stenotrophomonas, Serratia, Psychrobacter, Pseudomonas, Pantoea, Paenibacillus, Micrococcus, Lysinibacillus, Kocuria, Exiguobacterium, Duganella, Delftia, Corynebacterium, Bacillus, Arthrobacter and Acinetobacter* are able to exert a beneficial effect on plant growth.

Phosphate (P) and potassium (K) are the major essential macronutrients for biological growth and development. The most efficient phosphate solubilizing bacteria (PSB) belong to genera *Bacillus* and *Pseudomonas.* There are considerable populations of P- or K-solubilizing bacteria in soil and in plant rhizospheres. P-solubilizing bacteria (PSB) have ability to solubilize inorganic phosphate compounds (Goldstein 1995). K-solubilizing bacteria (KSB) were found to resolve potassium, silicon and aluminium from insoluble minerals (Hu et al. 2006). Zinc is a nutrient at low concentration but toxic at higher concentration. The solubilization of zinc might limit the growth of the bacteria at higher level. Zinc solubilization by bacteria has an immense importance in zinc nutrition to plants. Indole-3-acetic acid (IAA) is phytohormones, a type of best characterized auxin, which is essential for the growth and development of plants. The capacity to synthesize IAA is widespread among soil- and plant associated bacteria. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce the plant growth regulator IAA (Patten and Glick 2002).

In conclusion, utility of bacterial strains in the context of semi arid agro ecosystems is immense considering the unique crop growing situations and the climatic conditions of the drought agricultural systems. Such systems require situation-specific microbial inoculants that withstand extremities of alkali and retain their functional traits for plant growth promotion. The plant growth promotion potential of the bacterial strain dealt in this study requires further evaluation and validation before its use as bio-inoculants in the drought agro ecosystems, where alkali is a major determinant of plant and microbial activity. The selection of native functional plant growth promoting microorganisms is a mandatory step for reducing the use of energy intensive chemical fertilisers. The strain reported in this study seems to be an ideal candidate for promotion as bio- inoculants, due to its drought tolerance and multiple abilities of plant growth promotion traits.

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