CHAPTER 4 INCREASING YIELD OF WHEAT USING PHOSPHORUS MOBILISING BACTERIAL STRAINS IN GLASSHOUSE EXPERIMENTS

4.1 Introduction

Soil microorganisms can have a stimulative effect on plant growth and yield. Possible causes are N_2 fixation, production of phytohormones, plant growth promoting effect from bacteria and fungi, production of organic anions (Mishra and Banger, 1985), exudation of protons (Illmer and Schinner, 1995), production of siderophores (Bossier *et al.*, 1988; Suneja *et al.*, 1994), production of exopolysaccharides (Milas and Rinaudo, 1979; Kaci *et al.*, 2005), production of fructosyl polymers (Gouzou *et al.*, 1993; Bezzate *et al.*, 2000) and of mineral acids (Kapoor *et al.*, 1991). Some of these mechanisms such as excretion of protons or organic anions could be important for 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 fertiliser (Neyra and Dobereiner, 1977; van Berkum and Bohlool, 1980; Okon, 1982; Roesch *et al.*, 2005).

It has been established that bacterial inoculants can improve yields in a wide range of crop plants. The following yield increases are some examples of such results with cereals: maize, five to eight per cent (Helmeczi, 1962; Mishustin, 1966), spring and winter wheat eight to16 per cent (Rakhno and Ryys, 1963; Mishustin, 1966) and rice 24 to 42 per cent (Shende and Kokorina, 1964; Nguyen *et al.*, 2002; Nguyen *et al.*, 2003). Recently Rodrìguez and Fraga (1999) reported in a review paper that wheat yield increased up to 30 per cent with *Azotobacter* inoculation and up to 43 per cent with *Bacillus* inoculants, while a 10 to 20 per cent yield increase in the same crop was reported in field experiments using a combination of *Bacillus megaterium* and *Azotobacter chroococcum*.

Brown (1974) predicted that bacterial fertilizers will not replace mineral fertilizers, but the appropriate combinations of bacterial, mineral and organic fertilizers may enhance plant growth more than any one treatment alone.

In glasshouse experiments, inoculation with *Azospirillum* increased dry weight, plant height and number of spikelets per spike of wheat (Kapulnik *et al.*, 1981). It was found that the inoculation of corn (*Zea mays*) seeds with *A. brasilense* significantly enhanced the uptake of NO_3^-, K^+, and HPO_4^{-2} (Lin *et al.*, 1983).

The results discussed in Chapter 3 demonstrated that certain bacteria isolated from soil can mobilise insoluble P in liquid media. As the overall aim of this project is to demonstrate biofertiliser effects, it is important to find out whether the isolated bacteria (2.5) can mobilise insoluble P to a form available to plants, thus increasing crop yield. The objectives of this Chapter were to measure 1) the influence of bacteria on plant growth and yield in the glasshouse using wheat as a test crop, 2) to compare strains for their capacity for P mobilisation, 3) to compare strains for differential mobilisation using different types of insoluble P and 4) to examine the Plant Growth Promoting Rhizobacterial (PGPR) activities of P-mobilising bacteria for wheat.

4.2 Materials and methods

4.2.1 Materials

4.2.1.1 Soil

A P-depleted soil duplex soil (Alfisol) (Chan and Barchia, 2007) was collected from fallow land from the Plant Breeding Institute of the University of Sydney at Camden, NSW. The total amount of soil collected was about 100 kg in 5 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.

4.2.1.2 Potting mix

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

4.2.1.3 Plant 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 the

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).

4.2.1.4 Sources of phosphorus

Four different types of mineral P were used in the glasshouse 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 ($Ca(H_2PO_4)_2.H_2O$).

4.2.1.5 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 4.1, Stefaniak, 2003) were replaced by other chemicals to make the modified Hoagland (#2) solution (Table 4.2).

Both solutions were made in deionised water with the pH adjusted to 7.0. NH_4NO_3 was used instead of $(NH_4)_2SO_4$ 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.

Table 4.1 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 4.2 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 Tertrate)	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})

4.2.1.6 Glass house temperature

The glasshouse 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 Loggers, (Tinytalk; Part No: TK-0014) was used to monitor the daily temperature in the glasshouse.

4.2.1.7 Bacterial strains

4.2.1.7.1 P-mobilising bacteria from Australian soils

Six P-mobilising bacteria were used in this study. Five of them were described in Chapter 2 (2.5). They were FA001, FA002, FA003, FA005, and FA010. Of these five strains FA001 and FA010 were selected as the best P-mobilisers based on creating 'halo' zones around their colonies on agar plates containing insoluble tricalcium phosphate (2.3.3) and also mobilising the highest amount of insoluble P from different insoluble P substrates in a liquid medium over a five day period (3.3.1; 3.3.3; 3.3.4). The other three strains (described in Chapter 2) were randomly selected. Another P-mobilising strain JD12, isolated and identified as a P-mobiliser by the Department of Microbiology, the University of Sydney (Harris *et al.*, 2006), was included for comparison.

4.2.1.7.2 Other bacteria

Eleven bacteria held in the University of Sydney culture collection were used to study Plant Growth Promoting Rhizobacterial (PGPR) activity. They were *Pseudomonas*

fluorescens, Citrobacter fruendii, Klebsiella pneumoniae (4P), *Azospirillun brasilense* Sp 7s, *Azospirillum lipoferum* T10, *Azospirillum lipoferum* 596, *Azospirillum lipoferum* 687, *Herbaspirillium seropedicae, Rhizobium leguminosarum bv. trifoli.*

4.2.1.8 Fungus

One fungus, *Penicillium radicum* PR 70 was used in a study of its PGPR activity.

4.2.2 Methods

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

Two experiments were carried out, one commencing in October, 2003 (Experiment 1) and one commencing in June 2004 (Experiment 2).

4.2.2.1.1 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, and for Experiment 2, 168 pots were set up.

For Experiment 1, three P sources were used for six bacterial strains and a control (21 pots). There were seven replicates for each phosphate/bacterial strain combination, making a total of 147 pots. An additional seven pots were set up containing superphosphate. A total of 154 pots was set up in October 2003.

For Experiment 2, three P sources were used for six bacterial strains and a control (21 pots), with eight replicates for each phosphate/bacterial strain combination and the control. A total of 168 pots was set up in June 2004.

4.2.2.1.2 Addition of P to the soil

The standard P rate (15 kg P ha^{-1}) for wheat crops was used (http://www2.dpi.qld.gov.au, 2005). 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.

