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Growth and yield enhancement of *Triticum aestivum* L. by rhizobacteria isolated from agronomic plants

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Abstract

Growth and yield response of *Triticum aestivum* L. was evaluated by inoculating plants with rhizobacteria isolated from agronomic crops. Ten bacterial strains showing different plant growth promoting traits were isolated from the rhizosphere of *Momordica charantia*, *Zea mays*, *Oryza sativa* and *Hordeum vulgare*. Sequencing of 16S rRNA gene confirmed the affiliations of isolates with *Chryseobacterium*, *Acinetobacter*, *Bacillus* and *Enterobacter* genera. *In vitro* screening showed the ability of rhizobacteria for multiple plant growth promoting traits. For instance, highly significant phytase activity was observed for *A. calcoaceticus* McR-2 (4.6 U ml^{-1}) and *B. megaterium* ZmR-6 (4.2 U ml^{-1}). For 1-aminocyclopropane-1-carboxylate (ACC) deaminase, *B. megaterium* ZmR-4, *B. cereus* McR-3 and *B. megaterium* ZmR-4 recorded 930, 706 and 600 nmol h^{-1} activities, respectively. Similarly, highest auxin levels were quantified for *B. megaterium* ZmR-4 (140 µg ml^{-1}), *E. cloacae* FR (136 µg ml^{-1}) and *B. megaterium* ZmR-3 (134 µg ml^{-1}). In pot trials (axenic conditions), significant increases for shoot length and number of roots were observed with *E. cloacae* FR (22%) and *B. cereus* (46%), over control, respectively. At maturity, number of spikelets recorded highly significant increments with *E. cloacae* FR (54%), *B. cereus* McR-3 (29%) and *B. megaterium* McR-8 (28%). For seed weight, significant improvement of 62% was noted with *B. megaterium* ZmR-6. These findings suggested that rhizobacteria associated with agronomic plants harbor beneficial traits that can be used to enhance the growth and yield of *T. aestivum*. This study also reported the presence of agriculturally significant bacteria within the rhizosphere of *M. charantia*.

Keywords: *Triticum aestivum*; Phytase activity; ACC-deaminase; Auxin production; Plant growth promotion.
Abbreviations: ACC-1-aminocyclopropane-1-carboxylate; CFU-colony forming units; HCN-hydrogen cyanide; DF-Dworkin and Foster; EC-electrical conductivity; IAA-indole-3-acetic acid; PGPR-plant growth promoting rhizobacteria.

Introduction

Phytohormones production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and minerals solubilization are the prominent mechanisms by which plant growth promoting rhizobacteria (PGPR) directly contribute to the plant growth. Enhanced plant nutrition by PGPR is mainly through increased phosphorus uptake by solubilization of inorganic phosphates. Phosphorus (P), next to nitrogen, is the second important macronutrient required for plant growth and development. Even in phosphorus rich soils most of the P is present in insoluble form and only a small fraction of about 0.1% is available to plants. Additionally, more than 70% of the P fertilizers applied to soil converted into insoluble forms and become unavailable for crops (Stevenson and Cole, 1999; Hariprasad and Niranjana, 2009). Rhizobacteria secrete organic acids and phosphatases to convert the insoluble phosphate into soluble ions by a process known as mineral phosphate solubilization. Phytate (*myo*-inositol hexakisphosphate) accounts for 20-50% of the total soil organic phosphorus. Phytases are involved in the stepwise degradation of phytate to lower phosphate esters of myo-inositol and phosphorus. Mineralization of organic and inorganic phosphate has been reported from different bacterial genera including *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Citrobacter* and *Pantoea* (Hariprasad and Niranjana, 2009; Ramirez et al., 2010; Patel et al., 2010). Ethylene is a plant hormone that mediates a wide range of developmental processes in plants. However, higher concentrations of ethylene are inhibitory to plant growth. ACC is the immediate precursor of ethylene and for many plants; a burst of ethylene is required to break seed dormancy. After seed germination, a significant portion of the ACC can be exuded from plant roots and sustained levels of ethylene inhibit root elongation. It has been reported that bacteria contain an enzyme ACC-deaminase that hydrolyses ACC into ammonia and α-ketobutyrate. Hydrolysis of ACC on the surface of roots eliminates the potential inhibitory effects of higher ethylene concentrations and facilitates the formation of longer roots (Glick, 2005). Recently, inoculation with specific rhizobacteria has been shown to alter the endogenous levels of ethylene which subsequently led to changes in the growth and development of inoculated plants (Tittabutr et al., 2008; Akhtar and Ali, 2011; Noreen et al., 2012). Among the phytohormones indole-3-acetic acid (IAA) which is the most abundant type of auxin play very crucial role in plant growth enhancements (Ali et al., 2009a; Kochar and Srivastava, 2012). Rhizobacteria isolated from the rhizosphere of different crops have a greater potential to synthesize IAA as secondary metabolites because of the relatively rich supply of precursor L-tryptophan. In present study, we have demonstrated the genetic variability and plant growth promoting potential of rhizobacteria isolated from the rhizosphere of *Momordica charantia*, *Zea mays*, *Oryza sativa*

Table 1. 16S rRNA gene sequencing of bacterial strains isolated from different agronomic plants.

