3.1 Introduction

The average total P content in soils is about 500 mg kg^{-1}, but only 0.1 per cent of this total P is usually in a form available to plants (Scheffer and Schachtschabel, 1992). In Australia, measurement of soil total P ranges from 200 to 5000 mg P kg^{-1} soil, also 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 $H_2PO_4^-$ are mainly calcium monohydrogen phosphate dihydrate ($CaHPO_4.2H_2O$), 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 *et al.*, 1962).

Attempts are being made to develop plants and/or plant-microbial associations that allow more efficient use of P from soil and fertiliser sources (Rodrìguez and Fraga, 1999; Richardson, 2001). Mismanagement of P fertiliser and transport triggers accelerated eutrophication (Tunney *et al.*, 1997; Brady and Weil, 2002). 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 *et al.*, 1989; Rodrìguez and Fraga, 1999).

Several mechanisms are involved in P mobilisation in soil. Two potential mechanisms result from the excretion of organic acids or anions produced by microorganisms (Duff and Webley, 1959; Salih *et al.*, 1989; Halder *et al.*, 1990). Organic acids are reported to solubilise unavailable P by decreasing the pH (Halder *et al.*, 1991; Yadav and Singh, 1991; Cunningham and Kuiack, 1992; Kpomblekou-A and Tabatabi, 1994; Wenzel *et al.*, 1994). Organic anions can chelate the cations bound to P (Lan *et al.*, 1995; Jones and Kochian, 1996; Kirk *et al.*, 1999). Chelation of cations is an important mechanism only where the organic anion structure favours chelation, such

as in citrate (Swenson *et al.*, 1949; Fox *et al.*, 1990; Kirk *et al.*, 1999; Whitelaw *et al.*, 1999; Nautiyal *et al.*, 2000). Ammonium (NH_4^+) assimilation by proton (H^+) exchange is also a common phenomenon associated with P mobilisation by bacteria (Dighton and Boddy, 1989; Parks *et al.*, 1990; Illmer and Schinner, 1995). Fungal P mobilisation is well documented (Whitelaw *et al.*, 1999; Reyes *et al.*, 2001) either by the production of organic acids, and chelation of cations by organic anions, or by expanding the surface area of the rhizosphere.

In this chapter experiments to determine whether the bacteria described in chapter 2 can mobilise insoluble P from liquid culture are reported. The aims were to investigate the P-mobilising rate of isolated bacterial strains to solubilise synthetic compounds representing the various types of phosphate compounds commonly found in soil. Possible mechanisms for P-mobilisation from these compounds were investigated.

3.2 Material and methods

3.2.1 Materials

3.2.1.1 Bacteria

Of the ten isolated bacteria described in Chapter 2, the six used in this experiment were chosen because haloes on agar plates indicated good rates of P mobilisation. These selected strains were FA001, FA002, FA003, FA004, FA009 and FA010; 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 mobilisers (2.3.3) and of the other four; one was from the Wee Waa soil (FA002), two from the Narrabri soil (FA003 and FA004) and one from the Wagga Wagga soil (FA009).

A Vietnamese bacterial strain designated 4P (*Klebsiella pneumoniae*), known to be a P-mobiliser, was also used in some experiments (Nguyen *et al.*, 2003).

3.2.1.2 Media, agar plate and insoluble P samples

A minimal medium containing per L, 0.5 g $(NH_4)_2SO_4$, 0.2 g NaCl, 10 g glucose, 1 mL of micronutrient solution containing H_3BO_3 (5 g L^{-1}), Na_2MoO_4 (5 g L^{-1}), $ZnSO_4$ (0.2 g L^{-1}) and $AlCl_3$ (0.15 g L^{-1}) was prepared. 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 N, 0.3 g NH_4NO_3 per L 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 ™, Bacto) and 15 g L^{-1} agar technical (Bacto). 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 ($Ca_3(PO_4)_2$; (Rhone Poulenc)), (b) rock phosphate (crude rock phosphate: $Ca_{10}(PO_4)_6X$ (where X ≈ Cl, F, OH), (c) aluminium phosphate ($AlPO_4$; Aldrich) and (d) ferric phosphate ($FePO_4.H_2O$; BDH).

3.2.2 Methods

3.2.2.1 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 sterilised by autoclaving at 121°C for 20 min. The sterilised phosphates were transferred to 10 mL aliquots of liquid medium sterilised in 25 mL McCartney bottles.

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

3.2.2.2 Preparation of bacterial cultures from freeze-dried ampoules and estimation of numbers of bacteria

Freeze-dried ampoules were opened aseptically, some sterile media added to suspend bacterial cells and using a loop, bacteria were streaked on minimal agar plates (2.2.2.2) and grown for 24 h at 28°C. The colonies were scraped from the plates using 2 to 4 mL sterile 0.85 per cent 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 (3.2.1.2). The plates were incubated at 28°C for 24 h or 48 h until distinct colonies had developed. The colonies were counted and calculated in colony forming units per mL of the original suspension (CFU mL^{-1}).

3.2.2.3 Setting up of 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 five days and cultures examined every day, a total of 75 cultures was 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 was 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 six weeks.

3.2.2.4 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 (3.2.2.2). 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, Sorvall RC 5 C; Haraeus-9304637) 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 analysed 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.

3.2.2.5 Preparation of bacterial cultures for experiments to measure organic acid production

3.2.2.5.1 Bacterial culturing

Freeze-dried ampoules of bacteria were opened aseptically; streaked on an agar plate; and grown for 24h at 28°C. Bacterial strains used in this experiment were FA001, FA002, FA003, FA004, FA009, FA010 and P4 (3.2.1.1). The cultures were scraped into a bottle using 0.85 per cent 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 incubated for 48h at 28°C. A control sample with no added bacteria was included. All assays were carried out in triplicate.

3.2.2.5.2 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 (BDH; 20-50 US mesh) for about 10-15 min to remove salt by adsorbing cations to obtain pure acid and the samples were filtered through 0.45µm 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 UV-Vis detector. The Polypore H column designed for organic acid separation was used with dilute sulphuric acid as a mobile phase (Appendix 3.1). A Polypore H column is a member of the family of 10 µm porous styrene divinylbenzene ion exchange reversed-phase resins for non-polar molecules. The column was equilibrated using 2.5 mM 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.

3.2.2.6 Statistical analysis

Data were analysed using statistical software Genstat (ver 7.0) (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 chapter.

3.3 Results

Inoculation of liquid media with different bacterial strains led to significant differences in the amount of P-mobilisation, pH, number of bacteria and organic acids in the medium while uninoculated controls remained almost unchanged throughout the experiment.

3.3.1 Solubilisation of phosphorus from $Ca_3(PO_4)_2$ in a medium containing $(NH_4)_2SO_4$ as the source of N

3.3.1.1 Phosphorus solubilisation

The phosphorus solubilised 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 3.1

Table 3.1 P-mobilisation (mg L^{-1}) 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.

All values are the mean of three replicates assays.

Strains	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x 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 0.05 level by LSD.

Table 3.1 shows the significant effect of using different bacterial strains on the concentration of soluble P in the liquid media with time of incubation. Mobilisation of soluble P can be calculated as soluble P at day one, less soluble P at day zero. One day after inoculation, the highest net increase in soluble P (total soluble P - soluble P at zero time) was in the culture inoculated with the strain FA001 (23.67 mg L^{-1} – 8.27 mg L^{-1} = 15.39 mg L^{-1}) followed by FA010 (16.73 mg L^{-1} – 8.27 mg L^{-1} = 8.45 mgL^{-}

^{1}). Low P levels were mobilised by the strains FA002, FA003, FA004, and FA009. After five days the highest amount of P was mobilised by strains FA001 (80.15 - 8.25 = 71.87 mg P L^{-1}), and FA010 (77.90 - 8.27 = 69.63 mg P L^{-1}). The P mobilised by strains FA002, FA003, FA004 and FA009 after five days was significantly less than for strains FA001 and FA010. There was no significant difference between P mobilised by strains FA001 and FA010, or between strains FA002, FA003, FA004 and FA009.

3.3.1.2 pH changes (-ΔpH)

The pH of the bacterial cultures declined over a five day incubation period from an initial pH of 7.64 as shown in Figure 3.1. The results shown in Figure 3.1 are shown in Table 3.2 as changes in the pH of the media over a five day period.