4.2.2.1.3 Seed sterilisation, sowing and thinning

Seed samples were sterilised 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 (50g) were placed on cloth gauze (approx. 12 cm x 12 cm) and the four corners 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/distilled water until there were no detergent suds remaining. The seeds were then soaked in $HgCl_2$ for 75 sec 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 sterilised 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.

4.2.2.1.4 Inoculation with bacterial strains

The bacterial strains used for these experiments were strains FA001, FA002, FA003, FA005, FA010 and JD12. A control treatment for each type of P was not inoculated and in Experiment 1, a positive control treatment containing superphosphate not inoculated was included. A 2 mL bacterial suspension prepared as described in Chapter 3 (3.2.2.2) 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, determined as described in Chapter 3 (3.2.2.4), is shown in Table 4.3.

Table 4.3 Number of live bacterial cells (CFU mL^{-1}) in bacterial suspensions used in Experiment 1 and Experiment 2 to examine the effects of P-mobilisers on wheat yields.

Experiments	Number of bacteria (CFU mL^{-1})					
Bacterial strains	FA001	FA002	FA003	FA005	FA010	JD12
2003 (Experiment 1)	$4.5x10^8$	$9.1x10^7$	$2.1x10^8$	$5.4x10^8$	$3.9x10^8$	$4.3x10^8$
2004 (Experiment 2)	$6.7x10^8$	$2.9x10^8$	$1.8x10^8$	$10.1x10^8$	$4.2x10^8$	$2.0x10^8$

4.2.2.1.5 Watering and nutrient supplying

For Experiment 1 and 2 normal tap water was applied for irrigation when necessary throughout the experiment. The first dose (5 mL pot^{-1}) of modified Hoagland's (#2) solution was applied 10 days after sowing in both Experiment 1 and 2. The second dose (5 mL pot^{-1}) of modified Hoagland's (#2) solution was applied 24 days after sowing for Experiment 1 and 28 days after sowing for Experiment 2.

4.2.2.1.6 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 (http://www.yates.com.au, 2003). In Experiment 2 the insecticide Malathion was applied 35 days after seed sowing (http://www.ipm.ucdavis.edu, 2003).

4.2.2.1.7 Plant parameters 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 at $70^{O}C$ were measured and samples stored in zip lock plastic bags at $4^{O}C$.

4.2.2.1.8 Plant phosphorus uptake/content

Plant material digestion

Plant P uptake in straw and grain was determined for Experiment 1 and 2 (Sale and Campbell, 1980). The grain and straw were dried at 70°C, and ground using a grinder (Restsch, Model: ZM1 35306, Selbys Scientific Ltd). One gram samples were transferred into conical flasks and 25 mL concentrated HNO_3 was added in a fume cupboard. Preliminary digestion was carried out for about 20 min at about 120°C. After completing the preliminary digestion the sample was cooled and 5 mL of 1:1 $HClO_4/HNO_3$ 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 min to complete the digestion (secondary digestion). The contents of the flask were transferred to a volumetric flask and the volume of the plant digest made to 25 mL.

Phosphorus assay

Vanadate reagent was prepared by dissolving 0.25 g of NH_4VO_3 in 50 mL boiling deionised 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 $(NH_4)_6Mo_7)_2.4H_2O$ 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 (Pharmacia Biotech; Model 80-2088-64). A standard curve was developed using 0 to 500 µg P mL^{-1} in 1:10 $HClO_4$/deionised water.

4.2.2.2 Experiment to determine plant growth promoting rhizobacterial effects

A glasshouse experiment (Experiment 3), commencing in October 2003, was conducted to determine plant growth promoting rhizobacterial (PGPR) effects using

two of the bacterial strains used in Experiment 1 and 2 (FA001 and FA010) and a range of other microorganisms (4.2.1.7). In this experiment normal Hoagland (#2) solution containing soluble P was used as the nutrient supply.

4.2.2.2.1 Establishment of Experiment 3 and growth of wheat

Wheat was grown in pots (4.2.2.1.1) each containing 1150g potting mix (sand:perlite:vermiculite = 3:2:1) (4.2.1.2). Twelve replicates were set up using a completely randomised design for 11 treatments (132 pots). The two P-mobilisers strains FA001 and FA010 were used (2.3.4) as well as six other bacteria, or mixed bacterial cultures, one fungus, and a control with no added microorganisms (Table 4.4).

Table 4.4 Microorganisms used to determine PGPR effects on wheat in a glasshouse experiment.

USYD CC = University of Sydney culture collection
BNF = Biological Nitrogen fixation

Symbol used	Treatments	Sources
T_1	BioGrow (HY yeast not included), combination of *Pseudomonas fluorescens, Citrobacter freundii* and *Klebsiella pneumoniae*	Nguyen *et al.*, 2002
T_2	*Azospirillum brasilense* Sp7s epiphyte	USYD CC
T_3	*Azospirillum lipoferum* + *Flavobacterium* (732) Jodie 12 P-solubiliser	USYD CC Harris *et al.*, 2006
T_4	*Azospirillum lipoferum* 596 good competitor PGPR	USYD CC
T_5	*Azospirillum lipoferum* 687 (w79)	USYD CC
T_6	*Herbaspirillum seropedicae* BNF + PGPR endophyte	USYD CC
T_7	*Rhizobium leguminosarum bv. trifolii*	USYD CC
T_8	*Penicillium radicum* PR 70	Wakelin *et al.*, 2004
T_9	FA001	Chapter 2
T_{10}	FA010	Chapter 2
T_{11}	Control	

The potting mix was soaked with 200 mL deionised water per pot just prior to seed sowing. In each pot three sterilised seeds were sown on the 3rd October 2003. Ten days after seed sowing, seedlings were thinned and in each pot one healthy plant was retained. Two mL of the bacterial suspensions of strains FA001 and FA010 prepared as for Experiment 1 (3.2.2.2) were used. All other bacterial cultures were prepared

and suspensions were made as described in Chapter 3 (3.2.2.2). Mixed bacterial suspensions (T_1, T_3 and T_6) were made by mixing equal quantities of the component, determined using spectrophotometer readings at 600nm. The fungal spore (T_8) suspension was prepared following the manufacturer's instructions (Appendix 4.1). Plants in each pot were inoculated around the root zone of the wheat plant 16 days after seed sowing. The bacterial and fungal spore numbers applied were not counted.

Nutrition was supplied as 350 mL of the Hoaglands (#2) original solution, to 10, 11 and 12 days old seedlings (150 mL + 100 mL + 100 mL). Thereafter moisture status was maintained by periodic application of deionised water. Thirty-three days after seed sowing some aphids were found on the plants; oyster oil was used to minimise the aphids' activity (http://www.yates.com.au, 2003).

