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Effects of bacterial inoculants and sources of phosphorus on yield and phosphorus uptake of wheat

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ABSTRACT

Phosphorus (P) mobilizing bacteria play an important role in the availability of soil and fertilizer P for all crops including wheat. Two greenhouse experiments were conducted to evaluate the effects of six P mobilizing bacterial strains and three P sources tricalcium phosphate {$[Ca_3(PO_4)_2]$, calcium hydrogen phosphate [$CaHPO_4.2H_2O$] and rock phosphate} on yield and P uptake of wheat. All the bacterial inoculants increased grain yield significantly over control in one greenhouse experiment while only three strains produced significantly higher grain yield over control in a second experiment. Difference among P sources were not significant in acquiring grain yield in experiment 1 while $Ca_3(PO_4)_2$ and $CaHPO_4.2H_2O$ produced significantly higher grain yield over rock phosphate in experiment 2. The differential pattern in results in two experiments might be due to difference in growth conditions. More greenhouse studies as well field experiments are recommended to confirm the beneficial effects of these P mobilizing bacterial strains on wheat.

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phosphorus mobilization; bacterial inoculants and wheat

Introduction

Soil microorganisms can have a stimulative effect on plant growth and yield. Possible causes are biological nitrogen (N_2) fixation, production of phytohormones and organic anions, exudation of protons (Illmer and Schinner, 1995), production of siderophores (Suneja et al., 1994), production of exopolysaccharides (Kaci et al., 2005) and production of mineral acids (Kapoor et al., 1991). Some of these mechanisms such as excretion of protons or organic anions could be important for phosphorus (P) mobilisation.

There are several well-established reports on the association of different types of free living bacteria with cereals, enhancing plant growth directly or indirectly. For example, *Azospirillum* spp., free-living nitrogen fixing bacteria associated with the roots of grasses have been studied for their potential in saving nitrogen fertilizer (Okon, 1982). It has been established that bacterial inoculants can improve growth and yield of wheat (Arzanesh et al., 2011; Baig et al., 2012; Turan et al., 2012). Wheat yield can be increased up to 30 per cent with *Azotobacter* inoculation and even up to 43 per cent with Bacillus inoculants (Rodrìguez and Fraga, 1999). Bacterial inoculants fertilizers will not replace mineral fertilizers, but the appropriate combinations of bacterial, mineral and organic fertilizers may enhance plant growth (Brown, 1974). Previous investigations demonstrated that inoculation with Azospirillum increased dry weight, plant height and number of spikelets per spike of wheat (Kapulnik et al., 1981).

Investigations demonstrated that certain bacteria isolated from soil can mobilise insoluble P in liquid media (Ahmed, 2008). This study was conducted as a follow up to evaluate the effect of bacterial inoculation on growth and yield of wheat using different types of insoluble P.

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Table 1. Size of 'halo' zone created on agar plate by five different bacterial strains.

	Strain				
	FA001	FA002	FA003	FA005	FA010
Halo zone diameter (mm)	18	11	8.5	11	18
Sd	± 0.5	± 1.0	± 0.5	± 1.0	± 1.0
Clarity	Very clear	Clear	Clear	Very clear	Very clear

The diameter of the 'halo' zone is the mean value calculated from three replicates.
Source: Ahmed (2008).

Materials and methods

Isolation and characterization of bacteria

P solubilizing bacteria were isolated from three wheat-growing soils. Details of the procedures for isolation and identification are available in Ahmed (2008), and not described in this paper. The strains were initially identified as P mobilisers based on the 'halo zone' created on agar plates. The bacteria were characterized by conventional (growth, morphology and biochemical reactions) method as well as using 16S rDNA technique (molecular classification). Details of the experimental findings are available in Ahmed (2008). The salient findings are included in this paper as Tables 1, 2 and 3.

Experiments to determine the effect of P-mobilizing bacteria on wheat yields

Two greenhouse experiments were carried out, one commencing in October 2003 and another commencing in June 2004.

Soil

A P-depleted soil (Alfisol) (Chan and Barchia, 2007) was collected from a fallow land of the Plant Breeding Institute of University of Sydney at Camden, New South Wales (NSW). The total amount of

Table 2. Results of conventional tests performed on five bacterial strains.

Test	FA001	FA002	FA003	FA005	FA010
Gram strain	−ve	−ve	−ve	−ve	−ve
Shape	R	R	R	R	R
Motility	+	+	+	+	+
Growth in air	+	+	+	+	+
Growth anaerobically	+	+	–	–	+
Catalase test	+	+	+	+	+
Oxidase test	–	–	+	–	–
Glucose (acid)	+	+	+	+	+
Carbohydrate (O/F/-)	F	F	O	O	F
MacConkey Medium	+ve	+ve	+ve	+ve	+ve

+ = strain positive to the test, - = strain negative to the test, R = rods, O = oxidation, F = fermentation.
Source: Ahmed (2008).

Table 3. Identification of isolated bacteria by 16S rDNA sequencing (forward and reverse).