Strains	Plant	Homology (%)	Identified as	Accessions
McR-1	*M. charantia*	98	*C. indologenes*	JF894157
McR-2	*M. charantia*	99	*A. calcoaceticus*	JF894158
McR-3	*M. charantia*	99	*B. cereus*	JF894159
McR-7	*M. charantia*	100	*B. subtilis*	JF894160
McR-8	*M. charantia*	99	*B. megaterium*	JF894161
ZmR-3	*Z. mays*	100	*B. megaterium*	JF894163
ZmR-4	*Z. mays*	100	*B. megaterium*	JF894164
ZmR-6	*Z. mays*	99	*B. megaterium*	JF894165
OsR-3	*O. sativa*	99	*B. megaterium*	JF894162
FR	*H. vulgare*	99	*E. cloacae*	JF894166

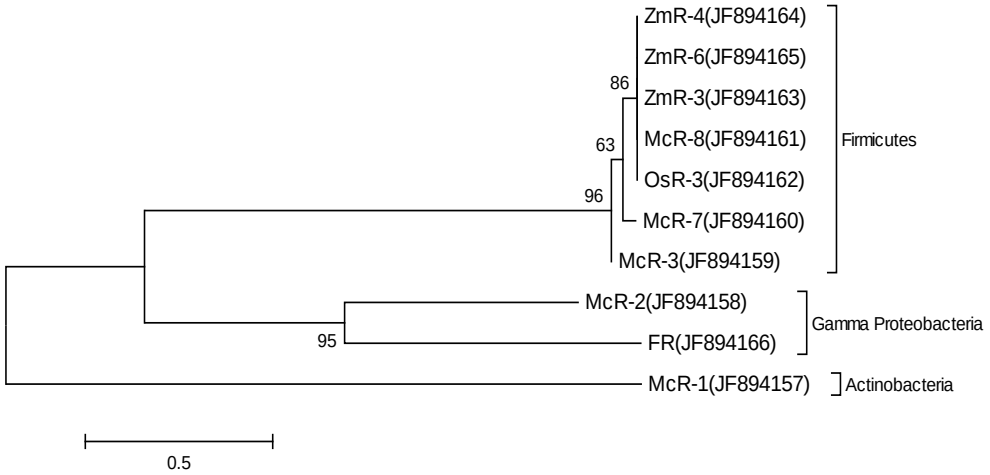

Fig 1. Phylogenetic tree showing the affiliations of 10 bacterial strains isolated from different agronomic plants. Phylogenies were inferred using neighbour-joining algorithm and tree was generated using software MEGA 4 (Tamura et al., 2007). Numbers at branches points indicates the percentage of 500 bootstrap resamplings. The scale bar represents mutations per nucleotide position. Affiliations: ZmR-4, *Bacillus megaterium*; ZmR-6, *B. megaterium*; ZmR-3, *B. megaterium*; McR-8, *B. megaterium*; OsR-3, *B. megaterium*; McR-7, *B. subtilis*; McR-3, *B. cereus*; McR-2, *Acinetobacter calcoaceticus*; FR, *Enterobacter cloacae*; McR-1, *Chryseobacterium indologenes*.

and *Hordeum vulgare*. Rhizobacteria were selected on the basis of auxin production and phytase or ACC-deaminase activity. Previous studies have reported the bacterial diversity of *Zea mays*, *Oryza sativa* and *Hordeum vulgare*. However, most of these studies provided little information about the plant growth promoting potential and genetic variability of bacteria associated with *M. charantia*. Therefore, in present work we reported the biofertilization of wheat (*Triticum aestivum* L.) by rhizobacteria isolated from the rhizosphere of agronomically important plants.

Results

Morphological and biochemical characterization

Microscopic examinations of stained bacterial smear showed that majority of the strains were gram positive rods. A few strains (McR-1, McR-2, FR) were gram negative rods. Majority of the strains exhibited circular colonies with entire margins and were creamy in color. All strains were catalase positive but showed variable results for oxidase, nitrate and citrate. Majority of the strains showed positive results for mannitol and glucose fermentation.

