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Evaluation of Phosphorus Mobilization Potentials of Six Bacterial Strains from Four Insoluble Phosphorus Sources

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*A laboratory experiment was conducted to evaluate the P mobilization potentials of six bacterial strains isolated from three wheat-growing soils of Australia. Four different forms of insoluble phosphorus (P) were used in this experiment. Two strains (*Pantoea ananatis *and* Pantoea *sp.) mobilized more P from calcium phosphate [$Ca_3(PO_4)_2$] when ammonium sulfate [$(NH_4)_2SO_4$] was used as a source of nitrogen (N) compared to ammonium nitrate (NH_4NO_3) as the N source. The remaining four strains showed increased P-mobilizing ability with nitrate as sources of N. Cultures containing* Burkholderia *sp. showed a greater net increase in soluble P from rock phosphate compared to $Ca_3(PO_4)_2$. Mobilization of P from aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$) was much lower than from calcium P sources in cultures containing all the bacterial strains tested.* Pantoea ananatis *and* Pantoea *sp. were significantly better than other strains in mobilizing P from $AlPO_4$ whereas* Pantoea *sp. was identified as a minor P mobilizer from $FePO_4$.*

Keywords Australian soils, bacteria, phosphorus mobilization, wheat

Introduction

In Australia, measurement of soil total phosphorus (P) ranges from 200 to 5000 mg P kg^{-1} soil, with an average of about 500 mg P kg^{-1} soil (Lindsay and Vlek 1977). After application of superphosphate to soil, the initial reaction products formed from dihydrogen phosphate ion ($H_2PO_4^-$) are mainly calcium monohydrogen phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$), calcium monohydrogen phosphate ($CaHPO_4$), colloidal ferric phosphate (colloidal Fe-P), and colloidal aluminium phosphate (colloidal Al-P). These relatively insoluble products are then slowly transformed to even less soluble forms such as calcium orthophosphate [$Ca_3(PO_4)_2$] in alkaline soils or crystalline ferric phosphate [($FePO_4$(crystalline)] and crystalline aluminium phosphate [$AlPO_4$(crystalline)] in acidic soils (Lindsay, Frazier, and Stephenson 1962).

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Attempts are being made to develop plants and/or plant–microbial associations that allow efficient use of P from soil and fertilizer sources (Rodrìguez and Fraga 1999; Richardson 2001). Mismanagement of P fertilizer and transport triggers accelerated eutrophication (Choudhury et al. 2007; Tunney et al. 1997; Brady and Weil 1996). Improving the efficiency of P uptake by plants through microbial associations would therefore be economically and environmentally beneficial. The existence of soil microorganisms capable of transforming unavailable P to available forms for plants is well documented (Kucey, Janzen, and Leggett 1989; Rodrìguez and Fraga 1999).

Several mechanisms are involved in P mobilization in soil. Two potential mechanisms result from the excretion of organic acids or anions produced by microorganisms (Salih et al. 1989; Halder et al. 1990). Organic acids are reported to solubilize unavailable P by decreasing the pH (Halder, Mishra, and Chakrabartty 1991; Cunningham and Kuiack 1992). Organic anions can chelate the cations bound to P (Jones and Kochian 1996; Kirk, Santos, and Findenegg 1999). Chelation of cations is an important mechanism only where the organic anion structure favors chelation, such as in citrate (Fox, Comeford, and McFee 1990; Kirk, Santos, and Findenegg 1999; Nautiyal et al. 2000). Ammonium (NH_4^+) assimilation by proton (H^+) exchange is also a common phenomenon associated with P mobilization by bacteria (Parks et al. 1990; Illmer and Schinner 1995). Fungal P mobilization is well documented (Whitelaw, Harden, and Helyar 1999; Reyes, Baziramakenga, and Bernier 2001) either by the production of organic acids and chelation of cations by organic anions or by expanding the surface area of the rhizosphere.

This study has been taken to investigate the P-mobilization potential of six isolated bacterial strains to solubilize synthetic compounds representing the various types of phosphate compounds commonly found in soil.

Materials and Methods

Bacteria

Of the 10 isolated bacteria described in Ahmed (2008), the six used in this experiment were chosen because halos created by them on agar plates indicated good rates of P mobilization. These selected strains were FA001 (*Pantoea ananatis*), FA002 (*Pantoea agglomerans*), FA003 (*Burkholderia* sp.), FA004 (*Burkholderia* sp.), FA009 (*Burkholderia* sp.), and FA010 (*Pantoea* sp.), two bacterial strains from each of the original three soils. The strains FA001 (from the Wee Waa soil) and FA010 (from the Wagga Wagga soil) were selected as the best P mobilizers (Ahmed 2008) and of the other four, one was from the Wee Waa soil (FA002), two were from the Narrabri soil (FA003 and FA004), and one was from the Wagga Wagga soil (FA009). A Vietnamese bacterial strain designated as 4P (*Klebsiella pneumoniae*), known to be a P mobilizer, was also used in one experiment (Nguyen et al. 2003).

Media, Agar Plate, and Insoluble P Samples

A minimal medium was prepared that contained, per L, 0.5 g ammonium sulfate [$(NH_4)_2SO_4$], 0.2 g sodium chloride (NaCl), 10 g glucose, 1 mL of micronutrient solution containing boric acid (H_3BO_3; 5 g L^{-1}), sodium molybdite (Na_2MoO_4; 5 g L^{-1}), zinc sulfate ($ZnSO_4$; 0.2 g L^{-1}), and aluminium chloride ($AlCl_3$; 0.15 g L^{-1}). In specified cases NH_4NO_3 was used instead of $(NH_4)_2SO_4$ as a source of N and in those cases to supply the

equivalent amount of nitrogen (N), 0.3 g NH_4NO_3 L^{-1} was included in the medium. The pH was adjusted to 7.0 and the solution was autoclaved for 20 min at 121 °C.

For bacterial counting, plates were prepared using 8 g L^{-1} nutrient broth (Difco™, Becton Dickinson and Company, Franklin Lakes, N.J.) and 15 g L^{-1} agar technical (Bacto Laboratories Pty. Ltd., Mt Pritchard, NSW, Australia). The suspension was autoclaved for 20 min at 121 °C and poured on the plates aseptically in a laminar flow cabinet.

Four different forms of insoluble P were tested in this experiment. They were (a) calcium phosphate [$Ca_3(PO_4)_2$], (b) rock phosphate (crude rock phosphate: $Ca_{10}(PO_4)_6X$ (where X ≈ Cl, F, OH), (c) aluminium phosphate ($AlPO_4$), and (d) ferric phosphate ($FePO_4 \cdot H_2O$).

Preparation of Liquid Media

The amounts of phosphate minerals calculated to provide 500 mg P L^{-1} in 10-mL samples of sterile medium were weighed into aluminium foil packets and sterilized by autoclaving at 121 °C for 20 min. The sterilized phosphates were transferred to 10-mL aliquots of liquid medium sterilized in 25-mL McCartney bottles.

For $Ca_3(PO_4)_2$, suspensions were made in media containing $(NH_4)_2SO_4$ and ammonium nitrate (NH_4NO_3) as source of N. For all other sources of P (rock phosphate, $AlPO_4$, $FePO_4 \cdot H_2O$), the medium containing NH_4NO_3 was used as source of N.