4.2.2.2.2 Harvesting of wheat plants

The grain and straw were harvested 95 days after sowing. The agronomic parameters of plant height, numbers of seed in each plant, seed weight and straw weight were measured after drying at 70^{O}C.

4.2.3 Data analysis

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

4.3 Results

4.3.1 Schedule summaries for Experiments 1, 2 and 3

4.3.1.1 Schedule summaries for Experiment 1, and 2

For Experiments 1 and 2, to examine the effect of P-mobilisation on wheat yields, time of seed sowing, bacterial inoculation, application of modified Hoagland (#2) medium (no P) and date of harvest are shown in Table 4.5.

Table 4.5 Time of seed sowing, inoculation, application of modified Hoaglands (#2) medium and date of harvest for Experiments 1 and 2.

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

4.3.1.2 Schedule summary for Experiment 3

For Experiment 3, to examine the bacterial PGPR effect on wheat yields, time of seed sowing, bacterial inoculation, application of original Hoagland (#2) medium (containing P) and date of harvest are shown in Table 4.6.

Table 4.6 Time of seed sowing, inoculation, application of original Hoaglands (#2) medium and date of harvest for Experiment 3.

Time of seed sowing	1st dose of original Hoaglands medium	Bacterial inoculation	2nd dose of original Hoaglands medium	Date of harvest
3 October, 2003	13 October 2003	16 October 2003	27 October, 2003	6 January, 2004

4.3.2 Soil properties

The physico-chemical properties of the soil used in these experiments, determined using the methods described in Chapter 2 (2.2.1) are shown in Table 4.7.

Table 4.7 The physico-chemical properties of soil used in the glasshouse experiment.

Soil properties	**amounts**
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 (%)	14.3
Soil texture	sandy loam

4.3.3 Variation of temperature in the glasshouse

The temperature in the glasshouse during the period when Experiments 1 and 3 were carried out is shown in Figure 4.1.

The mean maximum and minimum temperatures in Sydney in 2004 are shown in Table 4.8.

Table 4.8 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

(http://www.bom.gov.au, 2007)

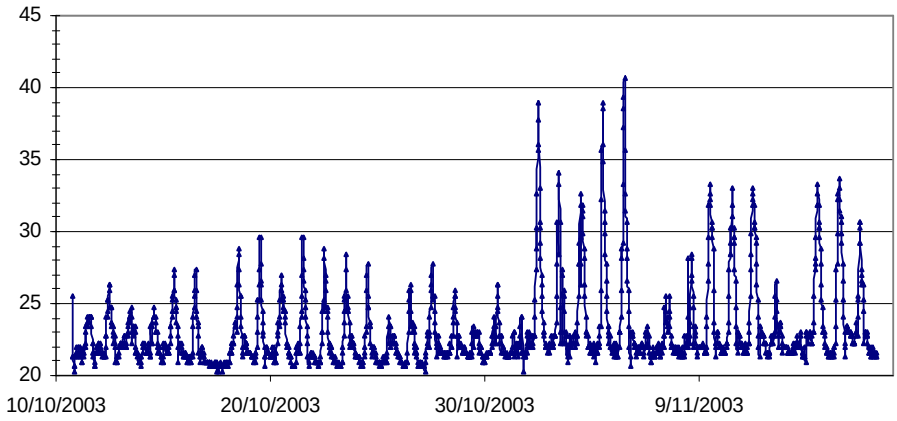

Figure 4.1a Temperature measurements in Parramatta Road Glasshouse from 10th October to 17th November 2003.

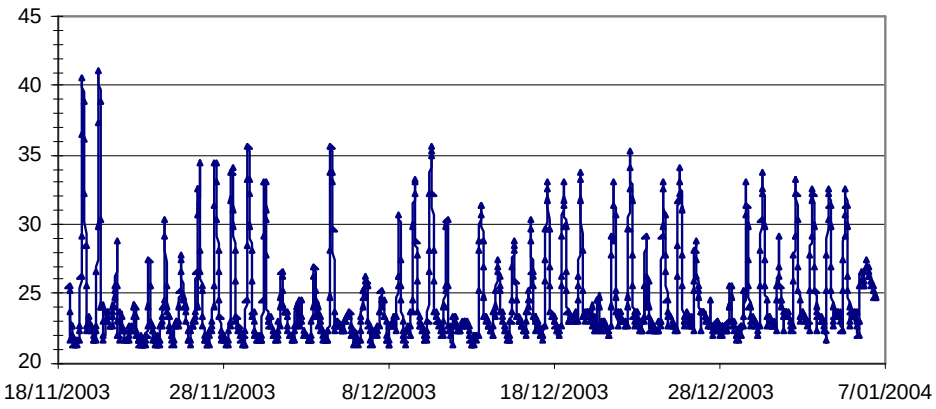

Figure 4.1b Temperature measurements in Parramatta Road Glasshouse from 18tn November 2003 to 7th January 2004.

4.3.4 Experiment 1: Phosphate mobilising activity (Oct 2003 – Jan 2004)

The grain yields, straw yields, plant heights, number of grain per spike, and P-uptake by grain and straw from wheat grown with three insoluble P sources and six potentially P-mobilising bacterial strains and a control set of wheat grown with added P and no bacteria, are shown below.

4.3.4.1 Grain yield

The results of grain yield are shown in Table 4.9.

Table 4.9 Grain yield of wheat (g pot^{-1}) in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Grain yield (g pot^{-1}) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean yield (P) [1]
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 yield (strain) [2]	0.430	0.425	0.412	

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 LSD.
ND = not determined

1 = Mean yield for three P-sources for the control and each treatment
2 = Mean yield for pots with six bacterial strains and two controls

There was no significant difference between the effects of different types of P on wheat grain yield in plants inoculated with different bacterial strains (Table 4.9). The interaction effect of strain and P-type was also insignificant at the 0.05 level, but it was at about the 0.06 level (Table 4.9). The mean grain yield for three P sources varied significantly with the bacterial strains used. Wheat plants with all strains and

SP produced a significantly higher grain yield than the control treatment. Wheat inoculated with the strains FA001 and FA010 produced the highest grain yields, significantly greater than all the other strains, but not significantly different from plants grown with SP.

4.3.4.2 Straw yield

The results for straw yield are shown in Table 4.10.