16S rDNA sequencing and phylogenetic analysis

Sequences of 16S rRNA gene were compared to online database (GenBank) through BLAST to identify the final taxonomic status of bacterial cultures. Strain McR-3 showed 99% homology with *Bacillus cereus* whereas McR-7 showed 100% similarity with *Bacillus subtilis*. On the other hand, McR-8, ZmR-3, ZmR-4, ZmR-6 and OsR-3 showed homology (up to 100%) with *Bacillus megaterium*. Bacterial strains McR-1, McR-2 and FR showed 98%, 99% and 99% homology, respectively, with *Chryseobacterium indologenes*, *Acinetobacter calcoaceticus* and *Enterobacter cloacae*. The sequences of all strains have been deposited in the GenBank under different accession numbers (Table 1). The phylogenetic tree constructed by using sequences of bacterial strains showed three major groups (Fig 1). The first group comprising of 7 isolates related to firmicutes and represented by genus *Bacillus*. The second group contained two isolates (McR-2, FR) that were placed in γ-proteobacteria. The third group was represented by McR-1 that included in group actinobacteria. Bootstrap values among the internal nodes of phylogenetic tree are high that supported the differentiation among different species.

Plant growth-promoting traits of rhizobacteria

Rhizobacteria showed variable potential for different plant growth promoting attributes. For instance, highly significant phytase activity was recorded for *A. calcoaceticus* McR-2 (4.6 U ml^{-1}) and *B. megaterium* ZmR-6 (4.2 U ml^{-1}) and *B. subtilis* McR-7 (1.8 U ml^{-1}) (Fig 2). For mineral phosphate solubilization, *A. calcoaceticus* McR-2 and *E. cloacae* FR

Table 2. Antibiotic susceptibility pattern of rhizobacteria. Zones indicates mean of 4 replicates.

	Antibiotics							
	Ery (15 µg)		Amp (10 µg)		Oxy (30 µg)		Car (100 µg)	
Strains	Zone size (mm)	Susceptibility	Zone size (mm)	Susceptibility	Zone size (mm)	Susceptibility	Zone size (mm)	Susceptibility
C.indologenes McR-1	0	R	0	R	14	R	0	R
A.calcoaceticus McR-2	16	I	0	R	21	S	0	R
B. cereus McR-3	25	S	0	R	19	S	0	R
B. subtilis McR-7	22	I	25	S	11	R	20	S
B.megaterium McR-8	24	S	16	S	27	S	16	S
B. megaterium ZmR-3	25	S	17	S	28	S	17	S
B. megaterium ZmR-4	25	S	21	S	29	S	19	S
B. megaterium ZmR-6	23	S	17	S	26	S	16	S
B. megaterium OsR-3	25	S	20	S	28	S	19	S
E. cloacae FR	0	R	0	R	20	S	6	R

Ery, Erythromycin; Amp, Ampicillin; Oxy, Oxytetracyclin; Car, Carbenicillin; S, Sensitive; R, Resistant; I, Intermediate

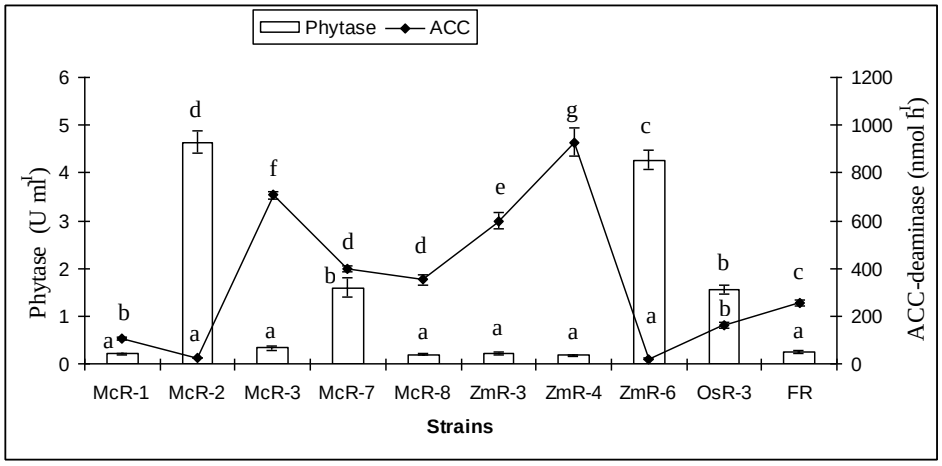

Fig 2. Phytase and ACC-deaminase activity of rhizobacteria. Mean±S.E. of 3 replicates. Different letters at various points indicate significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$).

showed positive results. For ACC-deaminase, *B. megaterium* ZmR-4, *B. cereus* McR-3, *B. megeterium* ZmR-3, *B. megaterium* McR-8, *B. subtilis* McR-7, *E. cloacae* FR, *B. megaterium* OsR-3 and *C. indologenes* McR-1 showed 930, 706, 600, 350, 340, 255, 160 and 104 nmol h^{-1} activities, respectively (Fig 2). All bacterial strains were very strong producers of auxin as indicated by colorimetric analysis. Maximum auxin production was observed for *B. megaterium* ZmR-4 (140 µg ml^{-1}), *E. cloacae* FR (136 µg ml^{-1}) and *B. megaterium* ZmR-3 (134 µg ml^{-1}) (Fig 3). For HCN production, a few strains (*B. subtilis* McR-7, *B. megaterium* McR-8, *B. megaterium* ZmR-6) were strongly positive while the rest were moderate.