Strain	Forward sequence	Reverse sequence
FA001	*Pantoea ananatis*	*Pantoea ananatis*
FA002	*Pantoea agglomerans*	NI
FA003	*Burkholderia* sp.	NI
FA005	*Burkholderia* sp.	*Burkholderia* sp.
FA010	*Pantoea* sp.	*Pantoea agglomerans*

NI = Not identified.

Table 4. The physico-chemical properties of soil used in the greenhouse experiment.

Soil properties analyzed	Results/inference
Total P (mg kg^{-1})	150
Total N (mg kg^{-1})	1900
%OM	5.1
Available P (mg kg^{-1})	3.9
pH	5.36
CEC (cmol c kg^{-1} soil)	5.58
Sand (%)	59.47
Silt (%)	26.22
Clay (%)	14.31
Soil texture	Sandy loam

soil collected was about 100 kg in five black plastic bags. Soil was collected from the top 15 cm of the paddock, air dried and sieved using a 3 mm mesh to remove plant debris. The soil was analysed for physico-chemical properties following some standard procedures. The physico-chemical properties of experimental soil are presented in Table 4.

Potting mixture

A composite mixture of sand, perlite and vermiculite in the ratio of 3:2:1 was prepared as potting mixture.

Wheat variety

The wheat used in these experiments was (*Triticum aestivum* v 'Dollar Bird'). This Australian hard wheat cultivar, released by the Plant Breeding Institute of University of Sydney, Camden is planted in substantial areas of southern NSW. It is an acid tolerant variety with stripe, leaf and stem rust resistance (Oliver and Allen, 1994).

Sources of phosphorus

Four different types of mineral P were used in the greenhouse experiments: a) tricalcium phosphate [$Ca_3(PO_4)_2$], b) calcium hydrogen phosphate ($CaHPO_4.2H_2O$), c) crude rock phosphate and d) super phosphate [$Ca(H_2PO_4)_2.H_2O$].

Nutrient solutions

Two nutrients solutions, Hoaglands (#2) and a modified Hoaglands (#2) were used in these experiments. All sources of P in the standard Hoagland (#2) medium (Table 5) were replaced by other chemicals to

Table 5. Composition of normal Hoagland's (#2) solution.

Chemical compounds	Mass
$(NH_4)H_2PO_4$	0.115 (g L^{-1})
H_3BO_3	2.86 (mg L^{-1})
$Ca_3(NO_3)_2.4H_20$	0.9447 (g L^{-1})
$CuSO_4.5H_20$	0.08 (mg L^{-1})
$C_{10}H_{12}O_8N_2FeNa.H_2O$ (Ferric tertrate)	3.7 (mg L^{-1})|
$MgSO_4$	0.2408 (g L^{-1})
$MnCl_2.4H_2O$	1.8 (mg L^{-1})
$H_2MoO_4.H_2O$	0.018 (mg L^{-1})
KNO_3	0.6066 (g L^{-1})
$ZnSO_4.7H_2O$	0.22 (mg L^{-1})

Table 6. Composition of modified Hoagland's (#2) solution.

Chemical compounds	Mass
NH_4NO_3	0.04 (g L^{-1})
H_3BO_3	2.86 (mg L^{-1})
$Ca_3(NO_3)_2.4H_20$	0.9284 (g L^{-1})
$CuSO_4.5H_20$	0.08 (mg L^{-1})
$C_{10}H_{12}O_8N_2FeNa.H_2O$ (Ferric tartrate)	0.0106 (g L^{-1})
$MgSO_4$	0.2408 (g L^{-1})
$MnCl_2.4H_2O$	1.8 (mg L^{-1})
$H_2MoO_4.H_2O$	0.01882 (mg L^{-1})
KNO_3	0.6066 (g L^{-1})
$ZnSO_4.7H_2O$	0.22 (mg L^{-1})

make the modified Hoagland (#2) solution (Table 6). Both solutions were made in deionised water with the pH adjusted to 7.0. Ammonium nitrate (NH_4NO_3) was used instead of ammonium sulfate [$(NH_4)_2SO_4$] as the main source of N in the modified solution because NH_4NO_3 keeps the pH of the medium in better balance as a result of anion and cation exchange throughout the nutrient uptake process.

Greenhouse temperature

The greenhouse used for these experiments was not dedicated to wheat growth and the temperature was set for a mean value of 26°C. From October 2003 to January 2004, a temperature recording device, a Hastings Data Logger, (Part No: TK-0014, Tinytalk, Chichester, UK) was used to monitor the daily temperature in the greenhouse.

Bacterial strains

P-mobilizing bacteria from Australian soils

Six P-mobilizing bacteria were used in this study. Five of them were described in a previous P mobilizing experiment (Ahmed, 2008). They were FA001, FA002, FA003, FA005, and FA010. Of these five strains FA001 and FA010 were selected as the best P-mobilizers based on creating 'halo' zones around their colonies on agar plates containing insoluble tricalcium phosphate and also mobilizing the highest amount of insoluble P from different insoluble P substrates in a liquid medium over a five day period (Ahmed, 2008). The size of the halo zone varied among the strains (Table 1). All of the bacteria were gram negative (Table 2). Results of 16S rDNA test for the classification of the bacteria are presented in Table 3. Another P-mobilizing strain JD12, isolated and identified as a P-mobilizer by the Department of Microbiology, University of Sydney (Harris et al., 2006), was included for comparison. Number of live bacterial cells varied among the strains (Table 7).