Table 4.10 Straw yield of wheat (g pot^{-1}) in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Straw yield (g pot^{-1}) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean yield (P) [1]
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 yield (strain)[2]	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

1 = Mean yield for three P-sources for the control and each treatment
2 = Mean yield for pots with six bacterial strains and two controls

There was no significant difference between the effects of different types of P on wheat straw yield in plants inoculated with different bacterial strains (Table 4.10). The interaction effect of strain and P-type was also insignificant. It was significant at about the 0.075 level (Table 4.10). There were significant differences ($P<0.05$)

between the mean straw yields of wheat inoculated with different bacterial strains. Wheat inoculated with all strains and SP produced significantly higher straw yields than the control treatment except for strain FA005. The SP treatment produced the highest straw yield (0.713 g pot^{-1}) significantly higher than the next best yields from wheat inoculated with the strains FA001 and FA010.

There was a strong relationship between grain yield (Table 4.9) and straw yield (Table 4.10) as shown in Figure 4.2 (R = 0.986).

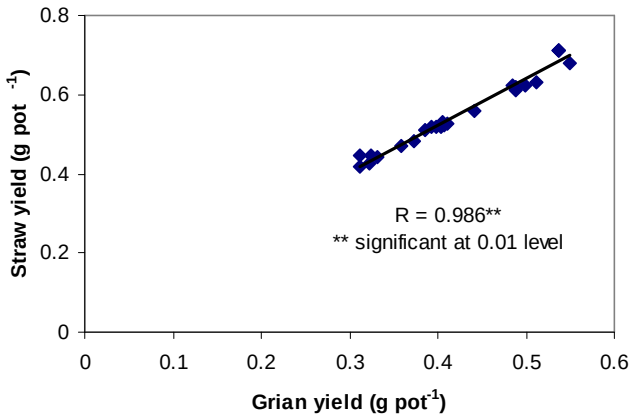

Figure 4.2 The relationship between grain yield and straw yield for wheat grown in Experiment 1 in pots containing three kinds of insoluble P and six bacterial strains.

4.3.4.3 Plant height (cm)

The results for plant heights are shown in Table 4.11.

Table 4.11 Plant height of wheat (cm) in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Plant height (cm) of wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean plant height (P) [1]
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 plant height (strain)[2]	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

1 = Mean plant height (cm) for three P-sources for the control and each treatment
2 = Mean plant height (cm) for pots with six bacterial strains and two controls

There was no significant difference between interaction effect of different strains and P on the height of wheat plants inoculated with different bacterial strains (Table 4.11). There were significant differences ($P < 0.05$) between the mean plant hight of wheat inoculated with different bacterial strains. Wheat inoculated with all bacterial strains and supplied with SP produced significantly higher plant height than the control treatment. Wheat supplied with SP produced significantly greater plant height (65.33 cm) than all other strains. There was a significant effect ($P<0.05$) on the mean plant height of wheat using different P-types. Wheat grown with $CaHPO_4.2H_2O$ and $Ca_3(PO_4)_2$ produced significantly greater plant height than wheat grown with rock phosphate (Table 4.11).

4.3.4.4 Number of grain per spike

The results for number of grain per spike are shown in Table 4.12.

Table 4.12 Number of grain per spike in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Number of grain spike^{-1} from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean No. of grain (P) [1]
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 no. of grain (strain)[2]	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 values strains 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

1 = Mean no. of grain per spike for three P-sources for the control and each treatment
2 = Mean no. of grain per spike for pots with six bacterial strains and two controls

There was no significant difference between the effects of different types of P on the number of grain per spike in plants inoculated with different bacterial strains (Table 4.12). The interaction effect of strain and P-types was also insignificant. There were significant differences ($P<0.05$) between the number of grain per spike of wheat inoculated with different bacterial strains. For all bacterial strains and SP there was a higher number of grain per spike than in the control treatment. The strain FA001 and SP produced the highest number of grain per spike and were significantly different from all other strains.

4.3.4.5 Phosphorus uptake

The results for grain P-uptake of wheat are shown in Tables 4.13 and 4.14.

Table 4.13 Phosphorus content in wheat grain in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Grain P uptake (mg/pot) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean P-uptake (P) [1]
Control	1.809 eA	1.744 dA	1.743 eA	1.766 e
FA001	2.593 bB	3.188 aA	2.580 bB	2.787 b
FA002	1.872 eC	2.624 bA	2.359 cB	2.285 c
FA003	2.267 cB	2.411 aA	2.054 dC	2.244 c
FA005	2.459 cA	1.810 dC	2.175 cB	2.148 d
FA010	2.685 bA	ND	2.668 bA	2.677 b
JD12	2.132 dA	2.028 cB	2.283 cA	2.148 d
Control (SP)	3.061 aA	3.061 aA	3.061 aA	3.061 a
Mean P-uptake (strain)[2]	2.360 A	2.409 A	2.365 A	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; <0.001 and <0.001, respectively. The LSD values for strains, P-type and strains x P-types interaction were 0.112, 0.068, and 0.193, 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

1 = Mean P-uptake for three P-sources for the control and each treatment
2 = Mean P-uptake for pots with six bacterial strains and two controls

Table 4.14 Phosphorus uptake in wheat grain calculated as percentage of P in grain (Experiment 1).

Strains	Grain P as a percentage of grain dry weight for wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean % P (P) [1]
Control	0.558aA	0.559bA	0.541bA	0.553 bc
FA001	0.530bB	0.581bA	0.584aA	0.565 b
FA002	0.470cB	0.541bA	0.585aA	0.532 c
FA003	0.587aB	0.588aA	0.619aB	0.598 a
FA005	0.606aA	0.582bA	0.606aA	0.598 a
FA010	0.550bA	ND	0.522bA	0.536 bc
JD12	0.524bB	0.544bA	0.583aA	0.550 b
Control (SP)	0.570aA	0.570bA	0.570aA	0.570 a
Mean %P (strain)[2]	0.549B	0.566A	0.576A	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; <0.001 and <0.001, respectively. The LSD values for strains, P-type and strains x P-types interaction were 0.029, 0.018, and 0.053, 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

1 = Mean %P for three P-sources for the control and each treatment
2 = Mean %P for pots with six bacterial strains and two controls

There were significant differences between the effects of different types of P ($P<0.05$) and bacterial strains ($P<0.05$) on the wheat grain P-uptake in plants inoculated with different bacterial strains (Table 4.13). The grain P-uptake by the plants that used SP was significantly higher than the amounts of P taken up by all other strains. The grain P taken up by the plants that used $CaHPO_4.2H_2O$ was significantly greater than that taken up by the other two sources of P. The interaction effect of strain and P-type was also significant ($P<0.05$) and the SP-treated grain P content was significantly higher than for all the bacterial strains, and the control, for all three types of P source except for the FA001 and FA003 strains using P from $CaHPO_4.2H_2O$.