Preparation of Bacterial Cultures

Freeze-dried ampoules were opened aseptically, some sterile media were added to suspend bacterial cells, and using a loop, bacteria were streaked on minimal agar plates and grown for 24 h at 28 °C. The colonies were scraped from the plates using 2 to 4 mL sterile 0.85% NaCl solution and the suspensions were transferred into sterile bottles aseptically using a pasteur pipette. These suspensions were used for inoculation of media containing various insoluble P materials.

After vortexing for 1 min and making 1 to 10 serial dilutions to 1 in 10^8, 0.1-mL samples of the diluted bacterial suspension (three replicates) were spread on nutrient broth/agar plates. The plates were incubated at 28 °C for 24 or 48 h until distinct colonies had developed. The colonies were counted and calculated in colony-forming units (CFU) per mL of the original suspension (CFU mL^{-1}).

Setting Up Culture/Inoculation and Incubation

For each bacterial strain there were three replicates for each of the five P-containing media, a total of 15 samples per strain. As the experiment was conducted over a period of 5 days and the cultures were examined every day, a total of 75 cultures were set up for each bacterial strain. Aseptically 0.1 mL of the initial bacterial suspension was added to the sterile 10-mL liquid medium samples. In addition to the six sets of cultures set up for the bacterial strains, a control set of cultures consisting of uninoculated media containing the same P sources as the inoculated treatments was set up. A total of 525 cultures were established. Another three replicates for each P-containing medium were set up to determine zero time values for soluble P and pH.

The bottles were incubated at 28 °C in a growth chamber with shaking at 140 rpm. The screw caps of the bottles were sightly loosened to allow gas exchange but to prevent airborne contamination. The experiment was carried out over a period of about 6 weeks.

Sampling and Assay for pH, Bacterial Count, and P Analysis

For each of the six bacterial cultures and the control set for each of the five media used, three replicates were sampled every day for five days (105 samples).

Bacterial Count. From each set of three replicates one was used for a bacterial count. The samples were diluted with 1 to 10 dilutions to a 1 in 10^8 dilution. The 1 in 10^6, 1 in 10^7, and 1 in 10^8 dilutions were used to determine bacterial counts. Three replicates of each dilution were counted. Because of the insoluble P in the cultures, the number of bacteria could not be determined by absorbance of the cultures.

pH Assay. The pH was measured immediately using a glass electrode (Peech 1965) for all samples.

Phosphorus Analysis. After removal of samples for the bacterial count, the remaining portions of the samples were centrifuged (20,000*g*, 10 min) to remove bacteria and insoluble P and their supernatants were carefully decanted. The supernatants were not assayed for any minor bacterial transfer but they were stored immediately at –28 °C for subsequent soluble P measurement. They were analyzed for inorganic P concentration colorimetrically by the molybdenum-blue method of Murphy and Riley (1962). The P content of the bacterial cells sedimented was not determined.

Preparation of Bacterial Cultures for Experiments to Measure Organic Acid Production

Bacterial Culturing. Freeze-dried ampoules of bacteria were opened aseptically, streaked on an agar plate, and grown for 24 h at 28 °C. Bacterial strains used in this experiment were FA001, FA002, FA003, FA004, FA009, FA010, and P4. The cultures were scraped into a bottle using 0.85% NaCl solution. The bacterial strains were added to 10-mL samples of the liquid medium containing $(NH_4)_2SO_4$ as the source of N and $Ca_3(PO_4)_2$ as the source of insoluble P, and the broths were incubated for 48 h at 28 °C. A control sample with no added bacteria was included. All assays were carried out in triplicate.

HPLC assay. Organic acids in the culture media were identified and quantified using high-performance liquid chromatography (HPLC). For analysis, broth cultures were shaken with about a teaspoon of Dowex 50W-X8 (Becton Dickinson and Company, Franklin Lakes, N.J.) for about 10–15 min to remove salt by adsorbing cations to obtain pure acid and the samples were filtered through 0.45-μm filter paper.

The analytical system consisted of a Shimadzu SIL-10AXL auto injector, Shimadzu SCL-10A system controller, and an LC-10AT VP pump fitted with a Gilson ultraviolet–visible (UV-vis) detector (Gilson, Inc., Middleton, Wisc., USA). The Polypore H column designed for organic acid separation was used with dilute sulfuric acid as a mobile phase. A Polypore H column is a member of the family of 10-μm porous styrene divinylbenzene ion exchange reversed-phase resins for nonpolar molecules. The column was equilibrated using 2.5 mM sulfuric acid (H_2SO_4) with a flow rate of 0.5 mL min^{-1}, and a UV detector at 210 nm was used for analysis of the standards and samples.

Standards of organic acids were prepared in 2.5 M H_2SO_4 solution. Internal standards (acetic, citric, malic, oxalic, succinic, and tartaric acids) were also used to confirm the identities of organic acids in the biological samples.

Statistical Analysis

Data were analyzed with statistical software Genstat (version 7; VSN International, Oxford, UK) (Payne et al. 2003) using one-way analysis of variance (ANOVA). A range of plots such as linear, logarithmic, polynomial, power, and exponential have been tried to get the best fit, and the one best fit has been presented in this article.

Results

Phosphorus Solubilization

The P solubilized by six strains of bacteria from $Ca_3(PO_4)_2$ suspended in a medium containing N as $(NH_4)_2SO_4$ is shown in Table 1. The effect of using different bacterial strains on the concentration of soluble P in the liquid media with time of incubation was significant. Mobilization of soluble P at day 1 was calculated as soluble P at day 1 and less soluble P at day 0. One day after inoculation, the greatest net increase in soluble P (total soluble P – soluble P at time 0) was in the culture inoculated with the strain FA001 (23.67 mg L^{-1} – 8.27 mg L^{-1} = 15.40 mg L^{-1}), followed by FA010 (16.73 mg L^{-1} – 8.27 mg L^{-1} = 8.46 mgL^{-1}). Low P levels were mobilized by the strains FA002, FA003, FA004, and FA009. After 5 days, the greatest amount of P was mobilized by strain FA001 (80.15 – 8.27 = 71.88 mg P L^{-1}) followed by FA010 (77.90 – 8.27 = 69.63 mg P L^{-1}). The P mobilized by strains FA002, FA003, FA004, and FA009 after 5 days was significantly less than for strains FA001 and FA010. There was no significant difference between P mobilized by strains FA001 and FA010, or among strains FA002, FA003, FA004, and FA009. Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 mobilized significantly more P than FA010 at days 1 and 3, whereas both the strains were statistically similar at days 2, 4, and 5.

Table 1
P mobilization from $Ca_3(PO_4)_2$ with time, using $(NH_4)_2SO_4$ as source of N, for six cultures containing bacterial strains and a culture without bacteria

	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	8.27 a A	8.31 d A	8.65 b A	8.72 c A	8.52 c A	8.25 c A
FA001	8.27 a E	23.67 a D	41.69 a C	73.54 a B	74.12 a B	80.15 a A
FA002	8.27 a C	11.15 c B	12.21 b B	12.54 c B	14.30 b AB	16.41 b A
FA003	8.27 a B	8.63 d B	9.21 b B	11.93 c AB	11.37 bc AB	14.47 b A
FA004	8.27 a B	8.77 d B	9.93 b AB	11.13 c AB	11.69 bc AB	12.73 b A
FA009	8.27 a B	8.83 d B	9.43 b B	11.80 c AB	12.53 bc AB	13.74 b A
FA010	8.27 a F	16.73 b E	44.32 a D	60.03 b C	71.41 a B	77.90 a A

Notes. F probability for treatments × time interaction was <0.001. The least significance difference (LSD) value for treatments × time interaction was 3.57 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 the 5% level by LSD.