Setting up pots with soil

For these experiments plastic pots were used (height 14 cm, diameter at base 10 cm, diameter at top 13 cm) and 1.4 kg air dried soil was placed in each pot. For experiment 1, 154 pots were set up. Three

Table 7. Number of live bacterial cells (CFU mL^{-1}) in bacterial suspensions used for inoculation in the previous two experiments to examine the effects of P-mobilizers on wheat yields.

	Number of bacteria (CFU mL^{-1}) Bacterial strains					
Experiment	FA001	FA002	FA003	FA005	FA010	JD12
2003 Experiment	4.5×10^8	9.1×10^7	2.1×10^8	5.4×10^8	3.9×10^8	4.3×10^8
2004 Experiment	6.7×10^8	2.9×10^8	1.8×10^8	10.1×10^8	4.2×10^8	2.0×10^8

Source: Ahmed (2008).

Table 8. Time of seed sowing, inoculation, application of modified Hoaglands (#2) medium and date of harvest for experiments 1 and 2.

Experiment number	Time of seed sowing	1st dose of modified Hoaglands medium	Bacterial inoculation	2nd dose of modified Hoaglands medium	Date of harvest
1	3 October 2003	13 October 2003	16 October 2003	27 October 2003	6 January 2004
2	1 June 2004	11 June 2004	17 June 2004	29 June 2004	10 October 2004

P sources were used for six bacterial strains and a control. There were seven replicates for each phosphate/bacterial strain combination, making a total of 147 pots. Additional seven pots were set up containing superphosphate. A total of 154 pots were set up in October 2003. For experiment 2, 168 pots were set up. Three P sources were used for six bacterial strains and a control, with eight replicates for each phosphate/bacterial strain combination and the control. A total of 168 pots were set up in June 2004. Time of seed sowing and other practices of these experiments are shown in Table 8.

Addition of P to the soil

The standard P rate (15 kg P ha^{-1}) for wheat crops was used. The amount calculated for each pot was 9.3 mg P. For experiment 1, the amounts of various P sources added to the pots were 47 mg $Ca_3(PO_4)_2$; 52 mg $CaHPO_4.2H_2O$; 52 mg rock phosphate and 35 mg superphosphate (SP). Forty-nine pots were set up for each of the three P sources $Ca_3(PO_4)_2$, $CaHPO_4.2H_2O$ and rock phosphate, and the P was mixed well with the soil. Seven pots were set up with SP that was mixed well with the soil. For experiment 2 the same quantities of $Ca_3(PO_4)_2$, $CaHPO_4.2H_2O$, and rock phosphate were added to three sets of 56 pots and mixed with the soil.

Seed sterilisation, sowing and thinning

Seed samples were sterilized using mercuric chloride ($HgCl_2$, 0.5 per cent) to remove or kill seed-borne plant pathogens, using safety measures (such as gloves and mask) as $HgCl_2$ is a hazardous chemical. Wheat seed samples (50 g) were placed on cloth gauze (approximately 12 cm × 12 cm) and the four corners were brought together and fastened with an elastic band. Subsequently the seed packages were placed in a Buchner flask with two drops of Tween-1 detergent, and then the seeds were rinsed seven to eight times with deionised water until there were no detergent suds remaining. The seeds were then soaked in $HgCl_2$ for 75 seconds and then rinsed using sterile deionised water approximately seven to eight times to remove all traces of $HgCl_2$. The seeds were removed carefully from the packet in the laminar flow cabinet and allowed to air dry. They were stored in covered petri dishes until required. Soil was soaked with 200 mL tap water per pot just prior to seed sowing. Three sterilized seeds were sown in each pot. After 15 days (10 days after germination) the germinated seedlings were thinned and in each pot the healthiest plant was retained.

Inoculation with bacterial strains

The bacterial strains used for these experiments were FA001, FA002, FA003, FA005, FA010 and JD12. A control (not inoculated) treatment for each type of P was in both the experiments, and a positive control treatment containing superphosphate not inoculated was included in experiment 1 only. A 2 mL bacterial suspension was inoculated in each pot in both experiments around the root zone of the wheat plant 13 days after seed sowing when root systems had developed. The estimated number of live bacterial cells added to the pots is shown in Table 7.

Watering and nutrient supplying

For experiments 1 and 2, normal tap water was applied for irrigation when necessary throughout the experimental time. The first dose of modified Hoagland's (#2) solution was applied 10 days after

sowing in both experiments 1 and 2. The second dose of modified Hoagland's (#2) solution was applied 24 days after sowing for experiment 1 and 28 days after sowing for experiment 2.

Pest control

In both experiments, some aphids were found on the plants, about a month after germination. In experiment 1, oyster oil was used for minimising the aphids' effects 33 days after seed sowing. In experiment 2 the insecticide malathion was applied 35 days after seed sowing.

