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ORIGINAL ARTICLE

Comparative effectiveness of *Bacillus* spp. possessing either dual or single growth-promoting traits for improving phosphorus uptake, growth and yield of wheat (*Triticum aestivum* L.)

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Abstract The aim of this study was to compare the effectiveness of *Bacillus* spp. simultaneously carrying dual traits such as P solubilization and ACC deaminase activity with *Bacillus* spp. having any one of these traits for improving growth, yield and P uptake by wheat crop. Six *Bacillus* strains having predominantly either ACC deaminase activity (KA1 and KA2) or P solubilizing activity (KP3 and KP4) or simultaneously both of these traits (KAP5 and KAP6) were evaluated for improving growth of wheat cv. Bhakar-2002 using rock phosphate (RP) as an exclusive P-source. Under axenic conditions, the bacterial strains with dual plant growth-promoting activities were superior in improving growth of wheat as compared to the strains possessing single trait. Similarly, these dual traits bacterial strains were more effective than single trait strains under soil conditions (pot trial) in increasing root weight (up to 3.9-fold) and root elongation (up to 3.8-fold), dry shoot weight (up to 37.6%), number of tillers (up to 56%), grain yield (up to 38.5%) and P uptake in grain (up to 77.4%) of wheat grown in the presence of P applied as diammonium phosphate (DAP), RP (rock phosphate) or RP-enriched compost. An almost similar trend was observed when the same trial was repeated under field conditions. Inoculation in the presence of RP-enriched compost resulted in promoting various growth parameters almost comparable to that recorded in the case of DAP. It was concluded that the simultaneous presence of two superior plant growth-promoting traits in the bacteria could have an additive effect not only on growth and yield of wheat but also on P uptake. The performance of *Bacillus* strains possessing dual traits was distinctly superior to that of the single trait strains. These bacteria exhibited an excellent effectiveness in utilizing RP as the source of P in the growth medium as well as in soil.

Keywords *Bacillus* · Phosphorus · ACC deaminase · Rock phosphate · Wheat

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Introduction

The supply of P in adequate amounts is very critical for normal plant growth and development (Zaidi et al. 2009). Phosphorus is one of the least bioavailable plant nutrients in soil (Raghothama and Karthikeyan 2005). That is why it is considered as one of the major nutrient constraints hampering crop yields (Oberson et al. 2011). In calcareous soils, 88–99% of the total inorganic P is Ca-bound, which is primarily responsible for low P in soil solution (Tisdale et al. 1993). In calcareous and alkaline soils like those of Pakistan, even the added soluble P (phosphatic fertilizer)

may get fixed/precipitated (Ahmad and Rashid 2004). Overall, P use efficiency (PUE) of the applied phosphate fertilizer is low because of the formation of insoluble complexes as it comes in contact with soil colloids (Vassilev and Vassileva 2003). Therefore, many soils throughout the world are deficient in available P even though the soils are quite rich in total P content (Adesemoye and Kloepper 2009).

Low availability of soil P warrants the regular application of soluble phosphatic fertilizers to maximize crop yields. However, the cost of manufactured phosphatic fertilizers has increased drastically over the time and is getting out of reach of low income farmers. As a consequence of these constraints, there seems no option except to develop strategies to enhance the availability of indigenous (non-available) soil P or PUE, and/or to use low grade rock phosphate (RP) as an effective source of P.

Specific plant growth-promoting bacteria (PGPB) are known to improve the PUE of indigenous and added P either by solubilizing insoluble complexes of P or by enhancing root surface area for greater P uptake through enhanced root–colloids contact and/or by releasing the phosphatases to release organically bound P (Rodriguez et al. 2006; Khan et al. 2007). Several studies have documented the solubilization of insoluble mineral phosphates by microorganisms (Vazquez et al. 2000; Gyaneshwar et al. 2006; Chen et al. 2008; Hameeda et al. 2008). It has also been reported that the bacterial strains isolated from alkaline soils solubilized phosphate even at high salt, pH and temperature regimes (Puente et al. 2004, 2009).

Rock phosphate is a cheap source of P which is used as a raw material in manufacturing phosphatic fertilizer (Bhatti and Yawara 2010). The extremely poor solubility of RP is the only constraint in its direct use as a soil amendment. A few studies have demonstrated that combined use of RP and P solubilizing bioinoculants could be a useful strategy to improve P nutrition of plants (Stamford et al. 2007; Hamdali et al. 2008). The potential of these P-solubilizing bioinoculants could be further enhanced if they are used in combination with compost (Vassilev and Vassileva 2003).

Several studies have documented that nutrient uptake by plants can be enhanced through developing more extensive root systems as a result of inoculation with PGPB carrying ACC deaminase activity (Shaharoona et al. 2008). This enzyme is known to suppress accelerated production of ethylene in growing roots, particularly when exposed to any kind of physical, biological or nutritional stress(es) (Glick 2004; Saleem et al. 2007). P deficiency has also been correlated with the production of elevated ethylene levels (Sobolewska and Plich 1986; Zhang et al. 2003). Borch et al. (1999) found that phosphorus-deficient bean roots produced twice as much ethylene per g dry weight as roots supplied with adequate P.