Antibiotic susceptibility of rhizobacteria

Antibiotic susceptibility pattern of rhizobacterial strains was observed against erythromycin (15 µg), ampicillin (10 µg), oxytetracyclin (30 µg) and carbenicillin (100 µg). Majority of the bacilli strains such as *B. cereus* McR-3, *B. megaterium* McR-8, *B. megaterium* ZmR-3, *B. megaterium* ZmR-4, *B. megaterium* ZmR-6 and *B. megaterium* OsR-3 were sensitive to erythromycin with zone of inhibition 25, 24, 25, 25, 23 and 25 mm, respectively (Table 2). On the other hand, *B. subtilis* McR-7 (25 mm), *B. megaterium* McR-8 (16 mm), *B. megaterium* ZmR-3 (17 mm), *B. megaterium* ZmR-4 (21 mm), *B. megaterium* ZmR-6 (17 mm) and *B. megaterium* OsR-3 (20 mm) were sensitive to ampicillin. Strains such as *A. calcoaceticus* McR-2 (21 mm), *B. cereus* McR-3 (19 mm), *B. megaterium* McR-8 (27 mm), *B. megaterium* ZmR-3 (28 mm), *B. megaterium* ZmR-4 (29 mm), *B. megaterium* ZmR-6 (26 mm), *B. megaterium* OsR-3 (28 mm) and *E. cloacae* FR (20 mm) were sensitive to oxytetracyclin. Bacterial strains *C. indologenes* McR-1, *A. calcoaceticus* McR-2, *B. cereus* McR-3 and *E. cloacae* FR were resistant to carbenicillin (Table 2).

Pot experiments

Growth experiments conducted under axenic conditions recorded significant increases in shoot length and root growth with bacterial inoculations (Table 3). Maximum increases of 22%, 20%, 20%, 17% and 16% for shoot length were recorded with *E. cloacae* FR, *C. indologenes* McR-1, *B. megaterium* OsR-3, *A. caloaceticus* McR-2 and *B. megaterium* ZmR-4, respectively.

Table 3. Effect of rhizobacteria on growth of T. aestivum under axenic conditions.

Strains	Shoot length (cm)	Root length (cm)	No. of roots/plant
Control	15.77±0.5 $^{(a)}$	13.10±0.6 $^{(a)}$	3.26±0.09 $^{(a)}$
C. indologenes McR-1	19.00±0.6 $^{(d)}$	14.80±0.8 $^{(a)}$	4.34±1.0 $^{(d)}$
A. calcoaceticus McR-2	18.45±0.5 $^{(cd)}$	20.20±0.5 $^{(b)}$	3.58±0.8 $^{(bc)}$
B. cereus McR-3	15.63±0.5 $^{(a)}$	17.98±0.9 $^{(a)}$	4.75±0.1 $^{(e)}$
B. subtilis McR-7	16.62±0.6 $^{(ab)}$	17.96±0.8 $^{(a)}$	3.59±0.07 $^{(bc)}$
B. megaterium McR-8	16.37±0.2 $^{(ab)}$	20.18±0.6 $^{(b)}$	3.47±0.05 $^{(abc)}$
B. megaterium ZmR-3	17.68±0.5 $^{(bcd)}$	17.04±0.8 $^{(a)}$	3.66±0.08 $^{(c)}$
B. megaterium ZmR-4	18.32±0.4 $^{(cd)}$	16.44±1.0 $^{(a)}$	3.37±0.07 $^{(ab)}$
B. megaterium ZmR-6	17.00±0.5 $^{(abc)}$	15.89±0.8 $^{(a)}$	4.27±0.07 $^{(d)}$
B. megaterium OsR-3	18.90±0.3 $^{(d)}$	19.18±0.6 $^{(a)}$	3.50±0.05 $^{(abc)}$
E. cloacae FR	19.23±0.3 $^{(d)}$	15.52±0.5 $^{(a)}$	3.62±0.1 $^{(bc)}$

Mean ± S.E. of 2 repeated experiments (72 plants). Different letters within same column in parenthesis indicate significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$).