Plant parameters measured at harvest

Plant height was measured and grain and straw were harvested 95 days after seed sowing in experiment 1 and 132 days after seed sowing in experiment 2. The number of seeds in each plant, seed dry weight and straw dry weight were measured and samples were stored in zip lock plastic bags at 4°C.

Plant phosphorus uptake

Plant material digestion

The grain and straw were dried at 70°C, and ground using a grinder (Restsch, Model: ZM1 35306, Selbys Scientific Ltd., Mulgrave, Australia). One gram samples were transferred into conical flasks and 25 mL concentrated nitric acid (HNO_3) was added in a fume cupboard. Preliminary digestion was carried out for about 20 minutes at about 120°C. After completing the preliminary digestion the sample was cooled and 5 mL of 1:1 perchloric acid/nitric acid ($HClO_4/HNO_3$) mixture was added to the flask. The mixture was reheated at 160°C until the vigorous reaction between the $HClO_4$ and the organic residue was completed. The temperature was than raised to 180°C for about 10 to 15 minutes to complete the digestion (secondary digestion). The contents of the flask were transferred to a volumetric flask and the volume of the plant digest was made to 25 mL with distilled water.

Phosphorus assay

Vanadate reagent was prepared by dissolving 0.25 g of ammonium metavanadate (NH_4VO_3) in 50 mL boiling deionised water (H_2O) to which 43 mL 70 per cent $HClO_4$ was added. The total volume was made to 1 L. Molybdate reagent was prepared by dissolving 12.5 g of $(NH_4)_6(Mo_7)_2.4H_2O$ in 1 L of deionised water. For assaying P, 1 mL samples were placed in 10 mL plastic containers to which 5 mL of the vanadate reagent was added and mixed. Then 4 mL of the molybdate reagent was added to a total of 10 mL. The absorbance was measured at 460 nm using 1 cm cells in a spectrophotometer (Model 80-2088-64; Pharmacia Biotech, Piscataway, NJ, USA). A standard curve was developed using 0 to 500 μg P mL^{-1} in 1:10 $HClO_4$/deionised water.

Data analysis

Data were analysed using statistical software Genstat (ver 7.0); using two ways analysis of variance (ANOVA) and ANOVA values were used for interpretation (Payne et al., 2003).

Results

Grain yield

In experiment 1, individual effect of P source as well as interaction effect of P source and strain was not significant (Table 9). All bacterial inoculation treatments as well as super phosphate (control SP) increased grain yield significantly over control. Wheat inoculated with the strains FA001 and FA010

Table 9. Effects of strain and source of P on grain yield (g pot^{-1}) of wheat in experiment 1.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	0.324	0.312	0.322	0.321 d
FA001	0.489	0.549	0.442	0.493 a
FA002	0.398	0.485	0.403	0.429 b
FA003	0.386	0.410	0.332	0.376 c
FA005	0.406	0.311	0.359	0.359 c
FA010	0.488	ND	0.511	0.500 a
JD12	0.407	0.373	0.392	0.391 bc
Control (SP)	0.537	0.537	0.537	0.537 a
Mean	0.430	0.425	0.412	

Two control sets of wheat were grown. One contained SP (super phosphate) but no bacteria, and no $Ca_3(PO_4)_2$, $CaHPO_4.2H_2O$ or rock phosphate. One contained $Ca_3(PO_4)_2$, $CaHPO_4.2H_2O$ or rock phosphate and no bacteria.

F probability for strains, P-types, and strains x P-types interaction were <0.001, 0.171, and 0.059, respectively. The LSD value for strains was 0.044 at 5% level of probability.

Means followed by a common small letter in a column are not significantly different at 0.05 level by least significance difference (LSD). ND = not determined

produced significantly higher grain yields over all the other strains, but not significantly different from plants grown with SP. In experiment 2, the interaction effect of P sources and inoculation was not significant while individual effects of P source and inoculation were significant (Table 10). Grain yield was significantly higher with $CaHPO_4.2H_2O$ and $Ca_3(PO_4)_2$ treated pots compared to rock phosphate treated ones. Pots inoculated with FA001 produced significantly higher grain yields over all the other strains except FA010. FA010 was statistically similar to FA001 and JD12 but superior to other strains.

Straw yield

Individual effect of P source as well as interaction effect of P source and strain was not significant in both the experiments (Tables 11 and 12). In both the experiments, FA001 and FA010 produced significantly higher straw yield over all other inoculants as well as control confirming their superior performance as shown in grain yield. In experiment 1, SP was significantly superior at all inoculants. All the inoculants except FA005 produced significantly higher straw yield over control in experiment 1 while only FA001 and FA010 were superior to control in experiment 2.

Plant height

The interaction effect of P source and inoculation was not significant on plant height in both the experiments (Tables 13 and 14). In experiment 1, $CaHPO_4.2H_2O$ and $Ca_3(PO_4)_2$ treated plants were

Table 10. Effects of strain and source of P on grain yield (g pot^{-1}) of wheat in experiment 2.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	0.394	0.385	0.380	0.386 c
FA001	0.509	0.563	0.507	0.526 a
FA002	0.413	0.422	0.408	0.414 c
FA003	0.401	0.448	0.408	0.419 c
FA005	0.400	0.466	0.407	0.424 c
FA010	0.524	0.528	0.455	0.502 ab
JD12	0.527	0.478	0.404	0.470 b
Mean	0.453 A	0.470 A	0.424 B	

A control set of wheat was grown for each type of phosphate containing no added bacteria.