Soluble P concentration in the media varied significantly with the bacterial strains when NH_4NO_3 was used as the source of nitrogen (Table 2). One day after inoculation the net increase in soluble P was the greatest with the FA001 strain, followed by the FA002 strain. The lowest amount of P was mobilized by the FA003, with a small but significantly greater amount in the culture containing the FA010 strain. After 5 days the P mobilized in the culture containing the FA001 strain was significantly greater than for all the other cultures. Interaction effect of strains and time after inoculation (days) was significant. For example, FA002 mobilized significantly more P than FA010 at day 1, whereas the opposite result was found at day 2.

The P solubilized by six cultures containing bacterial strains from rock phosphate suspended in a medium containing NH_4NO_3 as the source of N is shown in Table 3. One day after inoculation, the greatest amount of P ($12.72 - 0.32 = 12.40$ mg L^{-1}) was solubilized in the culture containing the FA001 strain. At 5 days after inoculation, cultures containing the FA001 and FA010 strains had significantly greater amounts of soluble P than the other cultures. Cultures containing all other strains also solubilized significantly greater amounts of P than was found in the control containing rock phosphate alone. Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 and FA009 were similar at day 1 whereas they were significantly different at days 2, 3, 4, and 5.

Phosphorus mobilization from $AlPO_4$ was generally poor. The mobilization of P in cultures containing different bacteria was much less than from $Ca_3(PO_4)_2$ and rock phosphate as sources of insoluble P. One day after inoculation the cultures containing strains FA001 and FA010 mobilized significantly greater amounts of P than other cultures (Table 4). Five days after inoculation, there was no significant difference in P mobilization between cultures containing these two strains. Interaction effect of strains and time after inoculation (days) was significant. For example, FA002 and FA003 were similar at day 1 whereas they were significantly different at days 2, 3, 4, and 5.

Soluble P concentration in liquid cultures with different bacterial strains varied significantly using $FePO_4$ as a source of P (Table 5). It was found that 1 and 5 days after

Table 2
P mobilization from $Ca_3(PO_4)_2$ with time, using NH_4NO_3 as source of N for six cultures containing bacterial strains and a culture without bacteria

	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	7.94 a A	8.50 d A	8.98 e A	8.38 e A	8.17 f A	7.81 e A
FA001	7.94 a E	24.70 a D	41.00 a C	44.98 a C	49.97 a B	55.58 a A
FA002	7.94 a C	19.28 b B	22.44 c AB	24.48 b A	24.70 c A	25.99 c A
FA003	7.94 a C	7.81 d C	10.46 e BC	13.77 d B	13.27 e B	23.11 c A
FA004	7.94 a B	7.81 d B	10.45 e B	19.91 c A	18.01 d A	20.63 d A
FA009	7.94 a D	8.64 d D	16.27 d C	26.40 b B	30.44 b A	30.77 b A
FA010	7.94 a C	13.35 c B	29.85 b A	28.91 b A	31.14 b A	29.84 bc A

Notes. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 4.33 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 the 5% level by LSD.

Table 3
P mobilization from rock phosphate with time, using NH_4NO_3 as source of N, for six cultures containing bacterial strains and a control culture without bacteria

	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.32 a A	0.38 c A	0.57 d A	0.71 e A	0.57 e A	0.59 e A
FA001	0.32 a F	12.72 a E	17.73 a D	25.40 b C	30.14 b B	38.25 a A
FA002	0.32 a B	5.89 b A	7.43 c A	8.96 d A	9.17 d A	9.90 d A
FA003	0.32 a D	3.14 bc C	12.57 b B	16.95 c B	15.04 c B	22.80 c A
FA004	0.32 a E	3.75 bc D	14.22 b C	14.78 cd BC	19.64 c B	29.79 b A
FA009	0.32 a C	7.86 a B	12.73 b AB	11.98 d AB	14.90 c A	14.30 d A
FA010	0.32 a E	7.72 b D	23.33 a C	31.02 a B	36.17 a A	39.71 a A

Notes. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 4.87 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 the 5% level by LSD.

Table 4
P mobilization from $AlPO_4$ with time, using NH_4NO_3 as source of N, for six cultures containing bacterial strains and a culture without bacteria

	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.23 a A	0.23 b A	0.23 b A	0.23 c A	0.23 c A	0.26 c A
FA001	0.23 a B	1.05 a A	1.18 a A	1.24 a A	1.19 a A	1.32 a A
FA002	0.23 a B	0.30 b B	0.53 b AB	0.62 b AB	0.71 b A	0.77 b A
FA003	0.23 a A	0.05 b A	0.05 c A	0.12 c A	0.15 c A	0.13 c A
FA004	0.23 a A	0.13 b A	0.07 c A	0.20 c A	0.20 c A	0.19 c A
FA009	0.23 a A	0.11 b A	0.10 c A	0.21 c A	0.54 b A	0.57 b A
FA010	0.23 a C	0.87 a B	1.06 a B	1.18 a B	1.37 a AB	1.53 a A

Notes. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.34 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 the 5% level by LSD.

inoculation, the culture containing the bacterial strain FA001 contained the greatest amount of soluble P (3.95 – 3.58 = 0.37 mg L^{-1} at day 1 and 7.84 – 4.26 = 3.58 mg L^{-1} at day 5), significantly more than the P released in cultures containing the other five strains. Five days after inoculation, the soluble P in the cultures containing the strains FA003, FA004, FA009, and FA010 was less than the 5-day control and initial soluble P (day 0) of the cultures containing these strains. Interaction effect of strains and time after inoculation (days) was significant. For example, FA002 and FA003 were similar at days 1 and 2, whereas they were significantly different at days 3, 4, and 5.

Table 5
P mobilization from $FePO_4$ with time, using NH_4NO_3 as source of N, for six cultures containing bacterial strains and a culture without bacteria

	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	3.53 a A	3.58 b A	4.05 a A	4.55 b A	4.01 c A	4.26 b A
FA001	3.53 a D	3.95 a D	4.50 a C	5.47 a B	5.42 a B	7.84 a A
FA002	3.53 a B	2.83 b C	2.95 b C	4.44 b A	4.81 b A	4.53 b A
FA003	3.53 a A	2.48 b B	3.36 b A	3.30 c A	3.23 d A	3.04 c A
FA004	3.53 a A	2.53 b B	2.38 c B	3.23 c A	3.23 d A	3.04 c A
FA009	3.53 a A	2.64 b B	2.61 c B	3.53 c A	3.06 d A	3.11 c A
FA010	3.53 a A	2.53 b B	2.81 c B	3.52 c A	3.02 d A	2.85 c B

Notes. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.51 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 the 5% level by LSD.

pH Changes (–ΔpH)

The pH of the bacterial cultures declined over a 5-day incubation period from an initial pH of 7.64 when $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ were used as sources of P and N, respectively. The changes are presented in Table 6. Interaction effect of strains and time after inoculation was significant in pH changes. For example FA009 was statistically different from FA004 at day 1 while both the strains are similar in days 2, 3, 4, and 5 (Table 6). The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for 5 days is shown in Figure 1. These data best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability ($R = 0.812$).