When P-uptake was calculated as a percentage of grain yield (Table 4.14) it was found that the % P in grain from the control was similar to the % P in grain grown with bacterial strains and SP (although there were statistically significant differences between some treatments). The differences in the percentage of P in grain from all bacterial treatments were less than the differences in grain yield for most bacterial

treatments. The per cent P in grain was significantly greater in grain from plants grown with $CaHPO_4.2H_2O$ and rock phosphate than with $Ca_3(PO_4)_2$.

The results for straw P-uptake of wheat are shown in Tables 4.15 and 4.16.

Table 4.15 Phosphorus content in wheat straw in Experiment 1 grown in the presence of six different bacterial strains and three types of insoluble P.

Two control sets of wheat were grown. One contained SP (control SP) 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 (control).

All results are the mean of seven replicates.

Strains	Straw P uptake (mg/pot) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean P-uptake (P) [1]
Control	0.932cA	1.041bA	0.946cA	0.973f
FA001	1.261bA	1.978aA	1.247bA	1.496b
FA002	0.862cC	1.852aA	1.128cB	1.281d
FA003	1.079bB	1.770aA	0.942cB	1.264d
FA005	1.089bA	1.066bA	1.029A	1.062e
FA010	1.267bA	ND	1.428bA	1.348c
JD12	1.127bA	1.025bA	1.231bA	1.128e
Control (SP)	1.768aA	1.768aA	1.768aA	1.768a
Mean P-uptake (strain)[2]	1.173B	1.500A	1.215B	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; <0.001 and <0.001, respectively. The LSD values for strains, P-type and strains x P-types interaction were 0.130, 0.079, and 0.225, 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

1 = Mean P-uptake for three P-sources for the control and each treatment
2 = Mean P-uptake for pots with six bacterial strains and a control

Table 4.16 Phosphorus uptake in wheat straw calculated as percentage of P in straw (Experiment 1).

Strains	Straw P as percentage of straw dry weight for wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean %P (P) [1]
Control	0.210aA	0.233bA	0.222aA	0.221a
FA001	0.206aB	0.291aA	0.223aB	0.240a
FA002	0.166bC	0.297aA	0.217aB	0.227a
FA003	0.211aB	0.335aA	0.214aB	0.253a
FA005	0.201aB	0.255bA	0.219aA	0.227a
FA010	0.205aA	ND	0.227aA	0.239a
JD12	0.215aA	0.212bA	0.238aA	0.221a
Control (SP)	0.248aA	0.248bA	0.248aA	0.248a
Mean %P (strain)[2]	0.208C	0.267A	0.226B	

F probabilities for strains, P-types and strains x P-types interaction were 0.188; <0.001 and <0.001, respectively. The LSD values for P-type, and strains x P-types interaction were 0.017, and 0.048, 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

1 = Mean %P for three P-sources for the control and each treatment
2 = Mean %P for pots with six bacterial strains and a control

There were significant differences between the effects of different types of P ($P<0.05$) and bacterial strains ($P<0.05$) on the wheat straw P-content in plants inoculated with different bacterial strains (Table 4.15). The interaction effects of the bacterial strain and P-types were also significant ($P<0.05$). The straw P-content from the plants grown with bacterial strains and SP was significantly greater than for the control treatment (Table 4.15). The straw P-content from the plants grown using SP was significantly greater than for plants grown with all bacterial strains. Considering the effect of P-type, the highest straw P-content was obtained with the $CaHPO_4.2H_2O$ significantly greater than for the other two P sources. In the case of interaction effects, the P-content was significantly higher using SP than with all bacterial strains and the control in $Ca_3(PO_4)_2$ and rock phosphate. Using $CaHPO_4.2H_2O$ as the P source significantly greater P-content was obtained with the wheat grown with SP and with the strains FA001, FA002 and FA003, than for the other strains and the control. The total P-content of the straw of plants inoculated with the strain FA001 was higher using $CaHPO_4.2H_2O$ than other two sources of P.

The mean P percentage in straw for three P sources was not significantly different for all bacterial treatments, SP and the control (Table 4.16). The percentage P content in the $CaHPO_4.2H_2O$ treated plant straw was significantly greater that for the other two types of P treated plant straw.

4.3.5 Experiment 2: Phosphorus mobilising activity by bacteria (June-October 2004)

The grain yields, straw yields, plant heights, number of grain per spike, and P-uptake by grain and straw from wheat grown with three insoluble P sources and six potentially P-mobilising bacterial strains; and a control set of wheat grown with added P and no bacteria, are shown below. This experiment is essentially a repetition of the experiment in 2003, to determine the reproducibility of the results.

4.3.5.1 Grain yield

The results for grain yield of wheat are shown in Table 4.17.

Table 4.17 Grain yield of wheat (g pot^{-1}) in Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

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

All results are the mean of eight replicates.

Strains	Grain yield (g pot^{-1}) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean yield (P) [1]
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 yield (strain) [2]	0.453 A	0.470 A	0.424 B	

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

1 = Mean yield for three P-sources for the control and each treatment
2 = Mean yield for pots with six bacterial strains and a control

There were significant differences between the effects of different types of P ($P<0.05$) on mean wheat grain yield in plants inoculated with different bacterial strains (Table 4.17). The interaction effect of strain and P-type was insignificant. There were significant differences ($P<0.05$) between the mean grain yields of wheat inoculated with different bacterial strains. Wheat inoculated with the strains FA001 and FA010 produced significantly higher grain yields than the control and other bacterial treatments. Considering P-sources the highest grain yield (0.470 g pot^{-1}) was obtained with $CaHPO_4.2H_2O$ but it was not statistically different to the yield with $Ca_3(PO_4)_2$ (Table 4.17).

4.3.5.2 Straw yield

The results for straw yield of wheat are shown in Table 4.18.

Table 4.18 Straw yield of wheat (g pot^{-1}) in Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

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

All results are the mean of eight replicates.

Strains	Straw yield (g pot^{-1}) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean yield (P) [1]
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 yield (strain) [2]	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.