F probabilities for strains, P-types and strains x P-types interaction were <0.001; 0.003 and 1.27, respectively. The LSD values for strains and P-type were 0.043 and 0.028, receptively at 5% level of probability.

Means followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.

Table 11. Effects of strain and source of P on straw yield (g pot^{-1}) of wheat in experiment 1.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	0.446	0.446	0.426	0.439 e
FA001	0.613	0.681	0.560	0.618 b
FA002	0.519	0.624	0.520	0.554 cd
FA003	0.512	0.528	0.441	0.494 d
FA005	0.529	0.418	0.470	0.473 de
FA010	0.618	ND	0.630	0.624 b
JD12	0.524	0.484	0.518	0.509 d
Control (SP)	0.713	0.713	0.713	0.713 a
Mean	0.559	0.556	0.535	

F probabilities for strains, P-types and strains x P-types interaction were <0.001, 0.108 and 0.075, respectively. The LSD value for strains was 0.051 at 5% level of probability.
Means followed by a common small letter in a column are not significantly different at 0.05 level by LSD. ND = not determined.

significantly taller than rock phosphate treated ones while there was no significant difference among the P sources in experiment 2. In experiment 1, SP produced the tallest plants followed by FA010 and FA001. All inoculants produced significantly taller plants compared to the control. In experiment 2, FA001 and FA010 gave significantly taller plants compared to all other inoculants and control.

Number of grain per spike

The interaction effect of P source and inoculation on number grain per spike was not significant in both the experiments (Tables 15 and 16). In experiment 1, there was no significant difference among the P sources while in experiment 2, $CaHPO_4.2H_2O$ and rock phosphate produced significantly higher number of grains compared to $Ca_3(PO_4)_2$. In experiment 1, FA010 and SP were superior to control and other inoculants while FA010 and JD12 were superior to control only. In experiment 2, FA001 and FA010 produced significantly higher number of grains over control and other inoculants while JD12 was superior to control only.

Phosphorus content and uptake by grain

The interaction effect of strain and P source was significant on P content (%) in grain in both the experiments (Tables 17 and 18) which was reflected in P uptake (mg pot^{-1}) by grain (Tables 19 and 20). In experiment 1, P uptake by grain was statistically similar among P sources in control, SP and FA010 treated plants while there were significant differences among the P sources in other inoculation

Table 12. Effects of strain and source of P on straw yield (g pot^{-1}) of wheat in experiment 2.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	0.522	0.512	0.520	0.518 b
FA001	0.585	0.645	0.692	0.641 a
FA002	0.541	0.570	0.514	0.552 b
FA003	0.515	0.590	0.527	0.544 b
FA005	0.509	0.600	0.529	0.546 b
FA010	0.617	0.623	0.615	0.618 a
JD12	0.599	0.556	0.517	0.554 b
Mean	0.555	0.585	0.559	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; 0.392 and 1.41, respectively. The LSD value for strains was 0.061 at 5% level of probability.
Means followed by a common small letter in a column are not significantly different at 0.05 level by LSD.

Table 13. Effects of strain and source of P on plant height (cm) of wheat in experiment 1.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	47.93	46.75	44.07	46.25 d
FA001	55.29	62.47	51.46	56.41 bc
FA002	52.57	53.86	50.18	52.20 c
FA003	49.51	56.07	49.29	51.62 c
FA005	52.79	51.89	49.86	51.51 c
FA010	58.42	ND	58.86	58.64 b
JD12	54.11	52.92	55.50	54.17 c
Control (SP)	65.33	65.33	65.33	65.33 a
Mean	54.49 A	55.61 A	53.07 B	

F probabilities for strains, P-types, and strains x P-types interaction were <0.001, 0.014 and 0.256, respectively. The LSD values for stains and P-types were 3.53, and 2.16, receptively at 5% level of probability.
Means followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD. ND = not determined.

treatments (Table 19). Inoculation in general increased P uptake significantly over control. With $Ca_3(PO_4)_2$ and rock phosphate, P uptake with SP was significantly higher over all other treatments while FA001 and FA003 were statistically similar to SP with $CaHPO_4.2H_2O$. FA001 was significantly superior to all other strains except FA010 with $Ca_3(PO_4)_2$ and rock phosphate while it was similar to FA003 with $CaHPO_4.2H_2O$. In experiment 2, P uptake by grain was significantly higher with $CaHPO_4.2H_2O$ compared to the other two P sources in control while the highest P uptake among the P sources was observed with $Ca_3(PO_4)_2$ by JD12 inoculation (Table 20). FA001 and FA010 were consistently superior to control irrespective of P sources while P uptake pattern by other inoculants varied among P sources.