Our hypothesis was that PGPB possessing simultaneously both P solubilizing plus ACC deaminase activities may improve P nutrition and plant growth more effectively than those carrying either one of these two growth-promoting traits. It is one of the few studies reporting potential of PGPB possessing two vital plant growth-promoting traits concurrently for improving growth, yield and P uptake of wheat, particularly in the presence of RP as P source.

Materials and methods

Isolation and identification of bacteria

About 35 bacterial isolates were collected from rhizosphere soils of wheat and maize, using the procedure described by Khalid et al. (2004). Briefly, plants of wheat and maize were uprooted along with good amount of non-rhizosphere soil, brought immediately to the laboratory in polythene bags were air-dried within 2 h. The non-rhizosphere soil was removed by gentle shaking, leaving behind only the rhizosphere soil (strongly adhering to the roots). The rhizosphere soil was collected from roots by dipping and gentle shaking in sterilized water under aseptic conditions. The soil suspension obtained was used to inoculate tryptic soy agar (TSA), a general purpose medium (Atlas 2004). Colonies exhibiting prolific growth with different morphological characters were selected for further streaking onto fresh plates. Further purification and multiplication of the isolates was done by streaking onto fresh agar plates.

Out of 35 isolates, 6 bacteria designated as KA1, KA2, KP3, KP4, KAP5 and KAP6 were selected on the basis of their P solubilizing and ACC deaminase activities. Strains KA1 and KA2 carried predominately ACC deaminase activity while KP3 and KP4 carried P-solubilizing activity. Two strains, KAP5 and KAP6, simultaneously possessed both P-solubilizing as well as ACC deaminase activities.

To identify the bacterial strains, extracted DNA was amplified for near full-length 16S rRNA genes by PCR using 27f (5'-agagtttgatcmtggctcag-3') and 1,492r (5'-agrtaccttgttacgactt-3') primers (Operon Biotechnologies, AL, USA). Nucleotide sequences were determined by sequencing (ABI PRISM® 377 DNA Sequencer). The BioEdit (Hall 1999) software package was used to obtain the consensus sequence. Sequences of closely related type strains used for constructing the phylogenetic tree were selected and retrieved using EzTaxon Server (http://147.47.212.35:8080/) The alignment and editing was performed using CLUSTAL X (v.1.8msw; Thompson et al. 1997) and BioEdit (Hall 1999) packages. Ambiguous positions and gaps were excluded during calculations. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al. 2011).

The stability of the relationship was assessed by bootstrap analysis (Felsenstein 2005), by performing 1,000 re-sampling for the tree topology of the neighbor-joining data. The 16S rRNA gene sequences were submitted to the GeneBank/EMBL/DDBJ databases and the accession numbers of the strains KA1, KA2, KP3, KP4, KAP5 and KAP6 are AB6388886–AB638891. Results of the sequence analyses showed that all these strains belonged to the genus *Bacillus* and that each of the strains matched with previously described database accessions to the species level with 99–100% similarities (Fig. 1).

Phosphate-solubilizing activity of bacteria

Phosphorus-solubilizing activity of bacteria was determined qualitatively and quantitatively according to the method described by Nautiyal (1999). In this assay, RP [$3Ca_3(PO_4)_2CaF_2$] was used in agar medium as an insoluble inorganic form of the phosphate. Thirty-five bacterial isolates were tested qualitatively by plate assay using NBRIP medium which contained (per liter): glucose, 10 g; RP, 5 g; $MgCl_2 \cdot 6H_2O$, 5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KCl, 0.2 g; $(NH_4)_2SO_4$, 0.1 g. Each bacterial culture was placed on the agar plates with the help of a loop and then the plates were incubated at 30°C for 7 days. A clear halo formed around some of the colonies after 7 days indicated that these isolates were positive for solubilizing activity of RP. The size of halo was considered for determining the qualitative efficiency of these isolates to solubilize RP. Two experiments were performed with three repeats for each bacterial isolate.

Quantitative estimation of phosphate solubilization of the same isolates in the broth medium was carried out using Erlenmeyer flasks (150 ml) containing 20 ml of NBRIP medium inoculated in triplicate. An autoclaved uninoculated medium served as a control. The flasks were incubated for 2 days at 30°C on an incubator shaker at 180 rpm. The cultures were harvested by centrifugation at 8,000 *g* for 10 min. Phosphate in the culture supernatant was estimated using the protocol described by Ryan et al. (2001). The supernatant was treated with a single solution reagent containing ammonium molybdate and ascorbic acid. A small amount of antimony was used for color development and intensity of the color was measured by spectrophotometer at 882 nm.

ACC deaminase activity of bacteria

Quantitative measurement of ACC deaminase activity of bacteria was carried out according to modified methods of Honma and Shimomura (1978) and Penrose and Glick (2003). α-Ketobutyrate (μM) produced by this reaction was determined by comparing the absorbance at 540 nm of the sample with a standard curve of α-ketobutyrate (Sigma-Aldrich, St. Louis, MO, USA) prepared from a stock solution of 100 mM in 0.1 M Tris-HCl (pH 8.5) and stored at 4°C. Standards containing 200 μl of known concentration of α-ketobutyrate were treated with 300 μl of the 2,4-dinitrophenylhydrazine reagent (0.2% 2,4-dinitrophenylhydrazine in 2 M HCl) and the contents were vortexed and incubated at 30°C for 30 min to achieve the denaturiztion of α-ketobutyrate as a phenylhydrazone. The color of phenylhydrazone was developed by the addition of 2 ml 2 M NaOH. After mixing, absorbance of the mixture was measured at 540 nm.