The change of pH (–ΔpH) was significantly different, comparing cultures containing different bacterial strains, when NH_4NO_3 was used as a source of N (Table 7). One day after inoculation, the greatest pH change ($-\Delta pH = 2.57$) was in the culture containing the strain FA001 while the lowest was in the culture containing the strain FA003 ($-\Delta pH = 0.63$). Five days after inoculation, the highest and the lowest pH changes were in the cultures containing the strains FA001 and FA004, respectively. The control treatment always had the lowest pH change over the 5-day period. Interaction effect of strains and time after inoculation (days) was significant. For example, FA003 and FA004 were similar at days 1, 2, 3, and 5 whereas they were significantly different at day 4. The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for 5 days is shown in Figure 2. These data best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability ($R = 0.954$).

The pH of six cultures containing bacterial strains in a liquid medium containing rock phosphate as source of P declined during a 5-day incubation period (Table 8). One day after inoculation the greatest (–ΔpH 2.39) and the least (–ΔpH 1.85) –ΔpH were observed with the strains FA010 and FA004, respectively. After 5 days of incubation, the greatest –ΔpH value was for culture containing strains FA001, which was statistically similar to

Table 6
pH changes with time in six cultures containing bacterial strains and a control culture containing $Ca_3(PO_4)_2$, and using $(NH_4)_2SO_4$ as a source of N over a 5-day period

	pH changes (−ΔpH)					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0 a C	0.38 e B	0.55 d AB	0.67 d A	0.71 d A	0.75 d A
FA001	0 a C	2.94 a B	3.31 a B	3.48 a AB	3.53 a A	3.52 a A
FA002	0 a D	1.96 b C	2.45 b B	2.52 b AB	2.64 b AB	2.70 b A
FA003	0 a E	0.76 d D	1.55 c C	2.48 b A	2.22 c B	2.53 bc A
FA004	0 a F	0.54 de E	1.67 c C	2.19 c B	2.51 b A	2.39 c AB
FA009	0 a E	1.10 c D	1.54 c C	2.32 bc B	2.60 b A	2.52 bc A
FA010	0 a D	2.84 a C	3.20 a B	3.37 a A	3.54 a A	3.57 a A

Notes. The pH of the minimal medium was adjusted to 7.0. After autoclaving the initial pH of all bacterial cultures and a control culture was 7.64. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.20 at the 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 the 5% level by LSD.

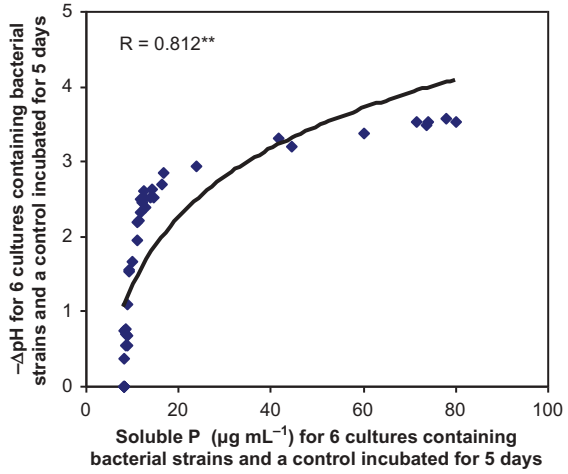

Figure 1. Relationship between soluble P and −ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for six cultures containing bacterial strains and a control culture incubated for 5 days when $(NH_4)_2SO_4$ was used as the source of N.

FA010 and FA004. Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 and FA010 were significantly different at days 2 and 3 whereas they were similar at days 1, 4, and 5. The relationship between the soluble P and the −ΔpH for each day for cultures containing six bacterial strains and a control incubated for 5 days is shown in Figure 3. These data best fitted a logarithmic regression, demonstrating a significant relationship at the 0.01 level of probability (R = 0.989).

Table 7
pH changes with time in six cultures containing bacterial strains and a control culture containing $Ca_3(PO_4)_2$, and using NH_4NO_3 as a source of N over a 5-day period

	pH changes (−ΔpH)					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0 a B	0.15 d B	0.30 e AB	0.28 d AB	0.38 e AB	0.42 d A
FA001	0 a C	2.57 a B	2.70 a B	2.77 a B	2.63 a B	3.14 a A
FA002	0 a B	2.16 b A	2.18 b A	2.27 b A	2.21 b A	2.24 b A
FA003	0 a F	0.63 c E	0.94 d D	1.77 c B	1.30 d C	2.06 bc A
FA004	0 a C	0.65 c B	0.76 d B	1.82 c A	1.94 c A	1.86 c A
FA009	0 a D	0.73 c C	1.71 c B	2.31 b A	2.26 b A	2.26 b A
FA010	0 a C	2.07 b B	2.12 b B	2.30 b AB	2.20 b AB	2.39 b A

Notes. The pH of the minimal medium was adjusted to 7.0. After autoclaving the initial pH of all bacterial cultures and a control cultures was 7.61. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.23 at the 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 the 5% level by LSD.

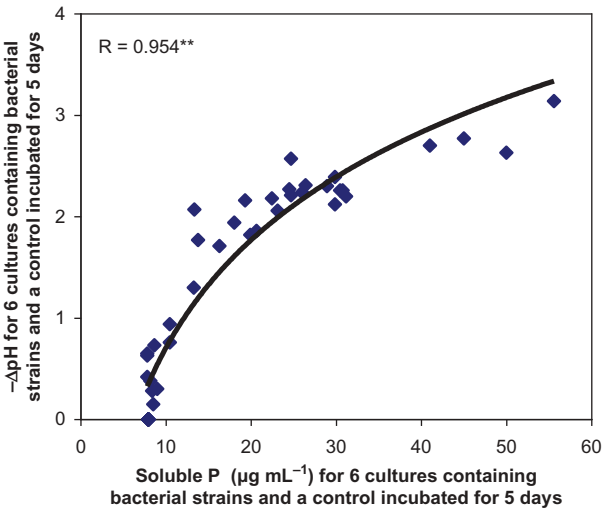

Figure 2. Relationship between soluble P and −ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for six cultures containing bacterial strains and a control culture incubated for 5 days when NH_4NO_3 was used as the source of N.

The pH of six cultures containing different bacterial strains in a medium containing $AlPO_4$ as source of P declined over a 5-day incubation period (Table 9). The overall decline in pH was similar to that in media with other P sources, but the variation between cultures containing different bacteria was less. There was no significant difference between the −ΔpH for bacterial strains FA001, FA002, FA004, and FA010 at 5 days after inoculation. Interaction effect of strains and time after inoculation (days) was significant. For example,

Table 8
pH changes with time in six cultures containing bacterial strains and a control culture containing rock phosphate using NH_4NO_3 as a source of N over a 5-day period

	pH changes (−ΔpH)					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0 a B	0.10 d B	0.08 B	0.36 d A	0.12 d B	0.07 d B
FA001	0 a D	2.26 a C	2.42 b C	2.66 b B	2.92 ab A	3.09 a A
FA002	0 a C	1.98 bc B	2.09 c A	2.18 c A	2.22 c A	2.25 c A
FA003	0 a D	2.00 bc C	2.41 b B	2.62 b B	2.71 b A	2.85 b A
FA004	0 a D	1.85 c C	2.51 ab B	2.62 b B	2.68 b A	2.89 ab A
FA009	0 a D	2.15 b C	2.25 bc B	2.37 c B	2.42 c A	2.61 c A
FA010	0 a D	2.39 a C	2.65 a B	2.91 a A	2.94 a A	3.05 ab A

Notes. The pH of the minimal medium was adjusted to 7.0. After autoclaving the initial pH of all bacterial cultures and a control culture was 6.79. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.21 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 5% level by LSD.