Phosphorus content and uptake by straw

The interaction effect of strain and P source was significant on P content (%) in straw in both the experiments (Tables 21 and 22) which was reflected in P uptake (mg pot^{-1}) by straw (Tables 23 and 24). In experiment 1, P uptake by straw was significantly different among P sources in FA002 and FA003 treated plants while the differences were not significant among the P sources in control, SP and other inoculation treatments (Table 23). FA001 and FA010 were consistently superior to control irrespective of P sources while P uptake pattern by other inoculants varied among P sources. In experiment 2, there was no significant difference among P sources regarding P uptake by straw with FA005 inoculation while there were significant differences

Table 14. Effects of strain and source of P Plant height (cm) of wheat in experiment 2.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	51.38	51.14	50.31	50.94 c
FA001	59.31	57.00	57.31	57.88 a
FA002	53.33	52.79	53.81	53.31 bc
FA003	51.81	54.71	52.64	53.06 bc
FA005	53.88	54.00	53.38	53.75 b
FA010	56.61	60.92	53.56	57.03 a
JD12	52.06	52.29	52.29	52.21 bc
Mean	54.05	54.69	53.33	

F probabilities for strains, P-types and strains x P-types interaction were <0.001, 0.312 and 0.554, respectively. The LSD value for strains was 2.69 at 5% level of probability.
Means followed by a common small letter in a column are not significantly different at 0.05 level by LSD.

Table 15. Effects of strain and source of P on number of grain per spike in experiment 1.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	14.57	11.43	11.29	12.43 c
FA001	17.29	20.43	16.86	18.19 a
FA002	15.43	16.14	12.71	14.76 bc
FA003	15.86	14.00	13.21	14.36 bc
FA005	15.29	13.57	12.71	13.86 bc
FA010	17.67	ND	16.74	17.21 b
JD12	15.00	16.00	15.86	15.62 b
Control (SP)	20.07	20.07	20.07	20.07 a
Mean	16.40	15.95	14.93	

F probabilities for strains, P-types, and strains x P-types interaction were <0.001, 0.102 and 0.763, respectively. The LSD value for strain was 2.36 at 5% level of probability.
Means followed by a common small letter in a column is not significantly different at 0.05 level by LSD. ND = not determined.

Table 16. Effects of strain and source of P on number of grain per spike in experiment 2.

	Source of P			
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean
Control	14.00	14.14	13.75	13.96 c
FA001	18.12	17.96	18.62	18.24 a
FA002	14.19	16.71	15.25	15.38 bc
FA003	14.88	16.00	16.00	15.62 bc
FA005	13.62	14.57	16.62	14.94 bc
FA010	16.56	21.33	18.25	18.72 a
JD12	14.19	17.33	16.29	15.97 b
Mean	15.08 B	16.88 A	16.40 A	

F probabilities for strains, P-types and strains x P-types interaction were <0.001, 0.015 and 0.102, respectively.
The LSD values for strains and P-type were 1.91 and 1.25, respectively at 5% level of probability.
Means followed by a common small letter in a column and a common capital letter in a row are not significantly different at P = 0.05 level by LSD.

among the P sources in control and other inoculants (Table 24). P uptake by straw was significantly higher with FA001 over control with $CaHPO_4.2H_2O$ and rock phosphate while it was statistically similar to control with $Ca_3(PO_4)_2$ although there was a slight difference. FA005 and FA010 were significantly superior to control with rock phosphate while the opposite results were noticed with $CaHPO_4.2H_2O$.

Table 17. Effects of strain and source of P on P content (%) in wheat grain in experiment 1.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	0.558aA	0.559bA	0.541bA
FA001	0.530bB	0.581bA	0.584aA
FA002	0.470cB	0.541bA	0.585aA
FA003	0.587aB	0.588aA	0.619aB
FA005	0.606aA	0.582bA	0.606aA
FA010	0.550bA	ND	0.522bA
JD12	0.524bB	0.544bA	0.583aA
Control (SP)	0.570aA	0.570bA	0.570aA

F probability for strains x P-types interaction was <0.001 with LSD value of 0.053 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD. ND = not determined.

Table 18. Effects of strain and source of P on P content (%) in wheat grain in experiment 2.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	0.494 bB	0.585 aA	0.451 cB
FA001	0.519 bA	0.525b cA	0.505 bA
FA002	0.477 cB	0.542 bA	0.522 bA
FA003	0.486 bB	0.548 bAB	0.569 aA
FA005	0.491 bB	0.492 cB	0.569 aA
FA010	0.465 cB	0.590 aA	0.522 bB
JD12	0.564 aA	0.553 bA	0.476 cB

F probability for strains x P-types interaction was <0.001 with LSD value of 0.036 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.

Table 19. Effects of strain and source of P on P uptake (mg pot^{-1}) by wheat grain in experiment 1.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	1.809 eA	1.744 dA	1.743 eA
FA001	2.593 bB	3.188 aA	2.580 bB
FA002	1.872 eC	2.624 bA	2.359 cB
FA003	2.267 cB	2.411 aA	2.054 dC
FA005	2.459 cA	1.810 dC	2.175 cB
FA010	2.685 bA	ND	2.668 bA
JD12	2.132 dA	2.028 cB	2.283 cA
Control (SP)	3.061 aA	3.061 aA	3.061 aA

F probability for strains x P-types interaction was <0.001 with LSD value of 0.193 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.ND = not determined.