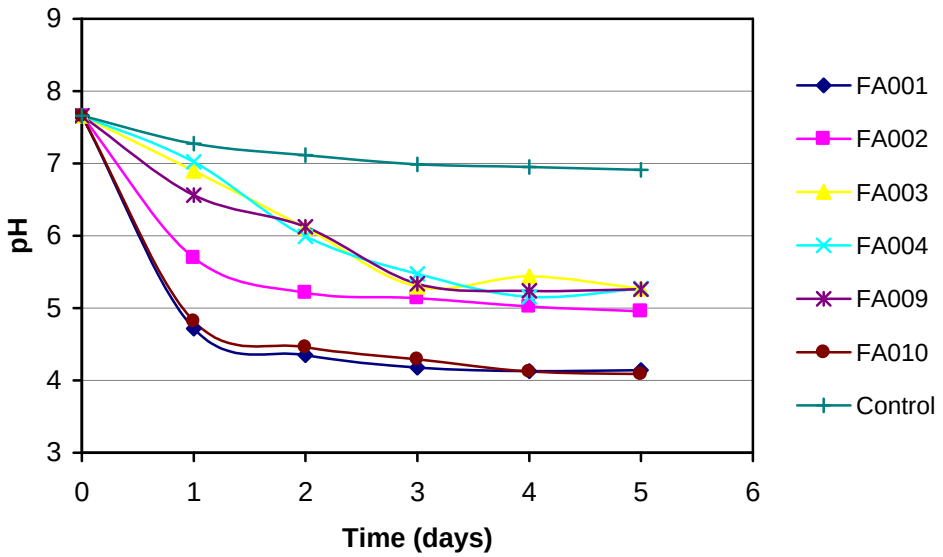

Figure 3. 1 The changes in pH in six cultures containing bacterial strains and a control culture containing $Ca_3(PO_4)_2$, and using $(NH_4)_2SO_4$ as the source of N over a five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial cultures and the control culture was 7.64.

Table 3.2 pH changes (-ΔpH) 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 five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial cultures and a control culture was 7.64.

Strains	pH changes (-ΔpH)					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.20 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

The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for five days (Tables 3.1 and 3.2) is shown in Figure 3.2. These data best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.812).

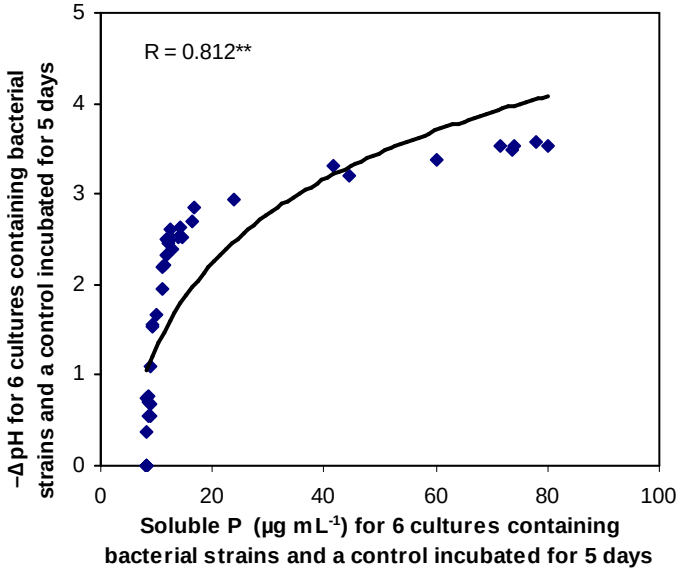

Figure 3.2 Relationship between soluble P and –ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $Ca_3(PO_4)_2$, and N as $(NH_4)_2SO_4$. The mean values were calculated from three replicates and zero day data were included. ** significant at 0.01 level of probability

The mean soluble P and the mean –ΔpH value for the five day incubation period were calculated for each bacterial culture and the control culture. The relationship between these means is shown in Figure 3.3 and best fitted a logarithmic regression demonstrating a significant relationship at the 0.05 level probability (R = 0.877).

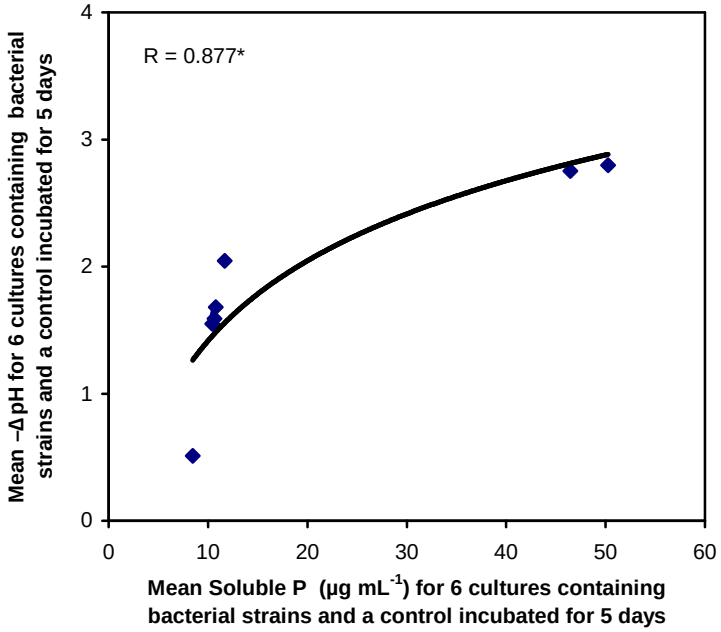

Figure 3.3 Relationship between mean soluble P and mean –ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for each strain for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $Ca_3(PO_4)_2$, and N as $(NH_4)_2SO_4$. The mean values were calculated from three replicates and zero day data were included. * significant at 0.05 level of probability

3.3.1.3 Bacterial counts

The bacteria in the cultures were counted immediately after inoculation and daily for 5 days of incubation, and the results are shown in Table 3.3.

Table 3.3 Number of bacteria (log_{10} CFU mL^{-1}) in cultures containing $Ca_3(PO_4)_2$, and $(NH_4)_2SO_4$ as source of N over a five day period.

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.

Strains	No. of Bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
	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	8.161a 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.141 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.

The highest and the lowest bacterial cell counts were found with the strains FA009 and FA003, respectively, one day after inoculation. After five days of inoculation the highest and the lowest number of bacterial cells were found with the strains FA004 and FA010, respectively.

3.3.1.4 Changes in soluble P, pH and bacteria number (CFU mL^{-1}) of six cultures containing bacterial strains over a five day incubation period

For each bacterial strain incubated in liquid medium containing $Ca_3(PO_4)_2$, and $(NH_4)_2SO_4$, data relating to soluble P, pH and bacterial number have been assembled and the results are shown in Figures 3.4 to 3.9. The actual P content has been multiplied by 5 and the bacterial numbers have been divided by 10^7 to get all in one figure.

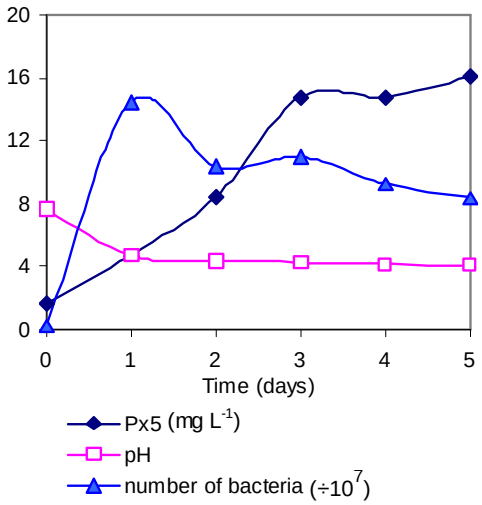

Figure 3.4 The number of bacteria, soluble P and pH of the strain FA001 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

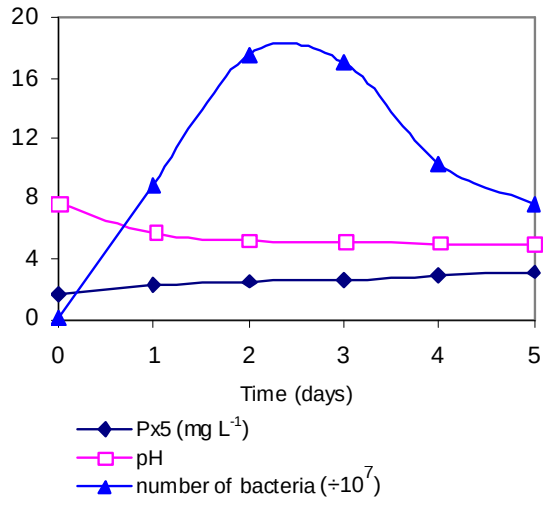

Figure 3.5 The number of bacteria, soluble P and pH of the strain FA002 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