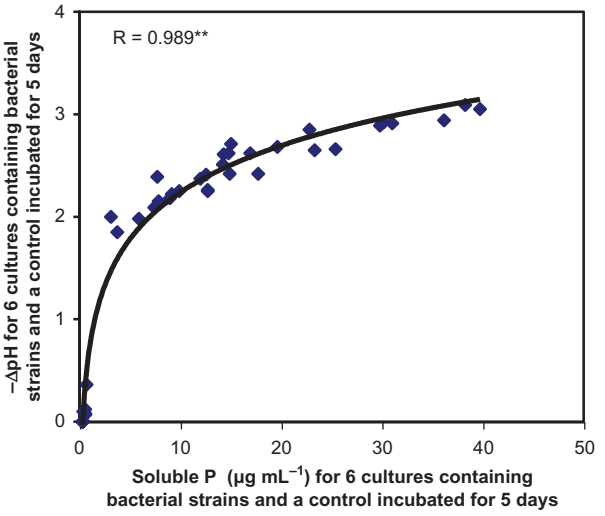

Figure 3. Relationship between soluble P and −ΔpH values in the liquid medium containing rock phosphate for six cultures containing bacterial strains and a control culture incubated for 5 days.

FA001 and FA002 were significantly different at day 1 whereas they were similar at days 2, 3, 4, and 5. The relationship between the soluble P and the −ΔpH for each day for cultures containing six bacterial strains and a control incubated for 5 days is shown in Figure 4. These data best fitted a linear regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.519).

Table 9
pH changes with time in six cultures containing bacterial strains and a control culture containing $AlPO_4$, using NH_4NO_3 as a source of N over a 5-day period

	pH changes (–ΔpH)					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0 a A	0.06 e A	0.12 d A	0.16 d A	0.18 d A	0.20 d A
FA001	0 a B	2.95 ab A	2.80 b A	2.84 ab A	2.82 ab A	2.95 a A
FA002	0 a C	2.32 c B	2.81 ab A	2.83 ab A	2.83 ab A	2.2 ab A
FA003	0 a C	1.85 d B	2.32 c A	2.46 c A	2.30 c A	2.32 c A
FA004	0 a D	2.22 c C	2.50 c B	2.73 b AB	2.69 b AB	2.90 a A
FA009	0 a B	2.34 c A	2.37 c A	2.44 c A	2.50 bc A	2.61 b A
FA010	0 a B	3.01 a A	3.07 a A	3.00 a A	3.04 a A	2.98 a A

Notes. The pH of the minimal medium was adjusted to 7.0. After autoclaving the initial pH of all bacterial and a control cultures was 6.85. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.26 at the 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 the 5% level by LSD.

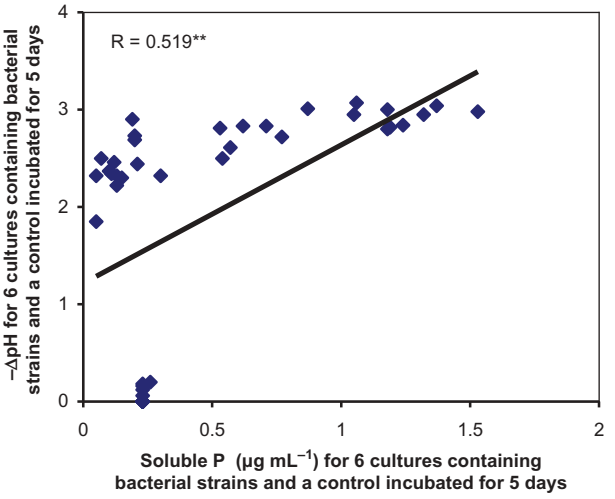

Figure 4. Relationship between soluble P and –ΔpH values in the liquid medium containing $AlPO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

The pH changes (–Δ pH) observed in six cultures containing different bacterial strains in the medium with $FePO_4$ are shown in Table 10. One day after inoculation, the greatest pH change (–ΔpH 2.67) was in the culture containing the strain FA002, followed by the culture containing the strain FA001 (–ΔpH 2.39), whereas the lowest pH change was found in the culture containing the strain FA003 (–ΔpH 0.56). Five days after inoculation the greatest and the lowest –ΔpH occurred with FA002 and FA009 strains, respectively.

Table 10
pH changes with time in six cultures containing bacterial strains and a control culture containing $FePO_4$ using NH_4NO_3 as a source of N over a 5-day period

	pH changes (−ΔpH)					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0 a A	0.09 d A	0.14 d A	0.16 d A	0.21 e A	0.21 e A
FA001	0 a C	2.39 b B	2.62 a A	2.58 a A	2.50 a A	2.72 a A
FA002	0 a B	2.67 a A	2.69 a A	2.81 a A	2.73 a A	2.76 a A
FA003	0 a C	0.56 c B	0.60 c B	0.67 c B	1.09 c A	1.32 b A
FA004	0 a C	0.62 c B	0.66 c AB	0.60 c B	0.84 d AB	0.91 c A
FA009	0 a B	0.69 c A	0.51 c A	0.54 c A	0.61 d A	0.58 d A
FA010	0 a C	2.14 b A	2.12 b A	2.08 b A	1.68 b B	1.33 b C

Notes. The pH of the minimal medium was adjusted to 7.0. After autoclaving the initial pH of all bacterial cultures and the control cultures was 7.74. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.27 at the 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 the 5% level by LSD.

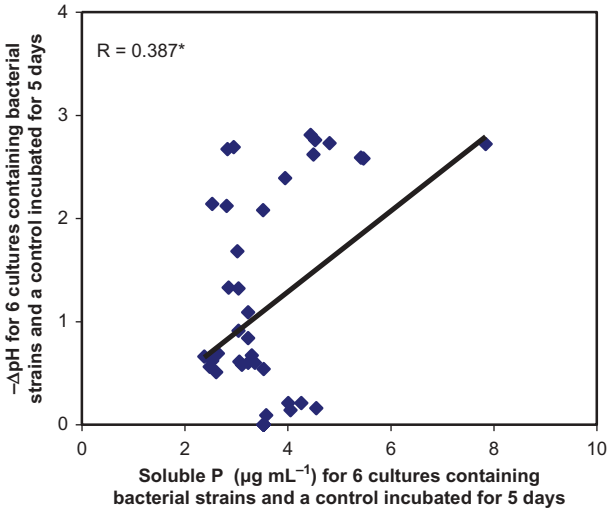

Figure 5. Relationship between soluble P and −ΔpH values in the liquid medium containing $FePO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 and FA002 were significantly different at day 1, whereas they were similar at days 2, 3, 4, and 5. The relationship between the soluble P and the −ΔpH for each day for cultures containing six bacterial strains and a control incubated for 5 days is shown in Figure 5. These data best fitted a linear regression demonstrating a significant relationship at the 0.05 level of probability (R = 0.387).