Effect of plant growth-promoting bacteria on wheat

The inoculum of each selected strain was prepared in 250-ml conical flasks containing TSB. After inoculation, the flasks were incubated at 28±1°C for 48 h under shaking (100 rpm) in an orbital shaking incubator (Model OSI-503 LD; Firstek Scientific, Japan). An optical density of 0.6 at λ 590 nm of broth medium containing PGPB was achieved by dilution to maintain a uniform cell density (10^8–10^9 CFU ml^{-1}) and used for seed inoculation.

Seeds of wheat (cv. Bhakar-2002) were surface-sterilized by momentarily exposing to 95% ethanol and immersing in 0.2% $HgCl_2$ solution for 3 min. The seeds were then subjected to several washings with sterile distilled water. Surface-disinfected seeds were inoculated with the broth mixed with 10% sugar solution, peat and clay (Kaolin) mixture (peat to clay ratio, 1:1 w/w). The seeds were shaken well until a fine coating appeared on seeds. Control seeds were treated with sterilized peat plus clay mixed with sterilized broth medium (without bacterial cells) and sugar solution. Inoculated seeds were placed overnight for drying under laboratory conditions at 26°C.

Leonard jar experiment under axenic conditions

Six selected bacterial strains differing in P-solubilizing and/or ACC deaminase activity were tested for their growth-promoting activity under axenic conditions by using a modified Leonard Jars assembly containing growth medium enriched with RP as the sole source of P. Sand was sieved through a 2-mm mesh sieve, dipped in 5% HCl solution and washed thoroughly with distilled water. Glass jars filled with 500 g sand and 1.0 g RP (powdered RP containing 1.2% moisture, 33.4% P_2O_5, 4.3% SiO_2, 48.2% CaO, 2.1% MgO and 1.94% Fe_2O_3, purchased from National Fertilizer, Faisalabad, Pakistan) were autoclaved at 121°C. Pre-germinated seeds of wheat at the rate of three seeds per jar were transplanted to autoclaved glass jars. Sterilized Hoagland solution without P was applied in the jars (treated

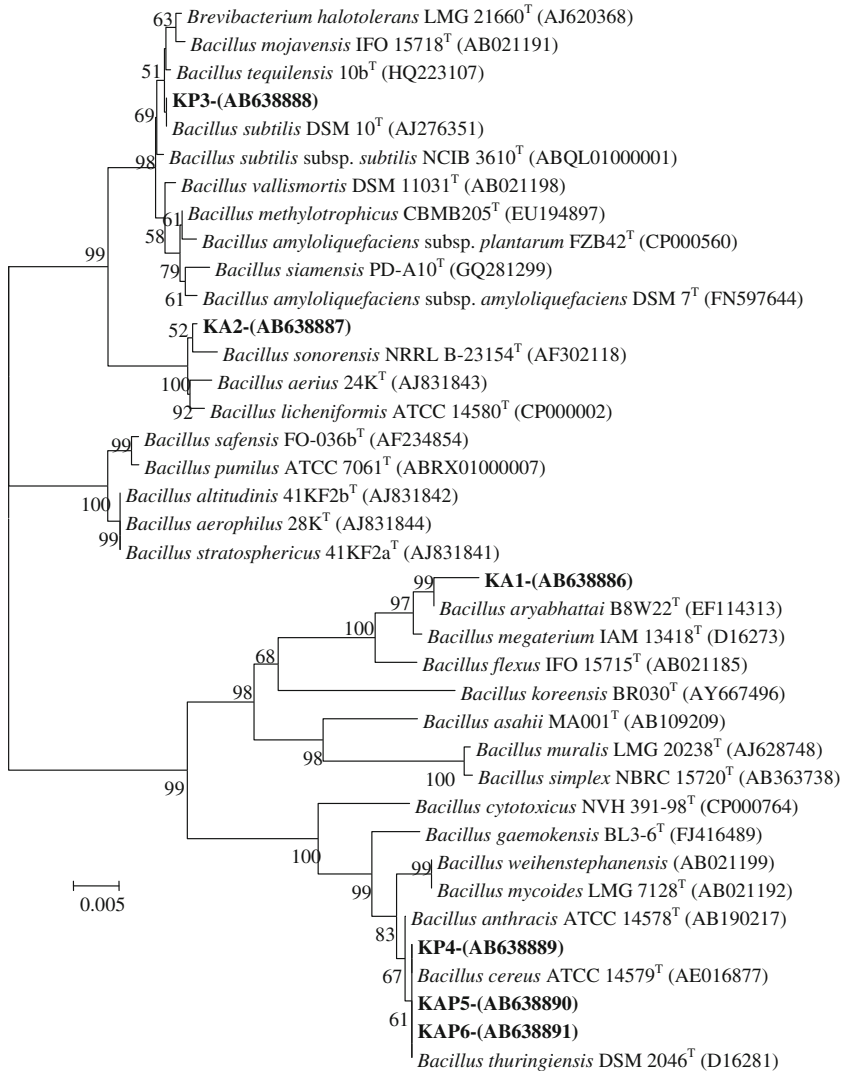

Fig. 1 Un-rooted phylogenetic tree showing inter-relationship of PGPB strains with closely related species of the genus *Bacillus* inferred from 16S rRNA sequences. The tree was generated using the Neighbor-Joining method and was constructed by MEGA-5 software. Bootstrap values (more than 50%), expressed as percentage of 1,000 replications, are indicated at the nodes. The *accession number* of each type strain is shown in *parentheses*