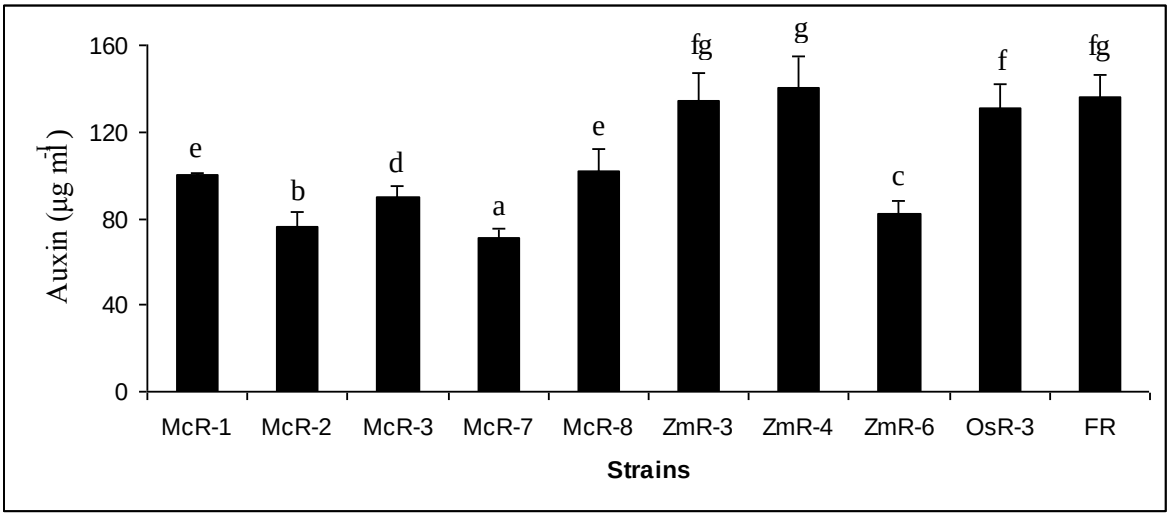

Fig 3. L-tryptophan dependent auxin production by rhizobacteria. Mean±S.E. of 3 replicates. Different letters at bars indicate significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$).

For root length, *A. calocoaceticus* McR-2 and *B. megaterium* McR-8 recorded 54% increments, over control. In case of number of roots, majority of the treatments showed significant enhancements with *B. cereus* McR-3 (46%), *C. indologenes* McR-1 (34%), *B. megaterium* ZmR-6 (31%) and *B. megaterium* ZmR-3 (12%). Growth responses under natural environmental conditions were also evaluated after harvesting plants at six week interval as well as at full maturity. After six weeks, *B. megaterium* ZmR-6, *C. indologenes* MCR-1 and *E. cloacae* FR showed, respectively, 93%, 81% and 72% increases in shoot fresh weight. Similarly, for dry weight highly significant improvements were recorded for *B. megaterium* ZmR-6 (43%) and *E. cloacae* FR (24%) over water treated control (Fig 4). At full maturity, bacterization of seeds mainly influenced yield parameters as compared to vegetative growth (Table 4). For shoot length, majority of the bacterial treatments showed comparable results to that of control. However, *B. megaterium* McR-8 and *B. megaterium* ZmR-6 showed significant improvements of 13% and 10%, respectively. On the other hand, number of tillers and spike length also recorded comparable growth response to that of control as indicated by statistical analysis. However, number of spikelets recorded highly significant increments with *E. cloacae* FR (54%), *B. cereus* McR-3 (29%), *B. megaterium* McR-8 (28%), *B. subtilis* McR-7 (27%) and *B. megaterium* ZmR-6 (26%). In case of seeds weight, significant improvements of 62%, 38%, 29%, 28% and 24%, respectively, were noted with *B. megaterium* ZmR-6, *E. cloacae* FR, *B. megaterium* OsR-3, *B. megaterium* ZmR-3 and *B. megaterium* ZmR-4, over control.

Discussion

The present work investigated the genetic variability and plant growth promoting activity of rhizobacteria isolated from the rhizosphere of different agronomic crops. Sequencing of bacterial strains from the rhizosphere of *Momordica charantia* showed the presence of *Chryseobacterium indologenes* (McR-1), *Acinetobacter calcoaceticus* (McR-2), *Bacillus cereus* (McR-3), *B. subtilis* (McR-7) and *B. megaterium* (McR-8). Isolates from *Zea mays* (ZmR-3, ZmR-4, ZmR-6) and *Oryza sativa* (OsR-3) also showed similarity with *B. megaterium*. On the other hand, strain FR associated with *Hordeum vulgare* was identified as *Enterobacter cloacae*. Phylogenetic analysis of 16S rDNA sequences resulted in the grouping of strains into three major clusters i.e. firmicutes, γ-proteobacteria and actinobacteria (Fig 1). Several workers have reported the presence of PGPR strains from the rhizosphere of agronomically important plants (Zinniel et al., 2002; Kundu et al., 2009; Someya et al., 2011). However, in present work, we first time reported the presence of *C. indologenes* and *A. calcoaceticus* within the rhizosphere of *M. charantia*. In this study, screening of bacterial strains for different plant growth promoting attributes revealed that isolates were strongly positive for phytase (up to 4.6 U ml^{-1}), ACC-deaminase (up to 930 nmol h^{-1}) and auxin production (up to 140 µg ml^{-1}).