Bacterial Counts

When $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ were used as sources of P and N, respectively, the bacterial count results were those shown in Table 11. The greatest and least bacterial cell counts were found with the strains FA009 and FA003, respectively, 1 day after inoculation. After 5 days of inoculation, the greatest and the least number of bacterial cells were found with the strains FA004 and FA010, respectively. Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 and FA009 were significantly different at day 4 whereas they were similar at days 1, 2, 3, and 5.

When $Ca_3(PO_4)_2$ and NH_4NO_3 were used as sources of P and N, respectively, the number of bacteria (CFU mL^{-1}) varied significantly throughout the experiment (Table 12), with most growth occurring in day 1. After 1 day of incubation, the maximum and the minimum bacterial live cell counts (CFU mL^{-1}) were found in cultures containing the FA001 and FA010 strains, respectively, while 5 days after inoculation the greatest and least bacterial live cell counts were found in cultures containing the FA003 and FA010 strains, respectively.

There was significant variation in the number of live cells (CFU mL^{-1}) in six cultures containing different bacterial strains in the medium containing rock phosphate as source of P (Table 13). One day after inoculation, the greatest (8.010 $\log_{10}$CFU mL^{-1}) and least (7.620 $\log_{10}$ CFU mL^{-1}) number of live cells were found in cultures containing the strains FA003 and FA001, respectively. Five days after inoculation, the greatest and the least bacterial live cell counts were found in cultures containing the strains FA002 and FA001, respectively. Interaction effect of strains and time after inoculation (days) was significant. For example, FA002 and FA003 were significantly different at days 1, 4, and 5 whereas they were similar at days 2 and 3.

All of the bacterial strains used in this experiment were able to grow to some extent in the liquid medium containing $AlPO_4$ as the source of P as shown in Table 14. One day after

Table 11
Number of bacteria in cultures containing $Ca_3(PO_4)_2$, and $(NH_4)_2SO_4$ as source of N over a 5-day period

	No. of bacteria ($\log_{10}$ CFU mL^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.000 e A	0.000 d A	0.000 e A	0.000 c A	0.000 c A	0.000 d A
FA001	6.320 ab C	8.161 a A	8.017 d B	8.043 b B	7.967 b B	7.920 b B
FA002	5.650 d D	7.939 b C	8.783 a A	8.343 a B	8.017 b C	7.879 b C
FA003	5.224 d D	7.033 c C	8.333 c A	8.067 b B	8.080 b B	8.070 a B
FA004	6.227 b E	7.839 bD	8.599 b A	8.393 a B	8.259 a BC	8.174 a C
FA009	6.391 a C	8.173 a A	8.110 d A	7.967 b B	8.120 a A	7.907 b B
FA010	6.000 c D	8.010 b A	7.720 c B	7.633 d BC	7.523 d C	7.440 c C

Notes. The bacterial count was determined after a 1 to 10 times dilution series; spreading a diluted culture on a nutrient agar plate; and incubating for 24 to 48 h at 28 °C. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.141 at the 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 the 5% level by LSD.

Table 12
Number of bacteria in cultures containing $Ca_3(PO_4)_2$ and NH_4NO_3 as source of N over a 5-day period

	No. of bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.000 d A	0.000 f A	0.000 d A	0.000 d A	0.000 d A	0.000 d A
FA001	7.447 a C	8.600 a A	8.087 ab B	8.143 a B	8.003 b B	7.987 ab B
FA002	7.487 a B	7.970 c A	7.933 b A	8.093 a A	7.980 b A	7.883 b A
FA003	7.147 b C	7.753 d B	8.320 a A	8.243 a A	8.280 a A	8.193 a A
FA004	7.377 a C	7.553 d C	8.303 a A	8.310 a A	8.307 a A	8.057 ab B
FA009	7.433 a C	8.280 b A	8.113 ab A	8.153 ab A	8.140 a A	7.817 b B
FA010	5.300 c C	7.493 e A	7.260 c A	7.100 c B	6.877 c B	2.560 c D

Notes. The bacterial count was determined after a 1 to 10 times dilution series; spreading a diluted culture on a nutrient agar plate; and incubating for 24 to 48 h at 28 °C. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.237 at the 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 the 5% level by LSD.

Table 13
Number of bacteria in cultures containing rock phosphate and NH_4NO_3 as source of N over a 5-day period

	No. of bacteria (log_{10} CFU mL^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.000 e A	0.000 d A	0.000 c A	0.000 c A	0.000 e A	0.000 f A
FA001	6.413 d D	7.620 b A	7.463 b B	7.413 c B	7.327 c C	5.977 e E
FA002	6.897 c C	7.777 b A	7.773 a A	7.643 a B	7.770 a A	7.547 a B
FA003	7.263 b E	8.010 a A	7.797 a B	7.640 a C	7.510 b D	7.413 b D
FA004	7.557 a C	7.897 ab A	7.807 a B	7.473 b C	7.467 bc C	7.320 bc D
FA009	6.960 c D	7.967 a A	7.727 a B	7.497 b C	6.927 d D	6.540 d E
FA010	6.867 c E	7.667 b A	7.567 b A	7.523 ab B	7.363 c C	7.230 c D

Notes. The bacterial count was determined after a 1 to 10 times dilution series; spreading a diluted culture on a nutrient agar plate; and incubating for 24 to 48 h at 28 °C. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.125 at the 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 the 5% level by LSD.

inoculation, the greatest bacterial growth (7.970 log_{10} CFU mL^{-1}) was found in the culture containing the FA003 strain, whereas the lowest total bacterial growth (7.370 log_{10} CFU mL^{-1}) was in the culture containing the FA010 strain. After 5 days of incubation the greatest number of bacteria was found in the culture containing the strain FA001. Interaction

Table 14
Number of bacteria in cultures containing $AlPO_4$ and NH_4NO_3 as source of N over a 5-day period

	No. of bacteria (log_{10} CFU mL^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.000 c A	0.000 c A	0.000 d A	0.000 e A	0.000 e A	0.000 d A
FA001	7.663 a B	7.943 a A	8.040 a A	8.037 a A	7.847 a A	7.530 a B
FA002	7.423 a B	7.830 a A	7.980 a A	6.677 d C	6.587 c C	6.593 b D
FA003	7.397 a B	7.970 a A	7.607 b B	7.483 b B	7.100 b C	6.663 b D
FA004	7.523 a B	7.837 a A	7.570 b A	7.247 c B	7.053 b C	6.717 b D
FA009	7.540 a B	7.963 a A	7.937 a A	7.663 b B	6.570 c C	6.610 b C
FA010	6.327 b C	7.370 b A	7.137 c A	6.783 d B	6.073 d C	6.000 c D

Notes. The bacterial count was determined after a 1 to 10 times dilution series; spreading a diluted culture on a nutrient agar plate; and incubating for 24 to 48 h at 28 °C. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.28 at the 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 the 5% level by LSD.