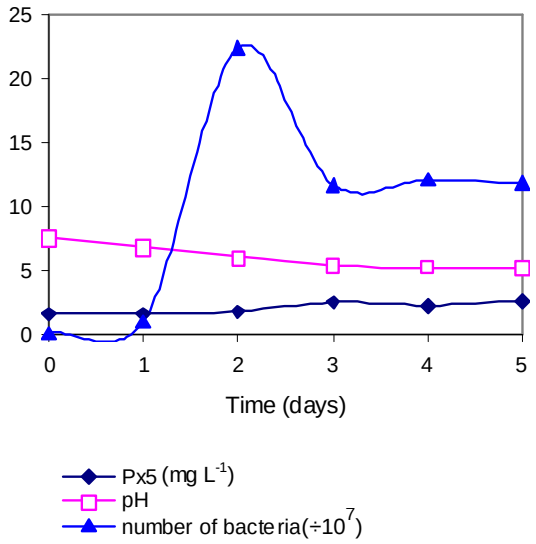

Figure 3.6 The number of bacteria, soluble P and pH of the strain FA003 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

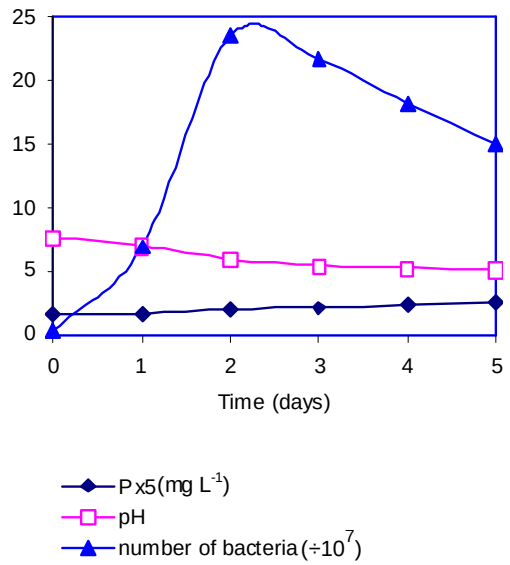

Figure 3.7 The number of bacteria, soluble P and pH of the strain FA004 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

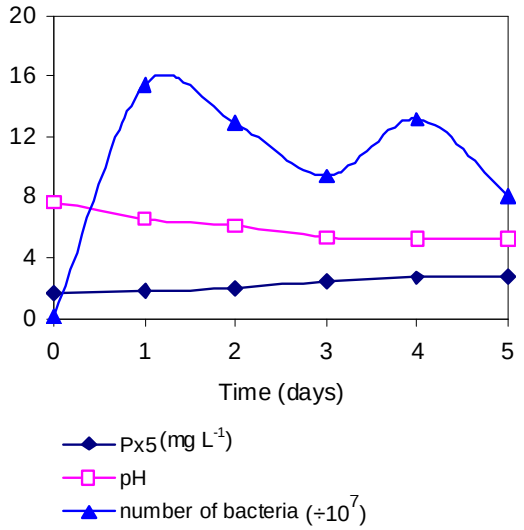

Figure 3.8 The number of bacteria, soluble P and pH of the strain FA009 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

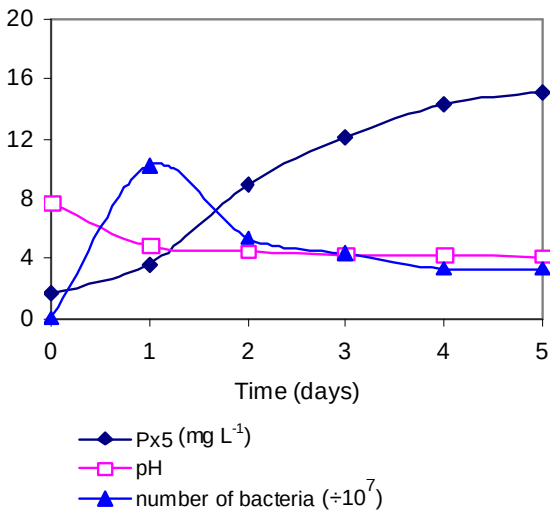

Figure 3.9 The number of bacteria, soluble P and pH of the strain FA010 in liquid medium containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ as the source of N over a 5 day incubation period.

3.3.1.5 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 (3.2.2.5). 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 3.4.

Table 3.4 Organic acids identified in 7 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.

All values are the mean of three replicate assays.

Strains	Organic acids in media (µg mL^{-1}/ 2 days)					
	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	<0.001	<0.001	<0.001	<0.001	<0.001
LSD at 0.05	0.251	0.871	0.049	0.335	0.273	0.260

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD

nd = not detected

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 highest amount of tartaric acid (7.92 µg mL^{-1}/ 2 days). The mobilisation of P by this strain has been shown to result from acid production (Rose, 2007).

3.3.2 Solubilisation of phosphorus from $Ca_3(PO_4)_2$ in a medium containing NH_4NO_3 as the source of N

3.3.2.1 Phosphorus solubilisation

Soluble P concentration in the media varied significantly with the bacterial strain when NH_4NO_3 was used as the source of nitrogen (Table 3.5).

Table 3.5 P- mobilisation (mg L^{-1}) 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.

All values are the mean of three replicates assays.

Strains	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 4.33 at 0.05 level of probability

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD

One day after inoculation the net increase in soluble P was the greatest with the FA001 strain followed by the FA002 strain. The lowest amounts of P were mobilised by the FA003, FA004 and FA009 strains, with a small, but significantly greater amount in the culture containing the FA010 strain. After five days the P mobilised in the culture containing the FA001 strain was significantly greater than for all the other cultures. In this nitrate-containing medium there was a significant difference in soluble P after five days of incubation between the cultures containing the FA001 and FA010 strains.

3.3.2.2 pH changes (-ΔpH)

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 (Figure 3.10; Table 3.6). One day after inoculation, the highest pH change (-ΔpH = 2.57) was in the culture containing the strain FA001 while the lowest was in the culture containing the strain FA003 (-ΔpH = 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 five day period.

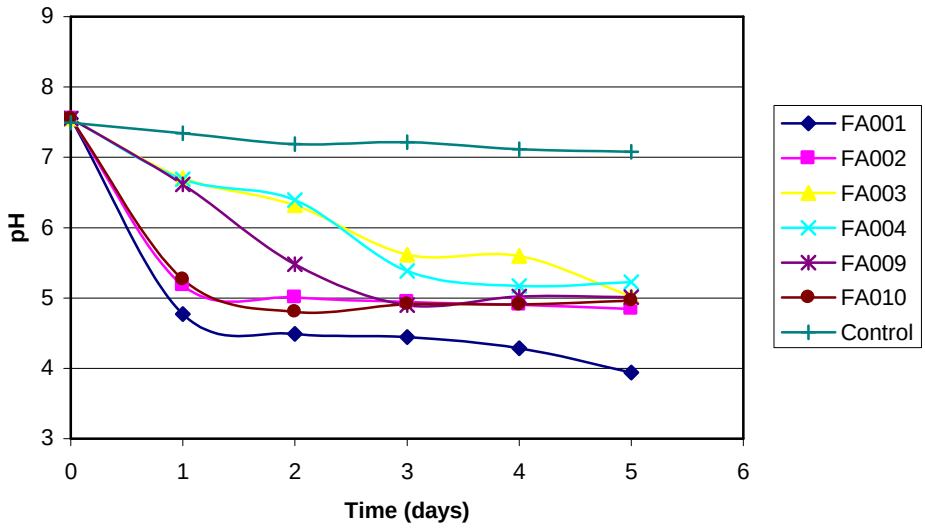

Figure 3.10 The changes in pH in six cultures containing bacterial strains and a control culture containing $Ca_3(PO_4)_2$ and using NH_4NO_3 as the source of N over a five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial culture and the control cultures was 7.49.

Table 3.6 pH changes (-ΔpH) 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 five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial cultures and a control cultures was 7.61.

Strains	pH changes (-ΔpH)					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.23 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for five days (Tables 3.5 and

3.6) is shown in Figure 3.11. These data best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.954).