with RP) for providing nutrients to wheat seedlings. The jars were arranged using a completely randomized design. Each treatment was repeated three times. The selected bacterial strains were used for inoculation of seeds. Jars were placed in a growth chamber at 25±1°C ,adjusted to 12-h light (1,000 μmol photons m^{-2} s^{-1}) at relative humidity of 70%. Data regarding root and shoot growth were recorded after 3 weeks.

Pot and field trials

The pot experiment was conducted on wheat in a net house of the University of Agriculture Faisalabad, Pakistan. The inoculated seeds of wheat were sown in pots containing 12 kg soil pot^{-1} on November 22, 2009. The soil used in the study was sandy clay loam (Typic haplocambids) having pH 7.6; electrical conductivity, 2.2 dS m^{-1}; organic matter, 0.52%; total nitrogen, 0.05%; available P, 7.3 mg kg^{-1}; and extractable K, 96.0 mg kg^{-1} soil.

Six strains possessing ACC deaminase (KA1 and KA2), phosphate-solubilizing activity (KP3 and KP4) and both of these activities (KAP5 and KAP6) were used for inoculation. Phosphorus at the rate of 85 kg ha^{-1} was applied at sowing time either as RP or as diammonium phosphate (DAP, 46% P_2O_5, 18% N). The following P fertilizers were used in the pot trial: F1=P as a DAP; F2=RP; F3=RP enriched compost. Recommended doses of N and K fertilizer (at the rate of 110 and 60 kg ha^{-1}, respectively) were applied as a basal dose in all treatments. A half dose of N was applied at the time of sowing while the remaining half does was applied at first irrigation. Each treatment was repeated four times for each source of P. The pots were arranged randomly at ambient temperature and light in the net house. The temperature range was 6–30°C during wheat growth. Wheat received an equivalent of 22 cm of rainfall. The crop was irrigated with canal water when needed to maintain 60% of field capacity. The crop was harvested in the second week of April 2010. Data regarding growth and

yield parameters were collected at the time of maturity. Available phosphorous was determined by following the procedure as described by Soltanpour and Schwab (1977), whereas the protocol described by Ryan et al. (2001) was followed for the determination of P in wheat plants.

The effectiveness of six bacterial strains was also examined under field conditions at the Research Farm of the University. The experiment was conducted in a factorial randomized complete block design with four replicates, using the same treatments and agronomic practices as used in pot experiment. However, a whole dose of PK and half N was broadcast at the time of soil preparation, and remaining half N was applied at tillering. Seeds of wheat were sown in plots (4 m×2.5 m) with single row seed drill keeping row-to-row distance of 25 cm. The total area under experiment was 280 sq meters. Data regarding growth and yield parameters were collected. Phosphorus contents in grain and straw samples were determined by using the same method as described above for the pot experiment.

Statistical analysis

Data regarding the effect of inoculation with PGPB on growth of wheat seedlings under axenic conditions was analyzed by applying ANOVA, using Satatistix 8 (v.8.1, © 1985–2005). Factorial ANOVA was applied for statistical analysis of the data of pot and field trials conducted at different sources of P-nutrition under soil conditions. The means were compared by using LSD test. Relationship between P-solubilizing activity or ACC deaminase activity of the PGPB against available P in soil, P uptake in plants and dry root weight was determined by using Pearson correlation analysis (Satatistix 8).

Results

PGPB strains with ACC deaminase and P solubilizing activities

ACC deaminase activity of all the 35 isolates ranged from 0.001 to 1.85 μM. *Bacillus* strain KAP6 showed a maximum ACC deaminase activity of 1.85 μM α-ketobutyrate (Fig. 2). Three PGPB strains, KA1, KA2 and KAP5, exhibited ACC deaminase activities from 1.1 to 1.27 μM. No such activity was detected in PGPB strains KP3 and KP4.

The collected bacterial isolates exhibited P solubilizing activity in the range of 113–753 mg l^{-1}. Maximum P solubilization activity (753 mg P l^{-1}) was detected in PGPB strain KAP5 and it was followed by KAP6 (698 mg P l^{-1}), KP3 (572 mg P l^{-1}) and KP4 (498 mg P l^{-1}) (Fig. 2). Two strains, KA1 and KA2, showed negligible P-solubilization activity from RP. PGPB strains KAP5 and KAP6 showed a highly promising dual activity, i.e. ACC deaminase and P-solubilizing activity.