Table 15
Number of bacteria in cultures containing $FePO_4$, and NH_4NO_3 as source of N over a 5-day period

	No. of bacteria (log_{10} CFU mL^{-1})					
	Time after inoculation (days)					
Strain	0	1	2	3	4	5
Control	0.000 d A	0.000 e A	0.000 c A	0.000 e A	0.000 d A	0.000 d A
FA001	7.066 a B	7.352 a A	7.297 b A	7.124 b A	7.102 b B	7.082 a B
FA002	6.704 bc C	7.012 b B	7.194 b A	7.239 b A	7.312 a A	7.084 a A
FA003	6.367 c B	6.586 c B	6.258 c C	6.873 c A	6.852 c A	6.697 b A
FA004	6.644 bc A	6.576 c A	6.562 c A	6.637 cd A	6.865 bcA	6.645 b A
FA009	6.717 b C	7.247 a B	7.676 a A	7.481 a A	7.493 a A	7.292 a B
FA010	6.475 c A	6.085 d B	6.545 c A	6.405 d A	6.443 d A	5.793 c C

Notes. The bacterial count was determined after a 1 to 10 times dilution series; spreading a diluted culture on a nutrient agar plate; and incubating for 24 to 48 h at 28 °C. F probability for treatments × time interaction was <0.001. The LSD value for treatments × time interaction was 0.236 at the 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 the 5% level by LSD.

effect of strains and time after inoculation (days) was significant. For example, FA001 and FA002 were similar at days 1 and 2 whereas they were significantly different at days 3, 4, and 5.

The growth of different strains significantly varied in the liquid culture containing $FePO_4$ as source of P (Table 15). One day after inoculation, the greatest (7.352 log_{10} CFU

Table 16
Organic acids identified in seven cultures containing bacterial strains after incubation for 2 d in a medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N

	Organic acids in media (μg mL^{-1}/2 days)					
Strain	Acetic	Citric	Malic	Oxalic	Succinic	Tartaric
FA001	Nd	5.090 a	Nd	0.651 ab	1.023 a	Nd
FA002	2.817 a	nd	Nd	Nd	nd	Nd
FA003	2.983 a	nd	Nd	Nd	nd	Nd
FA004	Nd	nd	Nd	0.670 ab	nd	1.576 b
FA009	Nd	nd	0.469 a	0.975 a	nd	Nd
FA010	Nd	5.780 a	Nd	0.582 b	1.040 a	Nd
4P	2.009 b	nd	Nd	0.534 b	nd	7.922 a
F Probability	< 0.001	ns	—	< 0.001	ns	< 0.001
LSD at 0.05	0.251	—	—	0.335	—	0.260

Notes. Means followed by a common letter in a column are not significantly different at the 5% level by LSD. nd, not detected; ns, nonsignificant.

mL^{-1}) and the least (6.085 $\log_{10}$CFU mL^{-1}) bacterial growth were found in cultures containing the bacterial strains FA001 and FA010, respectively. A similar result was found 5 days after inoculation. Interaction effect of strains and time after inoculation (days) was significant. For example, FA001 and FA009 were similar at days 1 and 5, whereas they were significantly different at days 2, 3, and 4.

Production of Organic Acids in Bacterial Cultures Containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$

After incubation for 48 h, the six cultures containing bacterial strains contained several organic anions. No organic anions were detected in the control (no bacteria). Although all strains produced some organic anions including known chelators, the identifiable anions differed in the cultures containing different bacteria. The results are presented in Table 16. Only the cultures containing the bacterial strains FA001 and FA010 contained citrate and there was no significant difference between the levels in the two cultures. The strain 4P (*Klebsiella pneumoniae*) produced the greatest amount of tartaric acid (7.92 μg mL^{-1}/2 days). The mobilization of P by this strain has been shown to result from acid production (Rose 2007).

Discussion

The mobilization of P from four insoluble P sources by liquid cultures containing six bacterial strains has been examined. The relationships between bacterial growth, pH changes, organic anion production, and P mobilization have been considered.

Four chemical compounds representative of phosphate minerals commonly found in soil have been used: calcium phosphate, rock phosphate (also a calcium mineral), aluminium phosphate, and iron phosphate. Six bacterial strains were identified as P mobilizers by their ability to produce clear haloes on agar plates containing insoluble $Ca_3(PO_4)_2$ have been tested for P-mobilizing capacity in liquid culture. The bacterial strains used

were identified as *Pantoea* spp., FA001, FA002, and FA010, and the *Burkholderia* spp., FA003, FA004, and FA009 (Ahmed 2008). For $Ca_3(PO_4)_2$, experiments were carried with $(NH_4)_2SO_4$ and NH_4NO_3 as the source of N in the culture medium. For the other three insoluble P compounds, the source of N in the medium was NH_4NO_3. Specific organic anion production was assayed only in cultures containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$.

In the five sets of cultures, three contained phosphates that were calcium salts and the other two contained aluminium and iron salts. Soluble P in the culture media containing different sources of mineral P differed and was in the sequence of $Ca_3(PO_4)_2/(NH_4)_2SO_4$, 8.27 mg L^{-1}; $Ca_3(PO_4)_2/NH_4NO_3$, 7.94 mg L^{-1}; $FePO_4$, 3.35 mg L^{-1}; rock P, 0.32 mg L^{-1}; and $AlPO_4$, 0.23 mg L^{-1}, before bacterial inoculation. It has been reported that aluminium and iron phosphates are less soluble than calcium phosphates (Lindsay, Peech, and Clark 1959; Whitelaw, Harden, and Helyar 1999). In these experiments soluble P from rock phosphate was very low. Overall the strains FA001 and FA010 mobilized P better than the other four strains, and strain FA001 was a better mobilizer than FA010. In the three cultures containing calcium salts, all bacterial strains mobilized significantly more P than the control culture (without bacteria).

In all cultures containing different sources of insoluble P and six different bacteria, the pH of the medium declined after inoculation. The maximum pH change occurred in the first day after inoculation for all different types of P-containing medium with the six different strains. Five days after inoculation the greatest pH decrease was with the cultures containing the bacterial strains FA001 and FA010 compared with the other four strains. The differences in $-\Delta$pH between cultures containing the strains FA001 and FA010 and the other strains was not as great as the difference between the P mobilized. There were some differences between the $-\Delta$pH in cultures containing the strains FA001 and FA010. The $-\Delta$pH in the culture containing the strain FA010 with $Ca_3(PO_4)_2$, and NH_4NO_3 as source of N, was lower than for the cultures containing the strain FA001. The $-\Delta$pH in the cultures containing $FePO_4$ was lower in the culture containing the strain FA010 than the strain FA001.

In this study the relationship between soluble P and $-\Delta$pH values for six cultures containing bacterial strains and a control culture were examined. For the media containing $Ca_3(PO_4)_2/(NH_4)_2SO_4$, $Ca_3(PO_4)_2/NH_4NO_3$, and rock P/NH_4NO_3, a significant relationship between the soluble P and $-\Delta$pH was found (Figures 1, 2, and 3). These relationships demonstrate the influence of the reduction in pH on bacterial P mobilization in these three media. In the case of $AlPO_4/NH_4NO_3$ and $FePO_4/NH_4NO_3$ media, the relationship between individual soluble P and $-\Delta$pH values for six cultures containing bacterial strains and a control culture was significant (Figures 4 and 5). These relationships for the aluminium and iron P-containing media suggest that $-\Delta$pH is less important for P mobilization from aluminium and iron salts than from Ca salts.