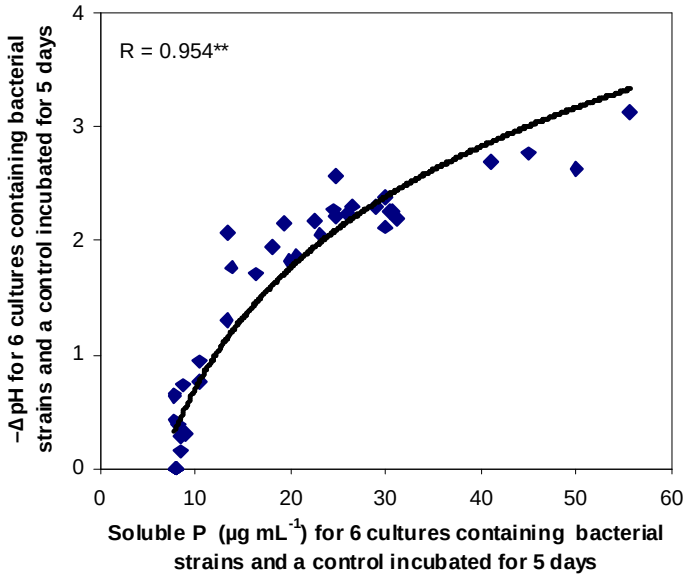

Figure 3.11 Relationship between soluble P and –ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $Ca_3(PO_4)_2$, and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ** = significant at 0.01 level of probability

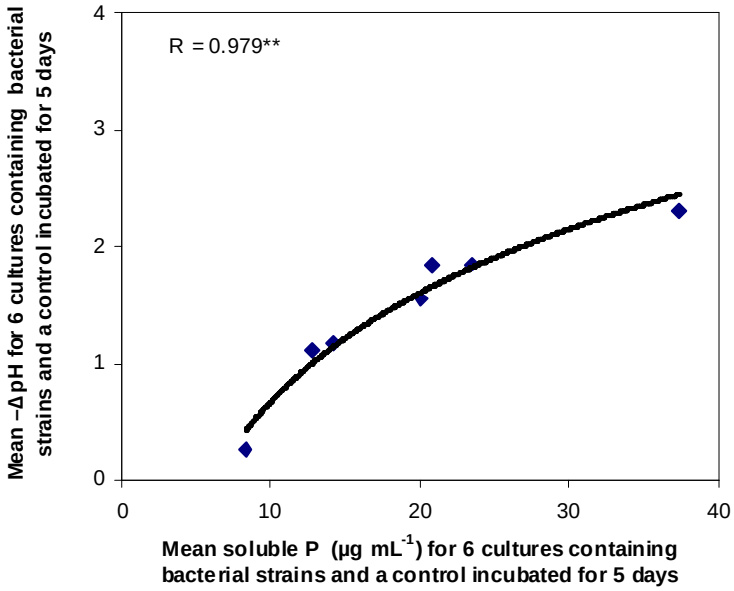

Figure 3.12 Relationship between mean soluble P and –ΔpH values in the liquid medium containing $Ca_3(PO_4)_2$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $Ca_3(PO_4)_2$, and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ** = significant at 0.01 level of probability

The mean soluble P and the mean –ΔpH value for the five day incubation period were calculated for each bacterial culture and the control culture. The relationship between these means is shown in Figure 3.12 and best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.979).

3.3.2.3 Bacterial counts

The number of bacteria (CFU mL^{-1}) varied significantly throughout the experiment when NH_4NO_3 was used as a source of N (Table 3.7), with most growth also occurring in day one. After one 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 five days after inoculation the highest and the lowest bacterial live cell counts were found in cultures containing the FA003 and FA010 strains, respectively.

Table 3.7 Number of bacteria (log_{10} CFU mL^{-1}) in cultures containing $Ca_3(PO_4)_2$, and NH_4NO_3 as source of N over a five day period.

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 ^{O}C.

Strains	No of Bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.237 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

3.3.2.4 Changes in soluble P, pH and bacteria number (CFU mL^{-1}) of six cultures containing bacterial strains over a five day incubation period

For each bacterial strain incubated in liquid medium containing $Ca_3(PO_4)_2$ and NH_4NO_3, data relating to soluble P, pH and number of bacteria have been assembled

and the results are shown in Figures 3.13 to 3.18. The actual P content has been multiplied by 5 and the bacterial numbers have been divided by 10^7 to get all in one figure.

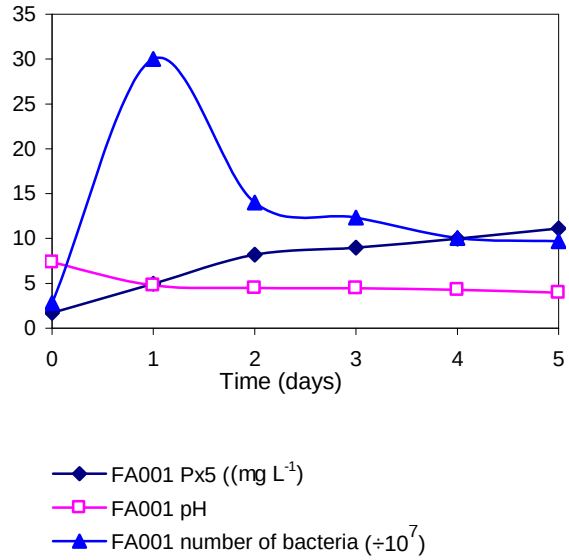

Figure 3.13 The number of bacteria, soluble P and pH of the strain FA001 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

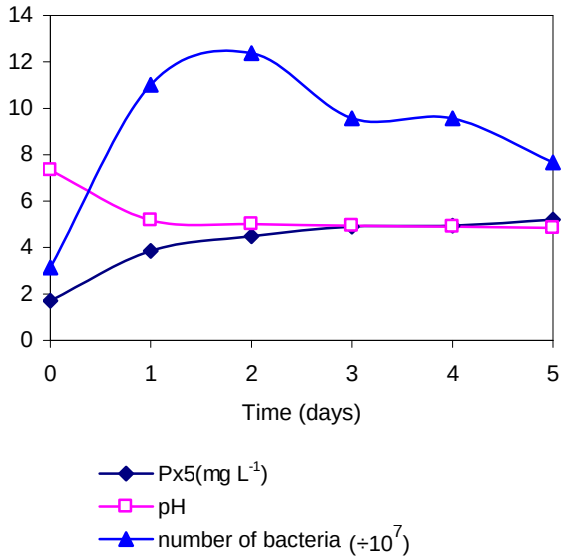

Figure 3.14 The number of bacteria, soluble P. and pH of the strain FA002 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

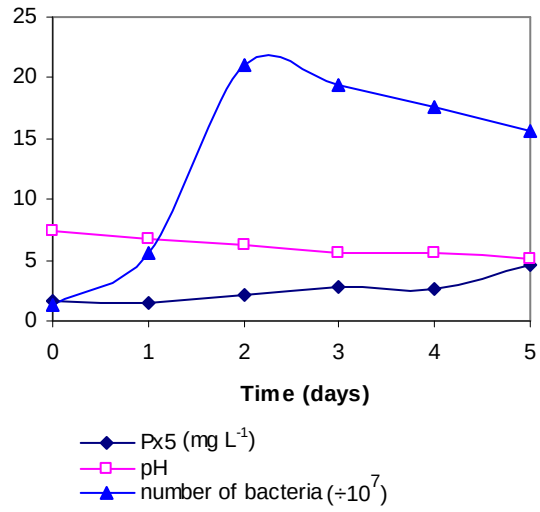

Figure 3.15 The number of bacteria, soluble P and pH of the strain FA003 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

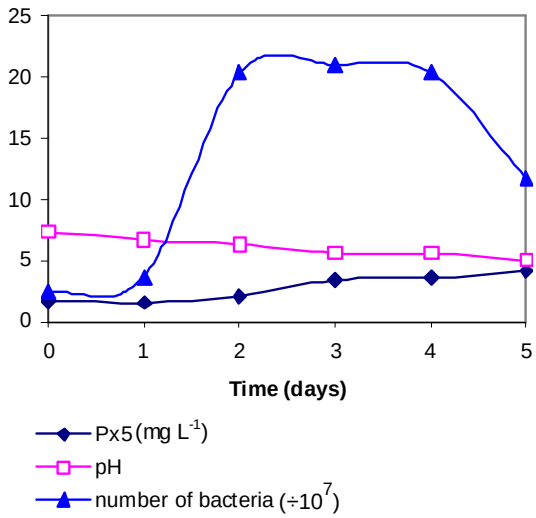

Figure 3.16 The number of bacteria, soluble P and pH the strain FA004 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