Table 4. Effect of rhizobacteria on growth and yield of *T. aestivum* at full maturity.

Strains	Shoot length (cm)	No. of tillers/Plant	Spike length (cm)	No. of spikelets	Weight of 200 seeds (g)
Control	50.00±1.0 (a)	1.10±0.05 (a)	7.39±0.23 (a)	24.69±1.4 (a)	7.37±0.5 (a)
C. indologenes McR-1	49.30±1.4 (a)	1.08±0.04 (a)	7.12±0.23 (a)	28.36±1.1 (abc)	8.20±0.4 (ab)
A. calcoaceticus McR-2	50.52±1.3 (a)	1.08±0.04 (a)	6.78±0.25 (a)	27.00±1.0 (ab)	8.76±0.5 (ab)
B. cereus McR-3	50.50±2.1 (a)	1.08±0.06 (a)	7.30±0.25 (a)	31.88±1.1 (c)	8.79±0.1 (ab)
B. subtilis McR-7	53.50±1.7 (ab)	1.05±0.03 (a)	7.68±0.22 (a)	31.22±1.1 (c)	8.70±0.3 (ab)
B. megaterium McR-8	56.40±1.3 (c)	1.11±0.05 (a)	7.63±0.16 (a)	31.61±1.3 (c)	8.72±0.1 (ab)
B. megaterium ZmR-3	51.70±1.6 (ab)	1.13±0.05 (a)	8.26±1.5 (a)	26.75±1.3 (ab)	9.46±0.8 (ab)
B. megaterium ZmR-4	50.30±1.0 (a)	1.22±0.07 (a)	6.88±0.23 (a)	24.55±1.4 (a)	9.16±1.4 (ab)
B. megaterium ZmR-6	54.90±1.8 (b)	1.11±0.05 (a)	7.75±0.31 (a)	31.11±1.6 (c)	11.93±3.2 (b)
B. megaterium OsR-3	51.00±1.4 (ab)	1.05±0.03 (a)	7.51±0.23 (a)	29.83±1.3 (bc)	9.49±0.9 (ab)
E. cloacae FR	48.55±2.1 (a)	1.05±0.03 (a)	7.43±0.25 (a)	38.08±1.1 (d)	10.15±0.5 (ab)

Mean ± S.E. of 6 replicates (30 plants). Different letters within same column in parenthesis indicate significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$).

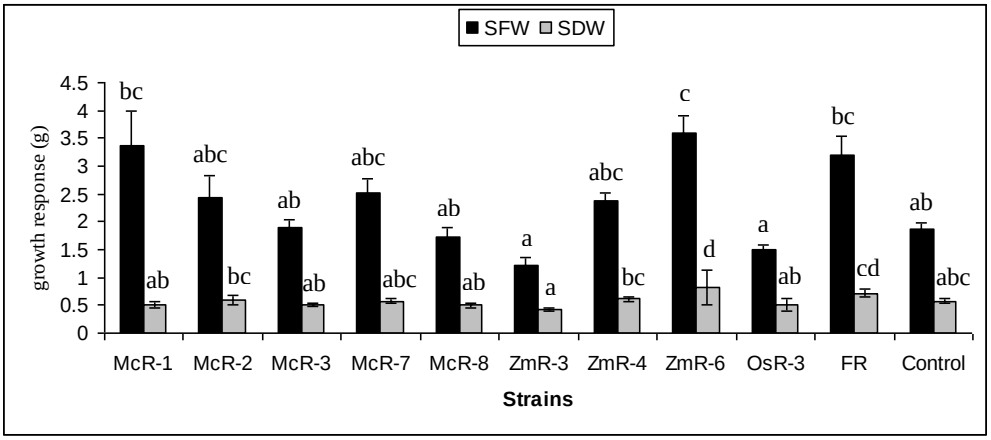

Fig 4. Effect of bacterial strains on shoot fresh and dry weight of *T. aestivum* after six weeks growth period at ambient conditions. Mean±S.E. of 6 replicates (30 plants). Different letters at bars indicate significant difference between treatments using Duncan's multiple range test ($P \leq 0.05$).