1 = Mean yield for three P-sources for the control and each treatment
2 = Mean yield for pots with six bacterial strains and a control

There was no significant difference between the effects of different types of P ($P<0.05$) on wheat straw yield in plants inoculated with different bacterial strains

(Table 4.18). The interaction effects of strain and P-type were also insignificant. There were significant differences ($P<0.05$) between the mean straw yields of wheat inoculated with different bacterial strains. Wheat inoculated with the strains FA001 and FA010 produced significantly higher straw yields than the control and other bacterial treatments (Table 4.18).

There was a strong relationship between grain yield (Table 4.17) and straw yield (Table 4.18) as shown in Figure 4.2 ($R = 0.861$).

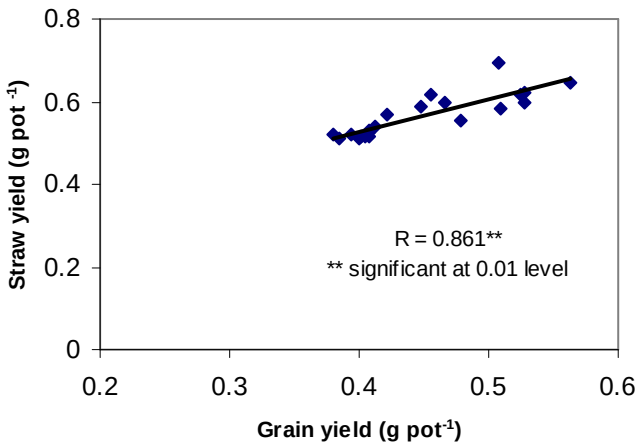

Figure 4.3 The relationship between grain yield and straw yield for wheat grown in Experiment 2 in pots containing three kinds of insoluble P and six bacterial strains.

4.3.5.3 Plant height (cm)

The results for plant height of wheat are shown in Table 4.19.

Table 4.19 Plant height of wheat (cm) in Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

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

All results are the mean of eight replicates.

Strains	Plant height (cm) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean plant height (P) [1]
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 plant height (strain) [2]	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.

1 = Mean plant height for three P-sources for the control and each treatment
2 = Mean plant height for pots with six bacterial strains and a control

There was no significant difference between the effects of different types of P ($P<0.05$) on wheat plant height in plants inoculated with different bacterial strains (Table 4.19). The interaction effects of strain and P-type were also insignificant. There were significant differences ($P<0.05$) between the mean plant height of wheat inoculated with different bacterial strains. The plant heights of all bacterial treatments were significantly greater than in the control treatment. Wheat inoculated with the strains FA001 and FA010 produced significantly higher plant heights than the control and other bacterial treatments (Table 4.19).

4.3.5.4 Number of grain per spike

The results for number of grain per spike of wheat are shown in Table 4.20.

Table 4.20 Number of grain per spike in Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

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

All results are the mean of eight replicates.

Strains	Number of grain per spike from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean No. of grain (P) [1]
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 No. of grain (strain)[2]	15.08 B	16.88 A	16.40 A	

F probabilities for strains, P-types and strains x P-types interaction were <0.001 and 0.015, 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 small letter in a row are not significantly different at P = 0.05 level by LSD.

1 = Mean grain number for three P-sources for the control and each treatment
2 = Mean grain number for pots with six bacterial strains and a control

There was significant difference between the effects of different types of P ($P<0.05$) on number of grain per spike in wheat plants inoculated with different bacterial strains (Table 4.20). Wheat using $CaHPO_4.2H_2O$ and rock phosphate produced significantly greater numbers of grain per spike than when supplied $Ca_3(PO_4)_2$ with samples. The interaction effect of strain and P-type was insignificant ($P<0.05$). There were significant differences ($P<0.05$) between the mean number of grain per spike of wheat inoculated with different bacterial strains. Wheat inoculated with the strains FA001 and FA010 produced significantly more number of grains per spike than the control and other bacterial treatments (Table 4.20).

4.3.5.5 Phosphorus uptake

The results for P-uptake of wheat grain are shown in Tables 4.21 and 4.22.

Table 4.21 Phosphorus content in wheat grain Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

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

All results are the mean of eight replicates.

Strains	Grain P uptake (mg/pot) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean P-uptake (P) [1]
Control	1.947 dB	2.251 dA	1.713 dC	1.968 e
FA001	2.639 bB	2.957 aA	2.560 aB	2.719 a
FA002	1.968 dB	2.288 cA	2.128 cA	2.128 d
FA003	1.948 dB	2.454 cA	2.321 bA	2.241 c
FA005	1.964 dB	2.291 dA	2.316 bA	2.190 c
FA010	2.437 cB	3.115 aA	2.373 bB	2.642 a
JD12	2.973 aA	2.643 bB	1.921d C	2.512 b
Mean P-uptake (strain)[2]	2.268 B	2.571 A	2.190 B	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; <0.001 and <0.001, respectively.
The LSD values for strains, P-type and strains x P-types interaction were 0.097, 0.064, and 0.169, 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.

1 = Mean P-uptake for three P-sources for the control and each treatment
2 = Mean P-uptake for pots with six bacterial strains and a control

Table 4.22 Phosphorus uptake in wheat grain calculated as percentage of P in grain (Experiment 2).

Strains	Grain P as a percentage of grain dry weight for wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean %P (P) [1]
Control	0.494 bB	0.585 aA	0.451 cB	0.509 a
FA001	0.519 bA	0.525b cA	0.505 bA	0.516 a
FA002	0.477 cB	0.542 bA	0.522 bA	0.513 a
FA003	0.486 bB	0.548 bAB	0.569 aA	0.534 a
FA005	0.491 bB	0.492 cB	0.569 aA	0.517 a
FA010	0.465 cB	0.590 aA	0.522 bB	0.526 a
JD12	0.564 aA	0.553 bA	0.476 cB	0.531 a
Mean %P (strain)[2]	0.499 C	0.548 A	0.516 B	

F probabilities for strains, P-types and strains x P-types interaction were 0.163; <0.001 and <0.001, respectively. The LSD values for P-type, and strains x P-types interaction were 0.014, and 0.036, 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.
1 = Mean %P for three P-sources for the control and each treatment
2 = Mean %P for pots with six bacterial strains and a control

Grain P-uptake by wheat was significantly influenced by different bacterial strains, sources of P and the interaction between strain and sources of P at 0.05 level of probability (Table 4.21). Considering the strains effect, all plants inoculated with different bacterial strains had significantly greater P uptake than the control. The P uptake was higher in the plants inoculated with the strain FA001 followed by the strain FA010 (Table 4.21). In the case of different sources of P, the highest P uptake was obtained using $CaHPO_4.2H_2O$. Considering interaction effect, the highest grain P-uptake was obtained with the treatment containing the strain JD12 from $Ca_3(PO_4)_2$. In the case of $CaHPO_4.2H_2O$, the highest grain P-uptake was with the strains FA001 and FA010 followed by the strain JD12. In the case of rock phosphate, the greatest P uptake was with the plants inoculated with the strain FA001 followed by FA010. The plants inoculated with the strain FA001 showed higher grain P-uptake with $Ca_3(PO_4)_2$ and $CaHPO_4.2H_2O$ than rock phosphate.