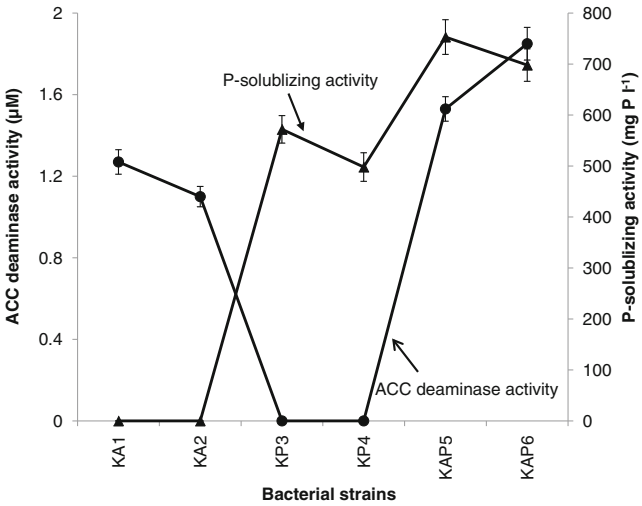

Fig. 2 Bacterial strains possessing either ACC deaminase activity (*KA1* and *KA2*) or P solubilizing activity (*KP3* and *KP4*) or both ACC deaminase and P solubilizing activities (*KAP5* and *KAP6*). *Vertical bars* represent standard error above and below the mean

Performance of selected PGPB under axenic conditions

All the six selected PGPB strains significantly increased the dry shoot weight ranging from 30 to 160% over uninoculated control (Table 1). The bacterial strains (i.e. KAP5 and KAP6) simultaneously possessing ACC deaminase and P-solubilizing activities were the most effective in promoting dry shoot weight followed by the strains with only P-solubilizing activity (KP3 and KP4). PGPB strains with only ACC deaminase activity (KA1 and KA2) were the least effective as they increased dry shoot weight up to 60% over uninoculated control.

Strains KAP5 and KAP6 were also found to be the most effective for promoting dry root weight (up to 4.1-fold) compared to the respective uninoculated control (Table 1). Among the single trait strains, KA1 and KA2 performed better than KP3 and KP4 strains in improving root dry weight. The same trend was observed in the case of root elongation of wheat seedlings. The maximum root elongation was recorded in response to inoculation with *Bacillus strain* KAP5, which was 2.2-fold greater than the respective uninoculated control.

Performance of selected PGPB in pot and field trials

The selected PGPB strains significantly increased the root growth of wheat as compared to the uninoculated control (Table 2). Effectiveness of inoculation varied at

Table 1 Relative efficacy of plant growth-promoting bacteria possessing ACC deaminase and/or phosphate-solubilizing activity for improving growth of wheat under axenic conditions

Bacillus strains[a]	Dry shoot weight (g plant^{-1})	Dry root weight (g plant^{-1})	Root elongation (cm)
Uninoculated	0.010 f[b]	0.035 f	8.2 e
KA1	0.016 d	0.062 c	12.6 b
KA2	0.013 e	0.055 cd	11.8 bc
KP3	0.019 c	0.048 de	10.7 cd
KP4	0.018 cd	0.044 e	9.9 d
KAP5	0.026 a	0.143 a	17.9 a
KAP6	0.023 b	0.127 b	17.4 a

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] Mean values sharing the same letter(s) in a column do not differ significantly at $p<0.05$, according to LSD test

each P-nutrition source. It was worth to note that root dry weight recorded in F3 (RP-enriched compost) in response to inoculation was at par or greater than that produced under F1 (DAP). PGPB strains KAP5 and KAP6 possessing dual activity showed better performance for all three P sources compared to the strains carrying a single trait. Inoculation with PGPB strain KAP5 resulted in maximum root dry weight in all three fertilizer treatments as it increased it by 2.3-, 5.5-, and 3.1-fold over the respective uninoculated control in F1, F2 and F3. Among the single trait strains, KA1 and KA2 having only ACC deaminase activity performed better in promoting root dry weight than those carrying P-solubilizing activity alone. Similarly, the effect of inoculation on root elongation of wheat grown in pots was also significant and followed the same trend as observed inthe case of root dry weight (Table 2). Strain KAP5 was once again the most effective PGPB in promoting root elongation.

Inoculation with PGPB strains also significantly increased the dry shoot weight in all the fertilizer treatments over the respective uninoculated controls (Table 2). The strains KAP5 and KAP6 with dual activity significantly increased dry shoot weight compared to single trait strains as well as uninoculated control where P was applied as DAP, RP and RP-enriched compost, respectively. The dry shoot weight in the presence of RP-enriched compost was comparable to that recorded in response to inoculation in the case of DAP. Contrary to the trend observed in case of root dry weight, the performance of the P solubilizing strains (KP3 and KP4) in terms of shoot weight production was better than the PGPB strains containing ACC deaminase (KA1 and KA2).