Our results are in agreement with previous findings that rhizobacteria may exhibit several plant growth promoting traits simultaneously (Raddadi et al., 2008; Gulati et al., 2009; Ali et al., 2010). Antibiotic sensitivity pattern showed that majority of the isolates were susceptible to different antibiotics. Sensitive bacterial strains can be used to develop safe biofertilizers for field applications as they are not showing multiple drug resistance. After screening, we used these rhizobacteria to inoculate *Triticum aestivum* under axenic and natural environmental conditions. Under axenic environment, maximum increases for shoot length (22%), root length (54%) and number of roots (46%) were observed with *E. cloacae* FR, *A. calocoaceticus* McR-2 and *B. cereus* McR-3, respectively. Experiments under natural environmental conditions recorded up to 54% and 62% increases for number of spikelets and seeds weight, respectively with *E. cloacae* and *B. megaterium* ZmR-6. Previously, we also reported the growth promoting effect of *Bacillus* strains exhibiting multiple plant growth promoting traits (Ali et al., 2009b; Akhtar and Ali, 2011). Gulati and coworkers (2009) reported the significant plant growth improvements with *Acinetobacter* exhibiting auxin production, ACC-deaminase activity and phosphate solubilization. Similarly, several studies suggested the role of bacterial auxin in enhancing the growth and yield of agronomically important crops (Ali et al., 2009a, b; Kochar and Srivastava, 2012). In another study, inoculation of plants with phytate mineralizing rhizobacteria significantly enhanced the phosphate availability within rhizosphere (Patel et al., 2010).

Materials and methods

Isolation of rhizobacteria

For bacterial isolation, one gram of soil was dissolved in flask containing 99 ml of autoclaved distilled water to prepare 10^{-2} dilution. One ml of soil suspension was added in next flask to make a dilution of 10^{-4} and so on. About 50 µl of each dilution was plated on nutrient agar in duplicate and incubated at 37°C for 24 h. After incubation, 40 bacterial strains that showed morphological variations were purified by many rounds of streaking. Finally, 10 strains were selected from the rhizosphere of *Momordica charantia* (McR-1, McR-2, McR-3, McR-7, McR-8), *Zea mays* (ZmR-3, ZmR-4, ZmR-6), *Oryza sativa* (OsR-3) and *Hordeum vulgare* (FR). Strains were further characterized morphologically and biochemically (Cappuccino and Sherman, 2002).

Identification and genetic variability of rhizobacteria

The final taxonomic status of the isolates was determined by 16S rRNA gene sequencing. Genomic DNA was extracted from bacterial cultures and a portion of the 16S rDNA was amplified according to the method described by Hasnain and Thomas (1996). About 1·5-kb DNA fragment containing 16S rRNA gene was amplified using forward 27f and reverse primer 1522r (Johnson, 1994). PCR amplification was performed by using 50 µl of Dream Taq™ Green PCR Master Mix (Fermentas) with 0.5 µg of chromosomal DNA template and 0.5 µM of each primer as described earlier

(Akhtar and Ali, 2011). After amplification, product was purified using QIAquick Gel Extraction Kit (QIAGEN) and sequenced using 27f and 1522r primers by ABI PRISM-3100 Genetic Analyzer (Applied Biosystems, USA). The sequences from 16S rDNA sequencing were aligned with multiple sequence alignment program ClustalW by using MEGA 4 software (Tamura et al., 2007) and phylogenetic tree was constructed by Neighbour-Joining (NJ) method (Saitou and Nei, 1987).

Phytase and phosphate solubilization assays

Phytase activity of rhizobacteria was determined in triplicate by growing strains in L-broth supplemented with 0.1% sodium phytate. Cultures were incubated at 37^{o}C on shaker with agitation at 200 rpm and extracellular phytase activity was recorded after 5 days (Hussin et al., 2007). Phosphate solubilization ability of rhizobacteria was also determined qualitatively by streaking strains on Pikovskaya agar plates (Pikovskaya, 1948).

ACC-deaminase activity

Induction of ACC-deaminase activity of rhizobacteria was carried out following the method of Penrose and Glick (2003). Bacterial cells grown in 25 ml L-both (in triplicate) were washed with 5 ml DF salts minimal medium and centrifuged. Cells were transferred to 7.5 ml DF salts minimal medium containing 45 µl ACC to a final concentration of 3 mM and incubated at 28^{o}C for 24 h on shaker. After incubation, the supernatant was removed and cell pellet was washed with 5 ml DF salts minimal medium. Bacterial cells were re-suspended in 7.5 ml DF salts minimal medium supplemented with ACC as mentioned above. The cultures were again incubated at 28^{o}C for 1 h on shaker and liberated ammonia was quantified as described in (Akhtar and Ali, 2011).