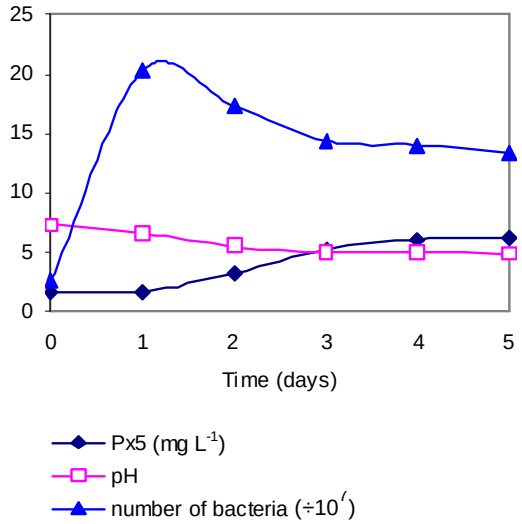

Figure 3.17 The number of bacteria, soluble P and pH of the strain FA009 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

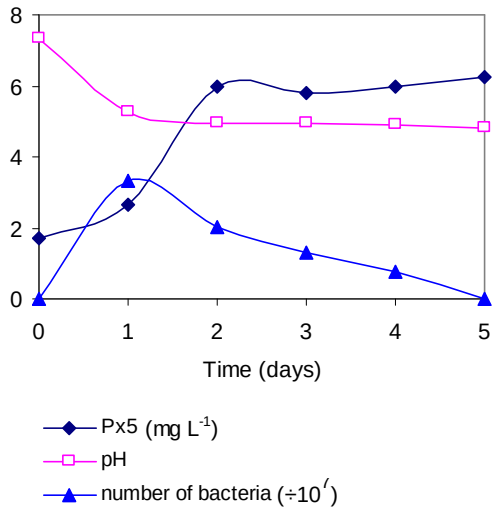

Figure 3.18 The number of bacteria, soluble P, and pH of the strain FA010 in liquid medium containing $Ca_3(PO_4)_2$, and NH_4NO_3 as the source of N over a 5 day incubation period.

3.3.3 Solubilisation of phosphorus from rock phosphate in a medium containing NH_4NO_3 as the source of N

3.3.3.1 Phosphorus solubilisation

The P solubilised 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 3.8.

Table 3.8 P-mobilisation (mg L^{-1}) 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.

All values are the mean of three replicates assays.

Strains	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
	0	1	2	3	4	5
Control	0.32 a A	0.38 c A	0.57 d A	0.71e 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 4.87 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

One day after inoculation, the highest amount of P (12.72 - 0.32 = 12.4 mg L^{-1}) was solubilised in the culture containing the FA001 strain. At five days after inoculation cultures containing the FA001 and FA010 strains had significantly more soluble P than the other cultures. Cultures containing all other strains also solubilised significantly more P than was found in the control containing rock phosphate alone (Table 3.8).

3.3.3.2 pH changes (-ΔpH)

The pH of six cultures containing bacterial strains in a liquid medium containing rock phosphate as source of P declined during a five day incubation period (Table 3.9). One day after inoculation the highest (-ΔpH 2.39) and the lowest (-ΔpH 1.85) -ΔpH were observed with the strains FA010 and FA004 (Table 3.9). After five days incubation, the greatest –ΔpH values were for cultures containing strains FA001 and FA010 (3.09 and 3.05).

Table 3.9 pH changes (-ΔpH) 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 five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial cultures and a control culture was 6.79.

Strains	pH changes (-ΔpH)					
	Time after inoculation (days)					
	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	2.51ab 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.21 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for five days (Tables 3.8 and 3.9) is shown in Figure 3.19. These data best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.989).

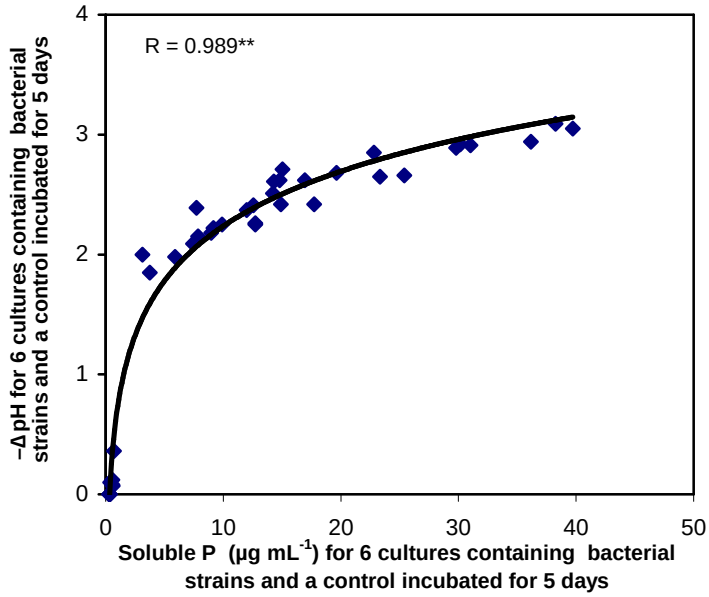

Figure 3.19 Relationship between soluble P and –ΔpH values in the liquid medium containing rock phosphate for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained rock phosphate, and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ** = significant at 0.01 level of probability

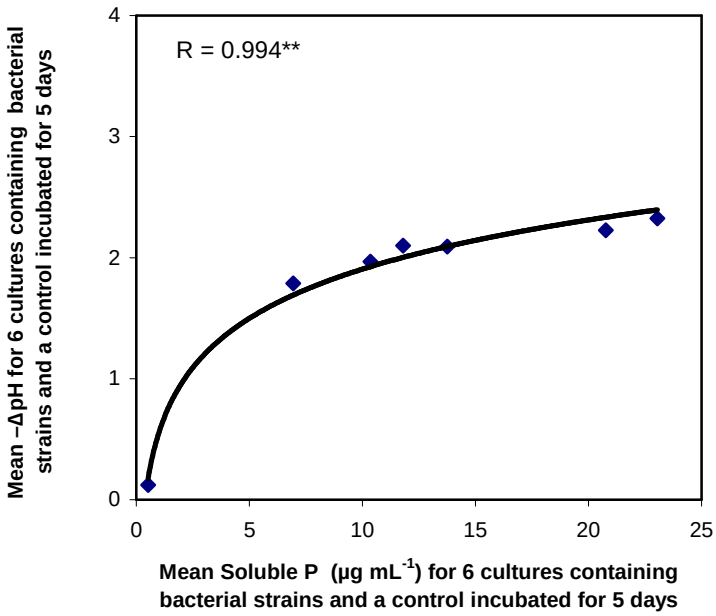

Figure 3.20 Relationship between mean soluble P and mean –ΔpH values in the liquid medium containing rock phosphate for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained rock phosphate and NH_4NO_3 as N. The mean value was taken from three replicates and zero day was also considered. ** = significant at 0.01 level of probability

The mean soluble P and the mean –ΔpH value for the five day incubation period were calculated for each bacterial culture and the control culture. The relationship between these means is shown in Figure 3.20 and best fitted a logarithmic regression demonstrating a significant relationship at the 0.01 level probability (R = 0.994).

3.3.3.3 Bacterial counts

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 unavailable P. One day after inoculation the highest (8.010 log_{10}CFU mL^{-1}) and the lowest (7.413 log_{10}CFU mL^{-1}) number of live cells were found in cultures containing the strains FA003 and FA001, respectively (Table 3.10). Five days after inoculation, the highest and the lowest bacterial live cell counts were found in cultures containing the strains FA002 and FA001, respectively.

Table 3.10 Number of bacteria (log_{10} CFU mL^{-1}) in cultures containing rock phosphate and NH_4NO_3 as source of N over a five day period.

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.

Strains	No of Bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
	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 ab 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.125 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

3.3.3.4 Changes in soluble P, pH and bacteria number (CFU mL^{-1}) of six cultures containing bacterial strains over a five day incubation period

For each bacterial strain incubated in liquid medium containing rock phosphate and NH_4NO_3, data relating to soluble P, pH and number of bacteria have been assembled and the results are shown in Figures 3.21 to 3.26. The actual P content has been

multiplied by 5 and the bacterial numbers have been divided by 10^7 to get all in one figure.