Discussion

In both experiments 1 and 2, for all P supplies grain yields with inoculations were higher than the yields in the control pots, suggesting that P for plant growth could be obtained by bacterial breakdown of insoluble P. This result suggests that P has been dissolved from insoluble P by bacteria and is consistent with other studies indicating increased yields as a result of P-mobilizing bacteria (Brown, 1974; Baldani et al., 1987; Rodrìguez and Fraga, 1999; Rudresh et al., 2005). In both experiments 1 and 2,

Table 20. Effects of strain and source of P on P uptake (mg pot^{-1}) by wheat grain in experiment 2.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	1.947 dB	2.251 dA	1.713 dC
FA001	2.639 bB	2.957 aA	2.560 aB
FA002	1.968 dB	2.288 cA	2.128 cA
FA003	1.948 dB	2.454 cA	2.321 bA
FA005	1.964 dB	2.291 dA	2.316 bA
FA010	2.437 cB	3.115 aA	2.373 bB
JD12	2.973 aA	2.643 bB	1.921d C

F probability for strains x P-types interaction was <0.001with LSD value of 0.169 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.

Table 21. Effects of strain and source of P on P content (%) in wheat straw in experiment 1.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	0.210aA	0.233bA	0.222aA
FA001	0.206aB	0.291aA	0.223aB
FA002	0.166bC	0.297aA	0.217aB
FA003	0.211aB	0.335aA	0.214aB
FA005	0.201aB	0.255bA	0.219aA
FA010	0.205aA	ND	0.227aA
JD12	0.215aA	0.212bA	0.238aA
Control (SP)	0.248aA	0.248bA	0.248aA

F probability for strains x P-types interaction was <0.001 with LSD value of 0.048 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD. ND = not determined.

Table 22. Effects of strain and source of P on P content (%) in wheat straw in experiment 2.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	0.224 bB	0.383 aA	0.214 bB
FA001	0.238 bB	0.350 aA	0.227 aB
FA002	0.208 bB	0.317 bA	0.225 aB
FA003	0.289 aA	0.285 bA	0.229 aB
FA005	0.285 abA	0.272 bA	0.272 aA
FA010	0.219 bA	0.262 cA	0.264 aA
JD12	0.231 bB	0.279 bA	0.229 aB

F probability for strains x P-types interaction was <0.001with LSD value of 0.047 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.

grain yields were better in pots inoculated with the bacterial strains FA001 and FA010 than other strains. The strains FA001 and FA010, together with several other bacteria, have been isolated from three soils as potential P-mobilizers (Ahmed, 2008).

The diameters of the halo zone in these two bacteria were higher than other bacteria included in this study (Table 1). Both bacteria were gram negative (Table 2), belong to the genus *Pantoea* as identified by the 16S rDNA technique (Table 3). They were identified as potentially useful P-mobilizing bacteria on the basis of "halo" formation on agar plates containing insoluble P-compounds. Studies of several bacteria in liquid cultures containing a range of insoluble P showed that the strains FA001 and FA010

Table 23. Effects of strain and source of P on P uptake (mg pot^{-1}) by wheat straw in experiment 1.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	0.932cA	1.041bA	0.946cA
FA001	1.261bA	1.978aA	1.247bA
FA002	0.862cC	1.852aA	1.128cB
FA003	1.079bB	1.770aA	0.942cB
FA005	1.089bA	1.066bA	1.029cA
FA010	1.267bA	ND	1.428bA
JD12	1.127bA	1.025bA	1.231bA
Control (SP)	1.768aA	1.768aA	1.768aA

F probability for strains x P-types interaction was <0.001 with LSD value of 0.225 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD. ND = not determined.

Table 24. Effects of strain and source of P on P uptake (mg pot^{-1}) by wheat straw in experiment 2.

	Source of P		
Strain	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate
Control	1.167 bB	1.959 bA	1.114 bB
FA001	1.391 abB	2.256 aA	1.568 aB
FA002	1.124 bB	1.806 bcA	1.157 bB
FA003	1.490 aA	1.682 cA	1.205 bB
FA005	1.448 aA	1.632 cA	1.439 aA
FA010	1.352 abB	1.629 cA	1.625 aA
JD12	1.382 abAB	1.548 cA	1.184 bB

F probability for strains x P-types interaction was <0.001 with LSD value of 0.267 at 5% level of probability.
Values followed by a common small letter in a column and a common capital letter in a row are not significantly different at 0.05 level by LSD.

were the best P-mobilizers (Ahmed, 2008). There was no significant difference in grain yield among the P sources in experiment 1 (Table 9). In experiment 2, $Ca_3(PO_4)_2$ and $CaHPO_4.2H_2O$ produced significantly higher grain yield than rock phosphate (Table 10). In both experiments, with all treatments, grain yields were low in comparison with yields reported by Harris et al. (2006). Reasons for this may include poor quality sandy soil and an nitrogen (N) supply to the plants that was not optimized. In addition, even in experiment 2 (the winter experiment) the greenhouse temperatures were not optimized for wheat growth possibly leading to some stress on the plants. In experiment 1 the positive control using superphosphate did not produce a significantly higher grain yield than the pots containing the FA001 and FA010 strains, suggesting that P availability was not the reason for the low yields.