The selected PGPB strains with dual activity were more promising in yielding the maximum number of tillers per pot than the strains carrying either of the two activities in all the fertilizer treatments (Table 3). Maximum increase in number of tillers at F1 (DAP), F2 (RP) and F3 (RP-enriched compost) was observed in response to inoculation with KAP5, which were 57.5, 131 and 73.8% greater than their respective uninoculated control. PGPB strains with dual activity also significantly increased the number of tillers in RP-amended soil compared to uninoculated DAP-amended soil. After dual activity strains, KP3 and KP4 with P-solubilizing activity were the most effective PGPB in increasing the number of tillers.

Maximum grain yield of wheat under different P sources was observed in the case of inoculation with PGPB strains possessing dual plant growth-promoting activity (Table 3). *Bacillus* strain KAP5 with dual activity caused the highest

Table 2 Relative efficacy of plant growth-promoting bacteria possessing ACC deaminase and/or phosphate-solubilizing activity for improving dry root weight and elongation, and dry shoot weight of wheat grown in pots

Bacillus strains[a]	Dry root weight (g pot^{-1})			Root elongation (cm)			Dry shoot weight (g pot^{-1})		
	F1[b]	F2	F3	F1	F2	F3	F1	F2	F3
Uninoculated	1.3 kl[c]	0.6 m	1.2 l	8.9 ijk	3.8 l	8.0 ijk	21.1 f	14.7 h	18.6 g
KA1	2.1 gh	1.9 hij	2.8 e	13.7 fg	12.5 fgh	18.1 de	24.0 cd	22.1 ef	23.1 de
KA2	1.9 hij	1.6 jk	2.6 ef	12.2 fgh	10.6 hi	17.3 e	22.2 fg	21.3 h	21.8 gh
KP3	1.7 ij	1.1 l	2.3 fg	11.2 ghi	7.3 jk	14.9 ef	25.6 c	24.2 c	24.6 cd
KP4	1.6 jk	1.0 l	2.0 ghi	10.3 hij	6.7 kl	13.0 fg	25.2 c	23.0 de	24.5 cd
KAP5	4.3 b	3.9 c	4.9 a	28.5 ab	25.7 b	32.0 a	30.2 a	29.1 ab	30.0 a
KAP6	3.8 c	3.2 d	4.4 b	24.8 bc	21.3 cd	29.1 a	28.8 b	28.5 b	29.2 ab

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] *F1*: NPK fertilizer; *F2*: NK fertilizer + rock phosphate; *F3*: NK fertilizer + rock phosphate enriched compost

[c] Mean values sharing the same letter(s) in a parameter do not differ significantly at $p<0.05$, according to LSD test

Table 3 Relative efficacy of plant growth-promoting bacteria possessing ACC deaminase and/or phosphate-solubilizing activity for improving thenumber of tillers and grain yield of wheat grown in pots

Bacillus strains[a]	Number of tillers pot^{-1}			Grain yield (g pot^{-1})		
	F1[b]	F2	F3	F1	F2	F3
Uninoculated	17.4 ghi[c]	10.0 k	14.1 j	13.1 i	8.7 k	11.5 j
KA1	20.5 ef	17.3 ghi	16.3 hi	15.5 cdef	14.1 ghi	15.0 defg
KA2	20.3ef	16.7 hi	15.7 ij	14.6 efgh	13.5 hi	14.4 fgh
KP3	21.6 de	18.8 fg	19.0 fg	16.4 c	15.6 cdef	16.3 c
KP4	21.0 e	17.7 gh	18.0 gh	16.2 cd	14.7 efgh	15.7 cde
KAP5	27.4 a	23.1 cd	24.5 bc	19.7 a	18.7 ab	19.4 ab
KAP6	26.0 ab	22.8 cd	24.0 c	19.0 ab	18.3 b	18.7 ab

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] *F1*: NPK fertilizer; *F2*: NK fertilizer + rock phosphate; *F3*: NK fertilizer + rock phosphate enriched compost

[c] Mean values sharing the same letter(s) in a parameter do not differ significantly at $p<0.05$, according to LSD test

increase in grain yield, which was 50.4, 115.0 and 68.7% greater than the respective uninoculated control for P sources of DAP, RP and RP-enriched compost. Among the single trait strains, KP3 possessing P-solubilizing activity performed better compared to other single trait strains.

Table 4 shows data regarding the effect of inoculation on P uptake in grain and straw of wheat at different sources of P applied to the soil in pots. PGPB strain KAP5 with dual activity significantly enhanced the P uptake in wheat grain and straw compared to strains carrying single traits as well as uninoculated controls. Using DAP, RP and RP-enriched compost as the P source, the highest increase (2.1, 3.2 and 2.3-fold over the respective uninoculated controls) in P uptake in wheat grain was observed in response to inoculation with *Bacillus* strain KAP5. Inoculation with strain KP3 and KP4 having only P-solubilizing activity resulted in more P uptake than those recorded in strains carrying ACC deaminase activity only. The same trend was observed in case of P uptake by wheat straw. Up to 4.3-, 6.4- and 4.5-fold increase in P uptake by wheat straw over the respective uninoculated controls was observed in response to bacterial inoculation under F1, F2 and F3. Overall, maximum inoculation response was observed in the case of RP-amended soil compared to uninoculated control.