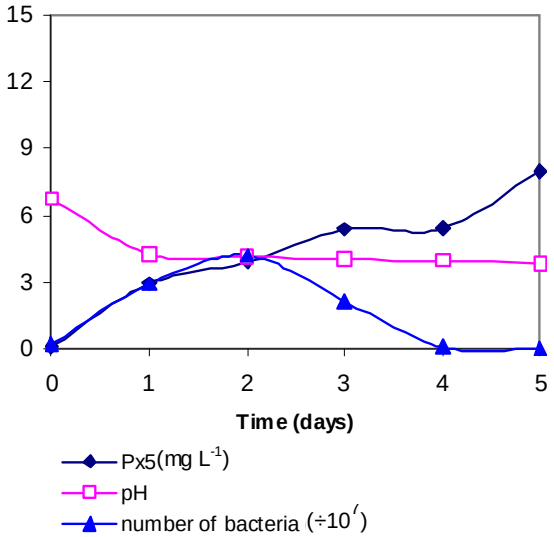

Figure 3.21 Rate of change in number of bacteria, available P, and pH of FA001 in liquid medium containing NH_4NO_3, and rock phosphate as source of N and P, respectively.

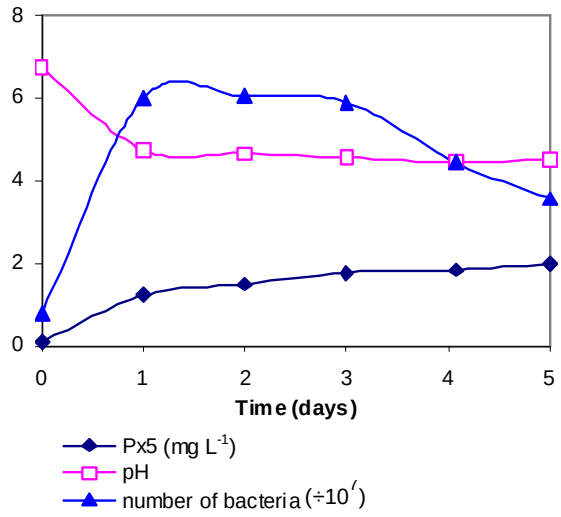

Figure 3.22 Rate of change in number of bacteria, available P and pH of FA002 in liquid medium containing NH_4NO_3, and rock phosphate as source of N and P, respectively.

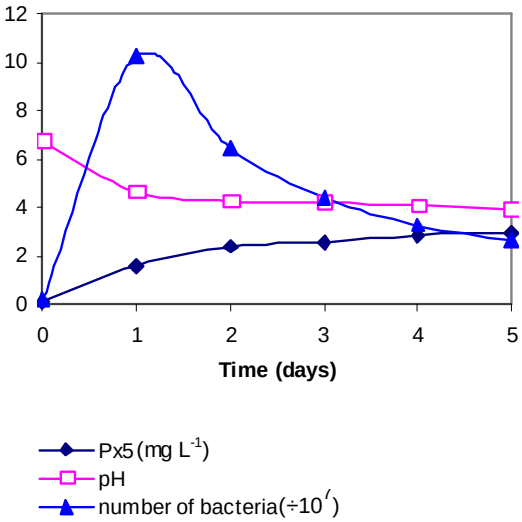

Figure 3.23 Rate of change in number of bacteria, available P, and pH of FA003 in liquid medium containing NH_4NO_3, and rock phosphate as source of N and P, respectively.

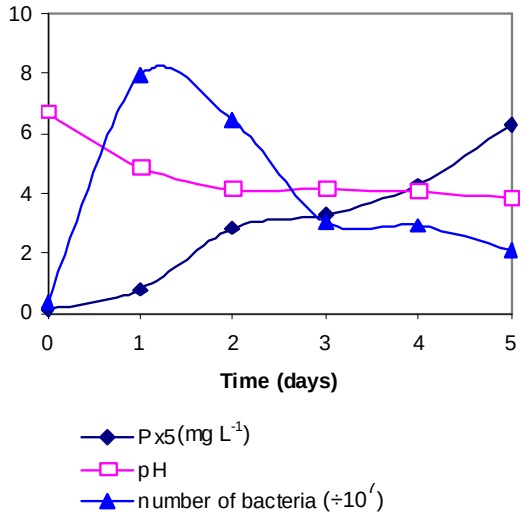

Figure 3.24 Rate of change in number of bacteria, available P and pH of FA004 in liquid medium containing NH_4NO_3, and rock phosphate as source of N and P, respectively.

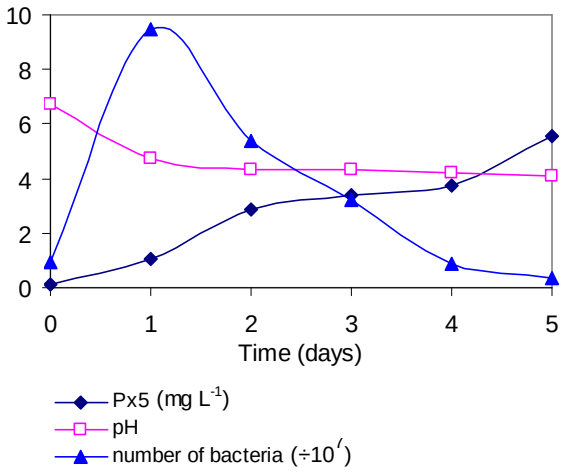

Figure 3.25 Rate of change in number of bacteria, available P and pH of FA009 in liquid medium containing NH_4NO_3 and rock phosphate as source of N and P, respectively.

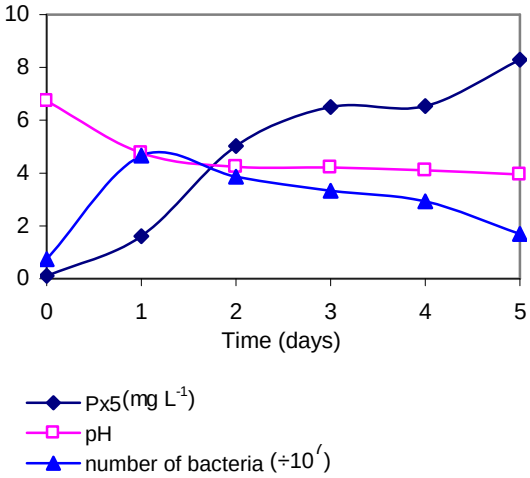

Figure 3.26 Rate of change in number of bacteria, available P and pH of FA010 in liquid medium containing NH_4NO_3, and rock phosphate as source of N and P, respectively.

3.3.4 Solubilisation of phosphorus from $AlPO_4$ in a medium containing NH_4NO_3 as the source of N

3.3.4.1 Phosphorus solubilisation

Phosphorus mobilisation from $AlPO_4$ was generally poor. The mobilisation 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 (3.3.1.1; 3.3.2.1; 3.3.3.1). One day after inoculation the cultures containing strains FA001 and FA010 mobilised significantly more P than other cultures (Table 3.11). Five days after inoculation, there was no significant difference in P mobilisation between cultures containing these two strains.

Table 3.11 P-mobilisation (mg L^{-1}) from $AlPO_4$ with time, using NH_4NO_3 as source of N, for six cultures containing bacterial strains and a culture without bacteria.

All values are the mean of three replicates assays.

Strains	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.34 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

3.3.4.2 pH changes (-ΔpH)

The pH of six cultures containing different bacterial strains in a medium containing $AlPO_4$ as source of P declined over a five day incubation period (Table 3.12). 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.

Table 3.12 pH changes (-ΔpH) 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 five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial and a control cultures was 6.85.

Strains	pH changes (-ΔpH)					
	Time after inoculation (days)					
	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	2.72 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.26 at 0.05 level of probability

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD

The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for five days (Tables 3.11 and 3.12) is shown in Figure 3.27. These data best fitted a linear regression demonstrating a significant relationship at the 0.01 level of probability (R = 0.519).

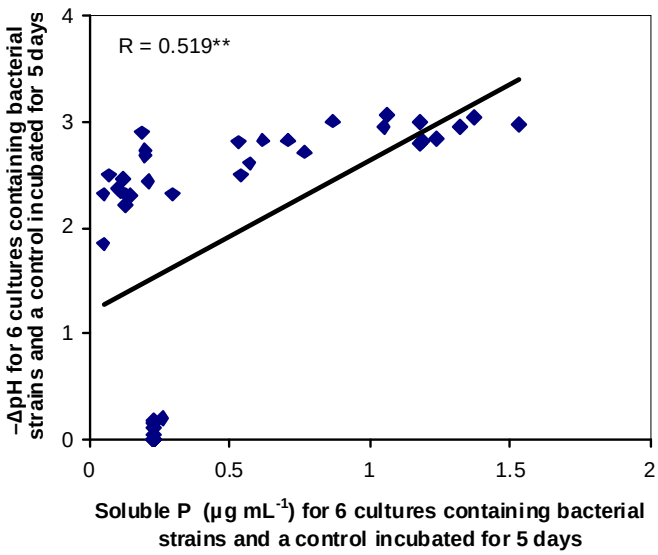

Figure 3.27 Relationship between soluble P and –ΔpH values in the liquid medium containing $AlPO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $AlPO_4$, and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ** = significant at 0.01 level of probability

The mean soluble P and the mean –ΔpH value for the five day incubation period were calculated for each bacterial culture and the control culture. The relationship between these means is shown in Figure 3.28 and best fitted a linear regression but with insignificant relationship at the 0.05 level probability (R = 0.50).