The greenhouse temperature was not fully controlled in this summer experiment. It fluctuated throughout the experimental period, and was not suitable for growing wheat plants. Mean maximum and minimum temperatures from October 2003 to January 2004 recorded in the greenhouse are presented in Table 25. In experiment 1, superphosphate (SP) was included without bacteria as a positive control. Experiment 2 was carried out in winter from June 2004 to October 2004. The greenhouse temperature was not monitored as it was known to be suitable for wheat. It is likely that conditions were better for wheat than in the October to January period as external temperatures at this time of the year are lower (Table 26).

In experiment 2 all measured parameters, grain yield, straw yield, plant height and number of grain per spike were higher for pots containing strains FA001 and FA010 than for the control and all other bacterial strains. It has been reported that plant growth and yield can be increased by P-mobilizing bacteria (Gaind and Gaur, 1991; Glick et al., 1995). Plant height is an important component of plant growth and P is one of the essential elements for plant growth and development (Giand and Gaur, 1991). In experiment 2, it was found that pots inoculated with all the bacterial strains produced higher plant height than the control and those with strains FA001 and FA010 had significantly greater height than the others. The nutrients provided in experiment 2 included insoluble P and it is assumed that the P was solubilized by the inoculated bacteria. In experiment 2 pots containing $Ca_3(PO_4)_2$ and $CaHPO_4.2H_2O$ produced significantly greater grain yield than those containing rock phosphate. It has been suggested that P is mobilized easily from $CaHPO_4.2H_2O$ and can supply more P than the other two sources of P (Whitelaw et al., 1999).

The results from experiment 1 which included wheat grown with SP were similar to these for experiment 2. The growth conditions were less favorable in experiment 1 and then may have been

Table 25. Mean maximum and minimum temperatures in the greenhouse from October 2003 to January 2004.

Temperature (°C)	October 2003	November 2003	December 2003	January 2004
Maximum	26.3	31.6	29.8	30.8
Minimum	20.6	21.3	21.8	22.6

Table 26. Mean maximum and minimum temperatures in 2004 in Sydney.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean max. °C	26.4	26.3	25.2	22.9	20.0	17.5	16.9	18.2	20.4	22.4	24.0	25.7
Mean min. °C	18.7	19.0	17.4	14.1	10.9	8.5	7.0	8.0	10.3	13.1	15.2	17.4

Source: http://www.bom.gov.au/climate/averages/tables/cw_066037.shtml (Accessed 23 August 2007)

responsible for the seasonal variation between these two experiments. Grain yields were similar in general to those in experiment 2 and there was no significant difference between yields with SP and from pots inoculated with FA001 and FA010. These results show that P mobilized from any of the three insoluble P sources supplied could be as useful for plant growth as SP.

The bacterial strains FA001 and FA010 were associated with increased wheat grain yield in P stress conditions (mineral phosphate) as shown in experiments 1 and 2 (Tables 9 and 10). These results indicate that the bacteria FA001 and FA010 have a PGPR effect as well as the ability to mobilize P from insoluble sources. It is well established that phosphate-mobilizing bacteria can demonstrate PGPR effects (Windham et al., 1986; Alagawadi and Gaur, 1998; Altmare et al., 1999; Narula et al., 2000; Rudresh et al., 2005).

Temperature control in the greenhouse was poor and was not ideal for wheat growth. Still positive effects of inoculation were observed. In fact it is not essential to have constant temperature to show PGP effects as temperature variation is normal in nature. The temperature range in experiment 1 (Table 25) was not favorable for wheat growth while the outside temperature during conducting experiment 2 (Table 26) was favorable for wheat growth. But, positive effects of inoculation were noticed in both the experiments. These results suggest that P mobilizing bacteria can benefit wheat crop even in unfavorable condition. This temperature factor should be considered in assessing the positive results of inoculation in several temperature regimes in the future studies.

The available P content in the experimental soil was only 3.9 mg kg^{-1} (Table 4). It is worthwhile to conduct similar greenhouse studies using the strains FA001 and FA010 in several soils having different level of available P content. Field experiments should also be conducted to confirm the results.

Conclusions

All the selected P-mobilizing bacteria increased the grain yield of wheat significantly in a greenhouse experiment (experiment 1) in 2003 and there were some increased yields in experiment 2 in 2004 as well. It can be assumed that these bacteria mobilized insoluble mineral P thereby resulting in increased yield. In both experiments, the bacteria FA001 and FA010 had the greatest influence on plant growth and yield. In general, the results in terms of grain yield were consistent with those obtained for P mobilization in vitro in previous experiments (Ahmed, 2008). Thus the hypothesis that the approach used in the isolation of P mobilizing strains could provide useful soluble P for plant growth was supported by this data. More greenhouse experiments may help to clarify the role of these bacteria in using insoluble P. The use of a different soil and better overall nutrient supply, should be considered, as the grain yields in experiments 1 and 2 were low. There should also be experimental tests to confirm these results under field conditions.

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