Under field conditions, inoculation with *Bacillus* strains also significantly increased the number of tillers and grain yield of wheat compared to uninoculated control (Table 5). Maximum number of tillers m^{-2} (204) were recorded in

Table 4 Relative efficacy of plant growth-promoting bacteria possessing ACC deaminase and/or phosphate-solubilizing activity for improving P uptake in wheat grain and straw grown in pots

Bacillus strains[a]	P uptake in grain (mg pot^{-1})			P uptake in straw (mg pot^{-1})		
	F1[b]	F2	F3	F1	F2	F3
Uninoculated	54.6 h[c]	28.9 j	43.3 i	21.4 kl	12.6 m	16.8 lm
KA1	68.5 def	59.5 gh	66.0 efg	29.4 hij	26.0 ijk	30.2 hi
KA2	63.7 fg	54.1 h	62.4 fgh	26.6 ijk	22.7 jkl	22.9 jkl
KP3	75.1 d	69.7 def	75.1 d	46.2 e	38.2 fg	41.5 ef
KP4	72.9 de	64.0 fg	70.9 def	35.0 fgh	32.6 ghi	35.1 fgh
KAP5	113 a	93.8 bc	101 b	92.6 a	81.5 b	76.3 bc
KAP6	96.1 bc	89.2 c	93.6 bc	77.4 b	60.7 d	69.5 c

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] *F1*: NPK fertilizer; *F2*: NK fertilizer + rock phosphate; *F3*: NK fertilizer + rock phosphate enriched compost

[c] Mean values sharing the same letter(s) in a parameter do not differ significantly at $p<0.05$, according to LSD test

Table 5 Relative efficacy of plant growth-promoting bacteria possessing ACC-deaminase and/or phosphate solubilizing activity for improving number of tillers and grain yield of wheat under field conditions

Bacillus strains[a]	Number of tillers m^{-2}			Grain yield (g m^{-2})		
	F1[b]	F2	F3	F1	F2	F3
Uninoculated	130 ijk[c]	87 o	104 n	360 gh	188 m	241 l
KA1	140 ghi	120 lm	128 jkl	391 ef	324 jk	340 hi
KA2	137 hij	106 n	117 m	383 f	312 k	332 ij
KP3	157 f	134 hij	143 gh	410 e	337 hij	363 g
KP4	151 fg	123 klm	131 ijk	402 e	335 ij	341 hi
KAP5	204 a	175 de	194 bc	580 a	442 d	530 bc
KAP6	200 ab	171 e	187 c	553 ab	431 d	508 c

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] *F1*: NPK fertilizer; *F2*: NK fertilizer + rock phosphate; *F3*: NK fertilizer + rock phosphate enriched compost

[c] Mean values sharing the same letter(s) in a parameter do not differ significantly at $p<0.05$, according to LSD test

response to inoculation with PGPB strain KAP5 when supplemented with DAP as a P source. This strain also produced highest number of tillers in case of RP and RP enriched compost. Next most effective strain in increasing wheat tillers was KAP6. PGPB strains with dual activity also significantly increased the number of tillers compared to single trait strains in DAP (up to 49%), RP (up to 65%) and RP plus compost (up to 65.8%) amended soils. Maximum grain yield of wheat under different P-sources was also observed in case of inoculation with PGPB strain KAP3 possessing dual plant growth-promoting activity. It was followed in descending order by *Bacillus* strain KAP6, KP3, KP4, KA1 and KA2. Similar kinds of results were obtained in case of P uptake by wheat grain and straw at different sources of P applied to the soil under field conditions (Table 6). PGPB strain KAP5 with dual activity significantly enhanced the P uptake in wheat grain and straw compared to the strains carrying single traits as well as uninoculated controls. The highest increase (4.5 fold over uninoculated controls) in P-uptake by wheat grain was observed in response to inoculation with *Bacillus* strain KAP5 using RP as the P source. The same trend was observed in case of P-uptake by wheat straw. Maximum increase up to 4.4 fold over uninoculated control was observed in response to PGPB strain KAP5 at F2 (RP). Overall, maximum inoculation response was observed in case of RP amended soil compared to uninoculated control.

There was a significant positive correlation ($R^2=0.79$–0.86) between ACC deaminase activity of the PGPB strains and root dry weight of wheat at all three P sources. The

Table 6 Relative efficacy of plant growth-promoting bacteria possessing ACC deaminase and/or phosphate solubilizing activity for improving P uptake in wheat grain and straw under field conditions

Bacillus strains[a]	P uptake in grain (g m^{-2})			P uptake in straw (g m^{-2})		
	F1[b]	F2	F3	F1	F2	F3
Uninoculated	0.94 k[c]	0.55 l	0.75 kl	0.73 jkl	0.40 m	0.54 lm
KA1	1.84 fgh	1.39	1.54 hij	1.08 ghi	0.81 ijkl	0.95 hji
KA2	1.74 fghij	1.40 j	1.41 ij	0.89 ijk	0.63 klm	0.80 ijkl
KP3	2.05 ef	1.50 hij	1.75 fghi	1.35 ef	0.95 hij	1.14 fghi
KP4	1.96 fg	1.51 hij	1.62 ghij	1.28 efg	0.94 hijk	1.07 ghi
KAP5	3.82 a	2.45 dd	3.17 bc	3.02 a	1.76 cd	2.25 b
KAP6	3.40 b	2.37 de	2.98 c	2.09 bc	1.45 de	1.88 bc