Decrease in pH can result from the production of organic acids by bacteria (Rodrìguez and Fraga 1999; Son et al. 2006; Lin et al. 2006). Organic acid production can affect P mobilization in two ways: by acidification and by chelation of cations by organic anions. The pH change due to the excretion of organic anions and protons (H^+) by bacteria is well recognized (Salih et al. 1989; Halder, Mishra, and Chakrabartty 1990; Nautiyal et al. 2000). Ammonium (NH_4^+) assimilation associated with proton (H^+) exchange and subsequent acidification is a common phenomenon (Parks et al. 1990; Kennedy 1992; Illmer and Schinner 1995). The greater pH change in the media containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ compared to NH_4NO_3, for all bacterial cultures (Tables 6 and 7) might be because of ammonium assimilation by proton (H^+) exchange and subsequent acidification of the medium. Greater P mobilization by the strains FA001 and FA010 in $(NH_4)_2SO_4$-

rather than NH_4NO_3-containing medium could be due to lowering of the pH by NH_4^+ assimilation. For all the other strains (FA002, FA003, FA004, and FA009), more P was mobilized in the media containing NH_4NO_3 rather than $(NH_4)_2SO_4$. These results suggest that there may be different mechanisms for P solubilization or different growth rates for these bacterial strains with different N sources. It has been reported that different strains of *Rhizobium* were able to solubilize hydroxyapatite in liquid culture without NH_4^+ and it was concluded that different mechanisms, other than acid production, were involved in P mobilization (Halder and Chakrabartty 1993). In this experiment (Tables 1 and 2), the utilization of NH_4NO_3 meant supplying fewer NH_4^+ ions than when $(NH_4)_2SO_4$ was used. The increased mobilization of P with these four strains was consistent with the finding of Halder and Chakrabratty (1993).

The Vietnamese strain 4P, known as a good P mobilizer (Nguyen et al. 2003), produced more than 7 μg mL^{-1} tartaric acid (Table 16). Tartrate is not as effective as citrate for P mobilization by chelation because citrate has three carboxyl groups and tartrate has only two carboxyl groups. It has been reported that 4P mobilized P by lowering the pH (Rose 2007). The P-mobilizing ability of this strain was not determined in this study.

In the case of the $AlPO_4$-containing medium, the greaetest number of bacteria was obtained 1 day (FA003, FA004, FA009, and FA010) and 2 days (FA001 and FA002) after inoculation. Although Al^+ can be toxic, in this medium bacterial growth was substantial for 2 days after inoculation. The Al^{3+}-detoxifying capacities of organic anions have been correlated with the relative positions of hydroxyl and carboxylic groups on their main carbon chains (Hue, Craddock, and Adams 1986). Strong chelation of Al^{3+} prevented inhibition of bacterial growth, facilitating P mobilization. In other reports it was explained that bacterial growth was not inhibited very much as citrate complexed Al, thus alleviating Al toxicity (Pohlman and McColl 1986; Lan, Comeford, and Fox 1995; Jones and Kochian 1996; Jones 1998). It was suggested that organic anions containing triple carboxyl groups such as citrate mobilize P from $AlPO_4$ better than organic anions containing a single carboxyl group such as acetate (Lan, Comeford, and Fox 1995; Jones and Kochian 1996).

In $FePO_4$-containing medium, there was good bacterial growth of the strain FA001 after 1 day of incubation. The number of bacteria in cultures containing the strains FA001, FA002, FA009, and FA010 increased significantly but the other strains were slow to grow. It has been reported that in media containing $FePO_4$ there is sufficient soluble P for initial bacterial growth (Molla et al. 1984; Seshadri et al. 2000). The initial soluble P in these cultures was a 10-fold greater level than in cultures containing rock phosphate, which supported good bacterial growth for all bacterial strains examined. The soluble P in the $FePO_4$-containing medium used in these experiments was probably sufficient for their growth in this medium. In most cultures the total number of viable bacterial cells (CFU mL^{-1}) decreased from 2 to 3 days after inoculation. This may be a consequence of the lower P values in the cultures from 1 day after inoculation. The decline in the total number of bacteria in the cultures over a 5-day incubation period may be caused by more acidic conditions rather than specific toxic effects. In addition, lack of nutrition might cause a reduction in bacterial numbers in the medium over the 5-day period. Lower pH conditions may have resulted in some release of soluble P from decaying bacterial cells.

The strain FA001 shows the lowest growth increase in bacterial number in culture containing all four P sources. Nevertheless, this strain was one of the two best P mobilizers of the six strains examined in this experiment. The greatest bacterial numbers were found with the strains FA004 in $Ca_3(PO_4)_2/(NH_4)_2SO_4$, FA003 in $Ca_3(PO_4)_2/NH_4NO_3$, FA002 in rock phosphate/NH_4NO_3, FA001 in $AlPO_4/NH_4NO_3$, and FA009 in $FePO_4/NH_4NO_3$ media. The greatest amount of soluble P was found with the strain FA001 in all different

P- and N-containing media except $AlPO_4/NH_4NO_3$. Thus it was clear in these five culture media containing different sources of P and N that the number of bacteria is not the only factor contributing to P mobilization. The P mobilization depends on the ability of the bacteria to produce acid and specific organic anions. This production and secretion of organic acid and anions is characteristic of individual bacterial strains.

Conclusion

The isolated bacterial strains could significantly mobilize insoluble P from $Ca_3(PO_4)_2$ and rock phosphate in minimal liquid medium. Strains FA001 and FA010 were the best P mobilizers. When $(NH_4)_2SO_4$ and NH_4NO_3 were used as sources of N with $Ca_3(PO_4)_2$ more P was mobilized by these two strains using $(NH_4)_2SO_4$ as a source of N, possibly because the assimilation of ammonium alone caused greater acid production (i.e., lower pH) in comparison to nitrate as source of N. Both strains FA001 and FA010 also produced citrate, a good cation chelator. Strains FA002, FA003, FA004, and FA009 showed increased P-mobilizing ability with nitrate as source of N with $Ca_3(PO_4)_2$ in comparison with the medium containing $(NH_4)_2SO_4$. Cultures containing strains FA004 and FA009 showed a greater net increase in soluble P from rock phosphate compared to $Ca_3(PO_4)_2$. Mobilization of P from $AlPO_4$ and $FePO_4$ was much lower than from calcium P sources in cultures containing all the bacterial strains tested. Strains FA001 and FA010 were significantly better than other strains in mobilizing P from $AlPO_4$ whereas FA010 was identified as a minor P mobilizer from $FePO_4$. In all cases, it was revealed that pH change and bacterial excretion of organic acids are more important than the number of bacteria for P mobilization.

The different rates of P mobilization with different bacteria, P sources, and N sources need to be examined further. The reason of more P was solubilized in NH_4NO_3-containing medium than $(NH_4)_2SO_4$-containing medium by the bacterial strains FA002, FA003, FA004, and FA009 is not clear. Studies with these bacteria and FA001 and FA010 using different sources of N may help in understanding the results reported in this paper. Organic anion production was measured for a limited number of anions in the medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$. It would be useful to investigate excretion of a wider range of anions (including α-ketogluconate) from cultures grown with other sources of P and N. As per the results presented here, all the bacterial strains are potential P mobilizers.

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