The per cent P in grain was not significantly different for any of the bacterial treatments and the control (Table 4.22). The percentage P content was significantly greater with $CaHPO_4.2H_2O$ as the P source than other two P sources.

The results for P-uptake of wheat straw are shown in Tables 4.23 and 4.24.

Table 4.23 Phosphorus content in wheat straw in Experiment 2 grown in the presence of six different bacterial strains and three types of insoluble P.

A control set of wheat was grown for each type of phosphate containing no added bacteria.
All results are the mean of eight replicates.

Strains	Straw P uptake (mg/pot) from wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean P-uptake (P) [1]
Control	1.167 bB*	1.959 bA	1.114 bB	1.413 b
FA001	1.391 abB	2.256 aA	1.568 aB	1.738 a
FA002	1.124 bB	1.806 bcA	1.157 bB	1.363 c
FA003	1.490 aA	1.682 cA	1.205 bB	1.459 b
FA005	1.448 aA	1.632 cA	1.439 aA	1.504 b
FA010	1.352 abB	1.629 cA	1.625 aA	1.535 b
JD12	1.382 abAB	1.548 cA	1.184 bB	1.372 c
Mean P-uptake (strain) [2]	1.336 B	1.787 A	1.327 B	

F probabilities for strains, P-types and strains x P-types interaction were <0.001; <0.001 and <0.001, respectively. The LSD values for strains, P-type and strains x P-types interaction were 0.154, 0.101, and 0.267, 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.

1 = Mean P-uptake for three P-sources for the control and each treatment
2 = Mean P-uptake for pots with six bacterial strains and a control

Table 4.24 Phosphorus uptake in wheat straw calculated as percentage of P in straw (Experiment 2)

Strains	Straw P as percentage of straw dry weight for wheat grown with different sources of P			
	$Ca_3(PO_4)_2$	$CaHPO_4.2H_2O$	Rock phosphate	Mean %P (P) [1]
Control	0.224 bB*	0.383 aA	0.214 bB	0.273 a
FA001	0.238 bB	0.350 aA	0.227 aB	0.271 a
FA002	0.208 bB	0.317 bA	0.225 aB	0.250 a
FA003	0.289 aA	0.285 bA	0.229 aB	0.268 a
FA005	0.285 abA	0.272 bA	0.272 aA	0.276 a
FA010	0.219 bA	0.262 cA	0.264 aA	0.248 a
JD12	0.231 bB	0.279 bA	0.229 aB	0.246 a
Mean %P (strain) [2]	0.242 B	0.307 A	0.231 B	

F probabilities for strains, P-types and strains x P-types interaction were 0.103, <0.001 and <0.001, respectively. The LSD values for strains, P-type and strains x P-types interaction were 0.018 and 0.047, 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.

1 = Mean %P for three P-sources for the control and each treatment
2 = Mean %P for pots with six bacterial strains and a control.

The effect of strain, P-types and their interaction was significant ($P<0.05$) on the straw P-uptake of wheat in Experiment 2 (Table 4.23). Considering the strains effect, the P-content in straw of all inoculated treatments was significantly greater than the control treatment. The highest P-content was obtained with the strain FA001 followed by the strains FA010, FA003 and FA005. Considering the P-types effect, the highest straw P-content was obtained in plants grown with $CaHPO_4.2H_2O$. Considering the interaction effect, the P-content of the straw of wheat plants inoculated with the strain FA001 was greater in all three different types of P. In contrast to the results for grain P uptake, the P-content of straw of wheat plants inoculated with the strain FA010 was greater using rock phosphate than for the other two sources of P.

As for the grain P percentage, the P percentages in straw were not significantly different for any of the bacterial treatments, or the control. The % P in straw from plants grown with $CaHPO_4.2H_2O$ was significantly greater than for the other two P sources.

4.3.6 Experiment 3: Plant growth promoting rhizobacterial activities (PGPR) (October 2003- January 2004)

The PGPR effect of 11 different treatments on the yield of wheat is shown in Table 4.25. These treatments contained a control treatment with no added microorganisms, individual microorganims, treatments with combinations of different microorganisms, and two P-mobilisers (strains FA001 and FA010) identified in this study (2.3.3).

Table 4.25 PGPR effects of different microorganisms on wheat in the glasshouse Experiment 3 in 2003.

Symbol used	Selection of strain combinations for pot Experiments:	Grain yield (g pot^{-1})	Number of grain pot^{-1}	Yield as percentage of control
T_1	Vietnamese strains (3) Bio Gro (HY yeast not included)	0.372 b*	14.50 b	98.70
T_2	*Azospirillum brasilense* Sp7s epiphyte	0.394 b	16.00 ab	104.51
T_3	*Azospirillum lipoferum* T10 + *Flavobacterium* (732) Jodie 12 P-solubilizer	0.414 ab	18.42 a	109.81
T_4	*Azospirillum lipoferum* 596 good competitor PGPR	0.461 ab	17.42 ab	122.28
T_5	*Azospirillum lipoferum* 687 (w79)	0.502 a	19.50 a	133.16
T_6	*Herbaspirillum seropedicae* BNF +PGPR endophyte	0.476 ab	17.17 ab	126.26
T_7	*Rhizobium leguminosarum bv. trifolii*	0.306 b	12.75 b	81.17
T_8	*Penicillium radicum* PR 70	0.385 b	14.33 b	102.12
T_9	FA001	0.416 ab	16.50 ab	110.34
T_{10}	FA010	0.414 ab	16.73 ab	109.81
T_{11}	Control	0.377 b	17.50 ab	100.00

F probability of grain yield and number of grain were 0.030 and 0.020, respectively and the LSD value of grain yield and number of grain were 0.1054 and 3.764, respectively 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

For some of the bacterial strains used in this experiment there was evidence of a PGPR effect on grain yield and number of grain produced per pot. The fungal strain *Penicillium radicum* PR70 had no significant effect on grain yield as weight or grain number. The grain yield (g pot^{-1}) in treatment T_5 was the only sample that was significantly higher than the control and all other treatments.