[a] *KA1* and *KA2*: ACC deaminase activity; *KP3* and *KP4*: phosphorus-solubilizing activity; *KAP5* and *KAP6*: dual activity (ACC deaminase + phosphorus-solubilizing activity)

[b] *F1*: NPK fertilizer; *F2*: NK fertilizer + rock phosphate; *F3*: NK fertilizer + rock phosphate enriched compost

[c] Mean values sharing the same letter(s) in a parameter do not differ significantly at $p<0.05$, according to LSD test

correlation (R^2=0.46–0.58) was non-significant between ACC deaminase activity of the PGPB and available P concentration in soil and P -uptake in grain. Conversely, P-solubilization activity of the PGPB strains showed a significant direct correlation with available P concentration in soil and P uptake in grains (R^2=0.80–0.90), while there was a non-significant correlation with root dry weight (R^2=0.34–0.61).

Discussion

This study compares the efficiency of bacteria carrying different traits for plant growth promotion for improving growth, yield and P uptake of wheat in the presence of different P sources. The results of this study clearly illustrated the potential of two PGPB strains, KAP5 and KAP6, carrying high P-solubilizing and ACC deaminase activities concurrently for promoting growth and yield of wheat compared to the PGPB possessing either one of these two traits. These PGPB strains belonged to genus *Bacillus* and they were able to promote root and shoot growth of wheat seedlings under axenic conditions, using RP as the source of P. They also significantly promoted yield and yield-contributing parameters of wheat under soil conditions, when P nutrition was supplied by applying DAP fertilizer, RP or RP-enriched compost. Probably, these PGPB provided double benefit to the plant:(1) by gradually releasing P from insoluble P complexes (RP) through P-solubilization activity, and (2) by improving root growth and root surface area for better uptake of P and other nutrients through ACC deaminase activity. The highest P uptake by wheat plants inoculated with dual traits PGPB (Tables 4 and 6) also supports the above premise. Further, root growth was much better in the case of plants inoculated with the dual traits PGPB than that of the single trait PGPB strains (Tables 1 and 2). This implies that the presence of two plant growth-promoting characters in a PGPB made it much superior to that of the single trait PGPB. Such PGPB could be the best candidates for the formulation of an effective inoculant. Some microbial communities have shown the ability to solubilize insoluble form of P in soil (Yadav and Dadarwal 1997; Khan et al. 2007). Similarly, PGPB are known to enhance root growth substantially because of their ACC deaminase activity which helps in regulation of accelerated synthesis of inhibitory levels of the plant hormone ethylene in roots (Glick 2005). Previously, PGPB with potential dual plant growth-promoting activities have not been compared with single trait PGPB for plant growth promotion. However, our results are in agreement with the findings of Zaidi and Khan (2005) who reported that inoculation with P-solubilizing bacteria plus mycorrhizal fungi enhanced the nutrient availability and P uptake by plants.

Comparison among the single trait PGPB showed that PGPB strains with P-solubilizing activity were more effective than PGPB with ACC deaminase activity in promoting shoot growth and yield of wheat. Contrarily, PGPB with ACC deaminase were shown to be promising in promoting root growth of wheat. A linear correlation was observed between P-solubilization activity of the *Bacillus* strains and available P concentration in soil and P uptake by wheat plants. Similar results have also been reported by other researchers that inoculation with P-solubilizing microorganisms improves growth and yield of wheat (Singh and Kapoor 1999). PGPB containing ACC deaminase have also been reported to increase root growth in several plants (Glick et al. 1998; Gosh et al. 2003; Mayak et al. 2004).

It was also observed that the tested PGPB strains significantly promoted growth and yield in all three kinds of P nutrition, i.e. DAP, RP and RP-enriched compost, compared to uninoculated controls. However, performance of the PGPB was more pronounced where RP was applied as the P source than that of DAP. Since RP is not a ready source of P, thus the applied PGPB might have released inorganic P from RP through their P-solubilizing activities, while P is readily available in the case of DAP application which diluted the impact of inoculation. Sharma and Prasad (2003) reported significantly less uptake of P with RP compared to DAP, but it increased significantly in the presence of P-solubilizing microorganisms. Barea et al. (2002) demonstrated that microbial inoculation along with RP application boosted the biomass production in addition to P accumulation in alfalfa.

Application of compost with RP had a positive impact on the efficiency of PGPB, wheat growth, yield and P uptake as compared to RP application alone. This might be due to contribution of the compost in stimulating activity of the inocula as well as indigenous microflora capable of solubilizing insoluble P (RP). Moreover, mineralization of organic material usually releases organic acids which might have further enhanced P solubilization from RP, rendering it available to the plant. Yazdani et al. (2009) found that application of composted farmyard manure along with P-solubilizing microbes significantly increased maize growth and seed corn yield.

Conclusion

This study demonstrated that the isolation of PGPB possessing dual plant growth-promoting traits such as P solubilization and ACC deaminase could be an effective strategy for the improvement of growth and yield of wheat crops and P use efficiency of phosphatic fertilizer in soil.

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