Bacterial auxin production

Bacterial auxin production in liquid culture was determined colorimetrically in the presence of precursor L-tryptophan. L-broth (25 ml) was supplemented with 200 µg ml^{-1} of L-Tryptophan and inoculated with 100 µl of bacterial suspension in triplicate. Cultures were incubated at 37^{o}C for 72 h on shaker at 150 rpm. After incubation, 1.5 ml of bacterial culture was centrifuged at 5000 *g* for 15 min and 1 ml of supernatant was taken in a test tube and mixed with 2 ml of salkowski reagent (Tang and Borner, 1979). Test tubes were kept in dark for 25 min for the development of pink color and auxin was quantified from standard curve constructed by using different concentrations of standard auxin (Noreen et al., 2012).

Hydrogen cyanide production

Hydrogen cyanide (HCN) activity of bacterial cultures was determined by growing strains on nutrient agar supplemented with glycine. Filter paper soaked in a solution of 2% sodium carbonate in 0.5% picric acid was placed at the top of agar surface. Plates were sealed with parafilm and incubated at 28^{o}C for 4 days and development of the orange to red color was taken as positive test for HCN production (Ahmad et al., 2008).

Antibiotic susceptibility pattern of rhizobacteria

Antibiotic susceptibility pattern of bacterial strains was evaluated by Kirby-Bauer method (Bauer et al., 1966). Lawn of bacterial strains was made by streaking on the entire surface of Mueller-Hinton agar plate. Four discs of each antibiotic that included erythromycin (15 µg), ampicillin (10 µg) oxytetracyclin (30 µg) and carbenicillin (100 µg) were placed at equal distance and incubated at 37^{o}C for 24 h. After incubation, zones of inhibition in millimeters were recorded and compared with the standard chart as mentioned in Cappuccino and Sherman (2002).

Plant inoculation experiments

(i)- Seeds sterilization and preparation of inoculum

Healthy seeds of *Triticum aestivum* L. var. Inqalab-91 were procured from Punjab seed corporation, Lahore, Pakistan. Seeds were surface sterilized with 0.1% $HgCl_2$ for 5 min followed by repeated washings with autoclaved distilled water. For inoculum preparation, bacterial strains were grown in 20 ml L-broth medium at 37^{o}C for 24 h. After incubation, cells were harvested and suspended in autoclaved distilled water to adjust optical density to 10^7 CFU ml^{-1}. Surface sterilized seeds were treated with bacterial cultures for 25 min. Control seeds were soaked in sterilized distilled water for the same duration.

(ii)-Pot trials

For experiments under axenic conditions, pots were filled with 110 g autoclaved and dried soil. Six pots were inoculated for each strain and experiment was repeated twice. Eight seeds were sown in each pot and finally, six uniform seedlings were maintained. All pots were incubated at 25^{o}C under photoperiod of 16 h. After two weeks, plants were harvested and different growth parameters such as shoot length, root length and numbers of roots were recorded. For experiments at ambient conditions, sterilized seeds of *T. aestivum* were treated with bacterial suspension as mentioned above. Garden soil (sandy loam) was collected and 10 kg soil was filled in each earthen pot (30 × 30 cm). The soil had a pH 7.3, electrical conductivity (EC) 44 ds m^{-1}, and 0.60% organic content. Seeds were planted 1 cm deep in the soil. Un-inoculated seeds were sown as control. Six pots for each bacterial treatment as well as control were used. Initially, 15 seeds in each pot were sown and after complete emergence plants were thinned out to 10 plants per pot. The pots were arranged in completely randomized design in the wire house of Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan at ambient light and temperature. After 6 weeks, 5 plants were removed from each pot to note fresh and dry weight of plants. At maturity, shoot length, number of tillers, spike length, number of spikelets and weight of 200 seeds per treatment were recorded.

Statistical analysis

For phytase, ACC-deaminase and auxin quantification three replicates were used for each strain. In plant-bacterial experiments, six pots for each strain were inoculated under axenic and ambient conditions. The experiments were repeated twice and all pots were arranged in completely randomized design. For all experiments, the data was subjected to analysis of variance (ANOVA) using software SPSS 17 program and means separated using Duncan's multiple range test ($P \leq 0.05$).

Conclusion

The results of present study showed a diverse type of PGPR strains within the rhizosphere of agronomic plants. Rhizobacteria isolated from different crops have variable potential for plant growth promoting traits. Majority of the strains of *Bacillus* showed significant auxin production and ACC-deaminase activity. Significant phytase activity was exhibited by *A. calcoaceticus* McR-2 and *B. megaterium* ZmR-6. Pot trials have shown that *B. megaterium* and *E. cloacae* were the most effective in improving growth of *T. aestivum*. Especially, the strains of *B. megaterium* were the most promising in enhancing yield parameters at full maturity. Further, this study also reported *M. charantia* as a potential source for the isolation of agriculturally important rhizobacteria.

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