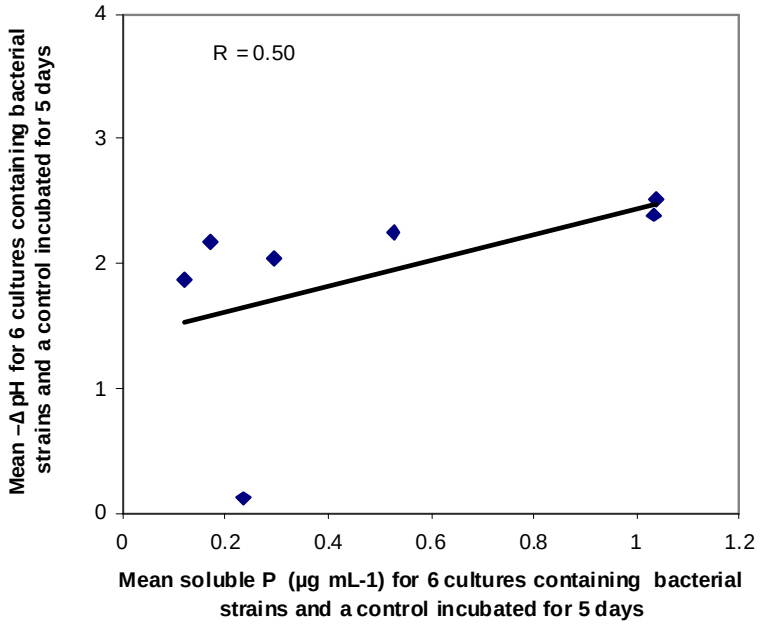

Figure 3.28 Relationship between mean soluble P and –ΔpH values in the liquid medium containing $AlPO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $AlPO_4$, and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ns = not significant

3.3.4.3 Bacterial counts

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 3.13.

Table 3.13 Number of bacteria (log_{10} CFU mL^{-1}) in cultures containing $AlPO_4$ and NH_4NO_3 as source of N over a five day period.

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.

Strains	No of Bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.28 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

One day after inoculation, the highest bacterial growth (7.663 log_{10}CFU mL^{-1}) was found in the culture containing the FA001 strain, whereas the lowest total bacterial growth (6.327 log_{10}CFU mL^{-1}) was in the culture containing the FA010 strain. After five days of incubation the highest number of bacteria was found in the culture containing the strain FA001.

3.3.5 Solubilisation of phosphorus from $FePO_4$ in a medium containing NH_4NO_3 as the source of N

3.3.5.1 Phosphorus solubilisation

Soluble P concentration in liquid cultures with different bacterial strains varied significantly using $FePO_4$ as a source of P (Table 3.14). It was found that one day and five days after inoculation, the culture containing the bacterial strain FA001 contained the highest amount of soluble P (3.95-3.53 = 0.42 mg L^{-1} at day 1 and 7.84 - 4.26 = 3 .53 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 culture containing the strain FA001 was greater than that of the other five strains but the soluble P in the cultures containing the strains FA003, FA004, FA009 and FA010 was below the five day control and initial soluble P (day zero) of the cultures containing these strains.

Table 3.14 P-mobilisation (mg L^{-1}) from $FePO_4$ with time, using NH_4NO_3 as source of N, for six cultures containing bacterial strains and a culture without bacteria.

All values are the mean of three replicates assays.

Strains	Soluble P (mg L^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.51 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

3.3.5.2 pH changes (- Δ pH)

The pH changes (-Δ pH) observed in six cultures containing different bacterial strains are shown in Table 3.15. One day after inoculation, the highest pH change (–ΔpH 2.67) was in the culture containing the strain FA002 followed by the culture containing the strain FA001 (–ΔpH 2.39), while the lowest pH change was found in the culture containing the strain FA003 (–ΔpH 0.56). Five days after inoculation the highest and the lowest –ΔpH occurred with FA002 and FA009 strains, respectively. The pH changes for the cultures containing the strains FA003, FA004, FA009 and FA010 were smaller than for cultures containing other P sources.

Table 3.15 pH changes (-ΔpH) 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 five day period.

The pH of the minimal medium was adjusted to 7.0 (3.2.1.2). After autoclaving the initial pH of all bacterial cultures and the control cultures was 7.74.

Strains	pH changes (-ΔpH)					
	Time after inoculation (days)					
	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	2.59 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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.27 at 0.05 level of probability.

*Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

The relationship between the soluble P and the –ΔpH for each day for cultures containing six bacterial strains and a control incubated for five days (Tables 3.14 and 3.15) is shown in Figure 3.29. These data best fitted a linear regression demonstrating a significant relationship at the 0.05 level of probability (R = 0.387).

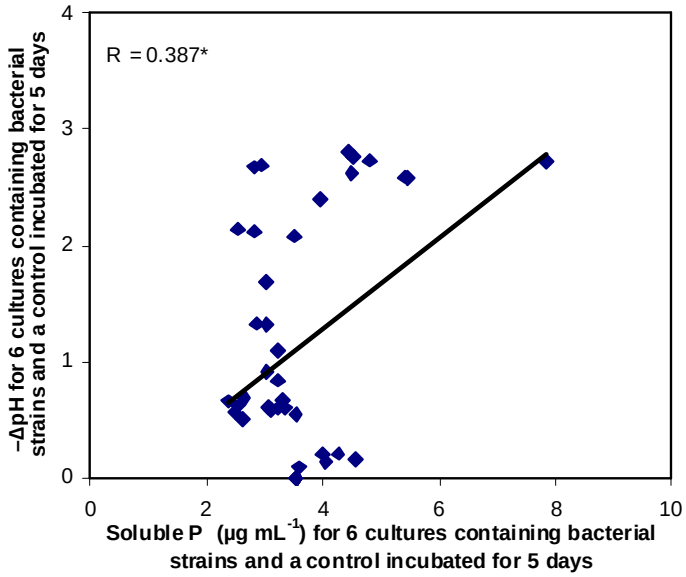

Figure 3.29 Relationship between soluble P and –ΔpH values in the liquid medium containing $FePO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $FePO_4$ and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. * = significant at 0.05 level of probability

The mean soluble P and the mean –ΔpH value for the five day incubation period were calculated for each bacterial culture and the control culture. The relationship between these means is shown in Figure 3.30 and best fitted a logarithmic regression with an insignificant relationship at the 0.05 level probability (R = 0.498).

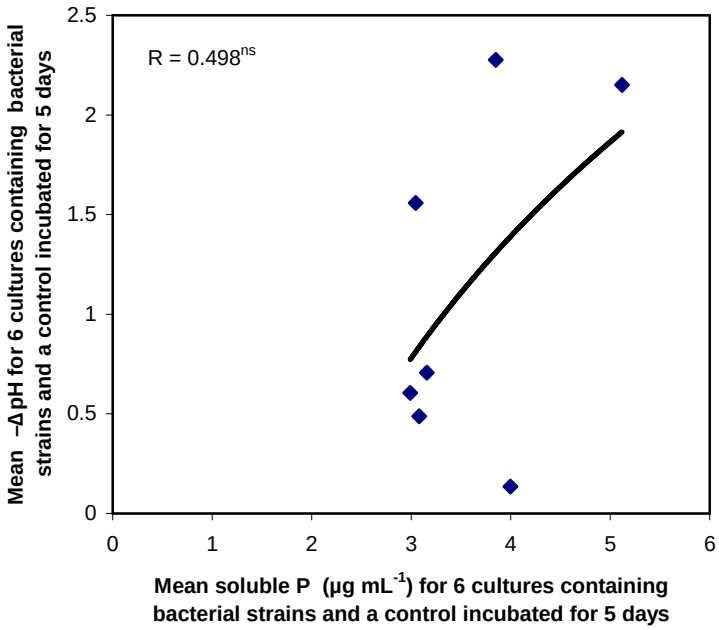

Figure 3.30 Relationship between mean soluble P and –ΔpH values in the liquid medium containing $FePO_4$ for six cultures containing bacterial strains and a control culture incubated for 5 days.