The variation in grain yield over the control lacking bacterial inoculant showed positive and negative PGPR effects on the grain yield of wheat (Table 4.25). The highest increase of grain yield (133% of the control) was obtained with the T_5 treatment. For the T_7 treatment the grain yield was only 81 per cent of the control. For treatments T_9 and T_{10} (FA001 and FA010) the grain yield was about 110 per cent of the control, although not significantly different. In this experiment roots were not examined, so that any root proliferation resulting from PGPR hormonal effects could not be observed.

4.4 Discussion

Two experiments were conducted to see whether bacteria could mobilise P from three insoluble P sources for use by wheat plants. Experiment 1 was carried out from October 2003 to January 2004 (Table 4.5). The glasshouse temperature was not fully controlled in this summer experiment. It fluctuated throughout the experiment (Figure 4.1), and was not suitable for growing wheat plants. In this experiment superphosphate (SP) was included without bacteria as a positive control. Experiment 2 was carried out in winter from June 2004 to October 2004. The glasshouse 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 4.8).

In both experiments grain yield (g pot^{-1}), straw yield (g pot^{-1}), plant height (cm), grain number $spike^{-1}$, and P-uptake into grain and straw were determined. The per cent P values were calculated for the grain and straw samples. For all P supplies and all bacterial strains grain yields were higher than in the control treatments without bacteria, 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-mobilising bacteria (Brown, 1974; Baldani *et al.*, 1987; Rodrìguez and Fraga, 1999; Rudresh *et al.*, 2005). In these experiments 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-mobilisers (2.3.4). Studies of several bacteria in liquid cultures containing a range of insoluble P showed that the strains FA001 and FA010 were the best P-mobilisers (3.4). There was no significant difference in grain yield versus P-type in Experiment 1 (Table 4.9). In Experiment 2, $Ca_3(PO_4)_2$ and $CaHPO_4.2H_2O$ produced significantly higher grain yield than rock phosphate (Table 4.17). 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 a N supply to the plants that was not optimised. In that experiment N, K and Fe were mixed with the sand-soil mixture (Harris *et al.* 2006), whereas in this experiment all

nutrients except P were added in solution form (Hoagland#2 solution). In addition, even in Experiment 2 (the winter experiment) the glasshouse temperatures were not optimised 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. In Experiment 2 the per cent P content in grain and straw was not significantly different for any of the bacterial treatments and the control. This contrasts with the results for the grain yields in the presence of bacterial strains which for strains FA001 and FA010 were significantly greater than for the control. These results suggest that plants inoculated with bacterial strains can obtain P from insoluble P sources and that $CaHPO_4.2H_2O$ is the best source.

In Experiment 2 all measured parameters, grain yield, straw yield, plant height and number of grain per spike were significantly greater for pots containing strains FA001 and FA010 than for the control and all other bacterial strains. A significant relationship was found between the grain and straw yield in Experiment 2 (Figure 4.3). It has been reported that plant growth and yield can be increased by P-mobilising bacteria (Gaind and Gaur, 1991; Sivan and Chet, 1992; 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; Brady and Weil, 2002). In Experiment 2, it was found that pots inoculated with all the bacterial strains produced significantly 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 solubilised 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 mobilised 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 favourable in Experiment 1 and may have been 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 mobilised from any of the three insoluble P sources supplied could be as useful for plant growth as SP.

In Experiment 3, normal Hoagland's (#2) solution was supplied, which contained soluble P with all other essential nutrients for wheat. The control treatment had no microorganisms but it contained normal Hoagland's (#2) solution. The treatment T_5 (containing *Azospirillium lipoferum 687*(w79)) produced a significantly better grain yield than all other treatments. In this study the T_1 treatment that included Vietnamese microorganisms produced a similar grain yield to the control. When this BioGro treatment was used in Vietnam there were significantly increased rice yields in 65 farmers' fields (Nguyen *et al.*, 2002). The reason for this difference could be differences in the paddy rice and wheat growth systems. It is well established that Rhizobia can increase the yield of legume crops because of their ability to fix atmospheric nitrogen in symbiotic relationships (Sogut, 2006; Ahmadi and Chaichi, 2007). It has also been reported that *Rhizobium sullae* increased wheat yield through soil aggregation, which enhanced root elongation and thereby increased nutrient uptake (Kaci *et al.*, 2005). Improved rice plant growth and higher grain yields were reported in crops treated with *Rhizobium leguminosarum* bv. *trifoli* (Yanni *et al.*, 1997). In this experiment there was no significant difference between wheat yields with *Rhizobium* inoculation (T_7). The grain yield and number of grain per pot were lower than for any other treatments.

In this study (Experiment 3) the isolated bacterial strains FA001 and FA010 appeared to have a positive PGPR effect, as the wheat yields were about 10 per cent above the uninoculated control. It has been reported that PGPR effects can increase the plant growth and yield of several crops (Gouzou *et al.*, 1993; Bezzate *et al.*, 2000; Egamberdiyeva and Hoflich, 2003; Kaci *et al.*, 2005; Suong *et al.*, 2005). The yield increases associated with these two strains in the P-mobilisation experiments are of the order of 30-50%. The PGPR effect alone is insufficient to explain these increases. The PGPR effect may enhance the P-mobilisation effect.

The bacterial strains FA001 and FA010 were associated with increased wheat grain yield in P stress conditions (mineral phosphate) (Table 4.9 and 4.17) as shown in Experiments 1 and 2. These results indicate that the bacteria FA001 and FA010 have a PGPR effect as well as the ability to mobilise P from insoluble sources. It is well established that phosphate mobilising 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; http://www.nysaes.cornell.edu, 2005).

4.5 Conclusion

All the selected P-mobilising bacteria increased the yield of wheat in a glasshouse experiment (Experiment 1) in 2003 and there were some increased yields in Experiment 2 in 2004. For all treatments per cent P dry weight in grain and straw was similar to the control treatments, although yields were higher. It can be assumed that in the glasshouse experiment these bacteria mobilise insoluble mineral P thereby resulting in yield and plant growth increases. 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 in Chapter 3 for P mobilisation *in vitro*. In the Experiment 3, it was shown that the bacterial strains that mobilise mineral P may also have PGPR effects. There was increased wheat yield (about 10% by FA001 and FA010) over the control although the differences were not significant. Thus the hypothesis that the approach used in the isolation of P mobilising strains described in Chapter 2 could provide useful soluble P for plant growth was supported by this data.

More glasshouse 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.