All cultures contained $FePO_4$ and NH_4NO_3 as N. The mean values were calculated from three replicates and zero day data were included. ns = not significant

3.3.5.3 Bacterial counts

The growth of different strains was significantly varied in the liquid culture containing $FePO_4$ as source of P (Table 3.16). One day after inoculation, the highest (7.066 $\log_{10}$CFU mL^{-1}) and the lowest (6.475 $\log_{10}$CFU mL^{-1}) bacterial growth was found in cultures containing the bacterial strains FA001 and FA010, respectively. A similar result was found five days after inoculation.

Table 3.16 Number of bacteria (log_{10} CFU mL^{-1}) in cultures containing $FePO_4$, and NH_4NO_3 as source of N over a five day period.

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.

Strains	No of Bacteria (log_{10}CFU mL^{-1})					
	Time after inoculation (days)					
	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

F probability for treatments x time interaction was <0.001. The LSD values for treatments x time interaction was 0.236 at 0.05 level of probability.

* Means followed by a common letter in a column are not significantly different at 0.05 level by LSD.

3.4 Summary of results for P mobilisation, pH change and bacterial growth

3.4.1 Summary of results for P-mobilisation

The summary of the results for P mobilisation in the five media inoculated with bacterial strains FA001 and FA010 are presented in Table 3.17. The increases in soluble P are shown at one and five days after inoculation with these two bacterial strains. The strains FA010 showed negative value in $FePO_4$. It could be P uptake by the bacteria was higher than its mobilisation and it should not be considered as a significant figure.

Table 3.17 Net P mobilisation (mg L^{-1}) in cultures containing the bacterial strains FA001 and FA010.

Medium	**FA001**		**FA010**	
	Increase in soluble P (mg L^{-1})			
	1 DAI	5 DAI	1 DAI	5 DAI
$Ca_3(P0_4)_2/(NH_4)_2SO_4$	15.40	71.88	8.06	69.63
$Ca_3(P0_4)_2/NH_4NO_3$	16.76	47.64	5.41	21.60
RockP/NH_4NO_3	12.40	37.93	7.40	39.39
$AlPO_4$ /NH_4NO_3	0.82	1.09	0.64	1.30
$FePO_4$ /NH_4NO_3	0.42	4.31	-1.00	-0.68

DAI = day after inoculation
RockP = rock phosphate

The results show the substantial differences between P released from the insoluble calcium phosphate sources and the aluminium and iron phosphates. The effect of the form of nitrogen is also evident from the results for soluble P in the media containing $(NH_4)_2SO_4$ or NH_4NO_3.

3.4.2 Summary of results for pH change

The summary of the results for pH change in the five media inoculated with bacterial strains FA001 and FA010 are presented in Table 3.18. The pH change in the medium is compared between the strains FA001 and FA010 with all other strains (FA002, FA003, FA004 and FA009) (Table 3.18).

Table 3.18 The average pH changes (–ΔpH) in cultures containing the bacterial strains, FA001 and FA010, and the other four strains, FA002, FA003, FA004 and FA009.

Medium	Mean –ΔpH 5 DAI	Mean –ΔpH 5 DAI
	Strains FA001&FA010	Strains FA002, FA003, FA004 & FA009
$Ca_3(PO_4)_2/(NH_4)_2SO_4$	3.56	2.54
$Ca_3(PO_4)_2/NH_4NO_3$	2.77	2.12
RockP/NH_4NO_3	3.07	2.65
$AlPO_4/NH_4NO_3$	2.97	2.64
$FePO_4/NH_4NO_3$	2.03	1.39

DAI = day after inoculation
RockP = rock phosphate

The results show substantial differences between the mean pH change value of the strains FA001 and FA010, and other strains (Table 3.18). These two strains (FA001 and FA010) have changed the pH more than all other strains. For the strains FA001 and FA010, the pH changes were greater in the $Ca_3(PO_4)_2$- and $(NH_4)_2SO_4$-containing medium than all other P and NH_4NO_3-containing media. The pH changes were less in the $FePO_4$-containing medium in all cases compared to other P-containing media.

3.4.3 Summary of results for bacterial growth

The summary of the results for the highest bacterial number in the five media inoculated with bacterial strains are presented in Table 3.19.

Table 3.19 Time for maximum bacterial growth in different media.

Inoculation numbers of live bacteria varied in the different P-containing media and with the different strains.
Bacterial number increased from two to more than 1000 times.

Medium	Soluble P ($mg\ L^{-1}$)	Highest bacterial number			
		1DAI	2DAI	3DAI	4DAI
$Ca_3(PO_4)_2/(NH_4)_2SO_4$	8.27	FA001, FA009, FA010	FA002, FA003, FA004		
$Ca_3(PO_4)_2/NH_4NO_3$	7.94	FA001, FA002, FA009, FA010	FA003, FA004		
$RockP/NH_4NO_3$	0.32	FA001, FA002, FA003, FA004, FA009 FA010			
$AlPO_4\ /NH_4NO_3$	0.23	FA003, FA004, FA009, FA010	FA001, FA002		
$FePO_4\ /NH_4NO_3$	3.35	FA001	FA009, FA010	FA003	FA002, FA004

DAI = day after inoculation

The results show that for of the strain FA001, in all media containing P and N the highest growth was obtained 24 h after inoculation except in the $AlPO_4$-containing medium, where the highest number was obtained after 48 h of inoculation. In case the of the strain FA010, in all media containing P and N the highest growth was obtained 24 h after inoculation except for the $FePO_4$-containing medium, where its highest number was obtained after 48 h of inoculation

The increase in bacterial numbers in relation to the original inoculation numbers varied substantially, as shown in Table 3.20.

Table 3.20. Maximum bacterial number after inoculation compared to number of bacteria used for the initial inoculation.

The bacterial number is the ratio of the maximum number and the inoculation number.

Medium	FA001	FA002	FA003	FA004	FA009	FA010
$Ca_3(PO_4)_2/(NH_4)_2SO_4$	69.3	1358.3	1285.3	235.5	60.5	102.3
$Ca_3(PO_4)_2/NH_4NO_3$	14.2	4.04	14.9	8.6	7.0	156.0
$RockP/NH_4NO_3$	16.1	7.6	5.6	2.9	10.2	6.3
$AlPO_4\ /NH_4NO_3$	2.4	3.6	3.7	2.1	2.6	11.0
$FePO_4\ /NH_4NO_3$	1.9	4.0	1.0	0.6	6.0	1.18

The highest bacterial population increase compared to the original inoculation number was found in the medium containing $Ca_3(PO_4)_2/(NH_4)_2SO_4$ for all strains. The ratio of

bacterial population after inoculation to original bacterial number was lower for all strain in the media containing $AlPO_4$ and $FePO_4$ with NH_4NO_3.

3.5 Discussion

The mobilisation 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 mobilisation 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 (3.2.1.2). Six bacterial strains identified as P-mobilisers by their ability to produce clear haloes on agar plates containing insoluble $Ca_3(PO_4)_2$ (2.3.4) have been tested for P-mobilising capacity in liquid culture. The bacterial strains used were identified as *Pantoea* spp, FA001 and FA010, and the *Burkholderia* spp, FA003, FA004, FA005, and FA009 (Chapter 2). 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}, $Ca3(PO_4)_2/NH_4NO_3$, 7.94 mg L^{-1} FePO4, 3.53 mg L^{-1}, rock-P, 0.32 mg L^{-1}, $AlPO_4$, 0.23 mg L^{-1} before bacterial inoculation (Table 3.19). It has been reported that aluminium and iron phosphates are less soluble than calcium phosphates (Lindsay *et al.*, 1959; Whitelaw *et al.*, 1999). In these experiments soluble P from rock phosphate was very low. Overall the strains FA001 and FA010 mobilised P better than the other four strains, and strain FA001 was a better mobiliser than FA010. In the three cultures containing calcium salts, all bacterial strains mobilised significantly more P than the control culture (without bacteria).

One day after bacterial inoculation, the net increase in soluble P in cultures containing $Ca_3(PO_4)_2$ and $(NH_4)_2SO_4$ or NH_4NO_3 was similar and slightly greater than for the rock phosphate and NH_4NO_3 culture. The amounts of soluble P in the cultures containing the bacterial strains FA001 and FA010 are compared in Table 3.17. One day after inoculation, for cultures containing aluminium and iron phosphates the soluble P in the cultures containing the strains FA001 and FA010 was about four per cent of that found in cultures containing Ca sources (Table 3.17). In the $AlPO_4$-containing medium, one day after inoculation, the cultures containing strains FA002, FA003, FA004 and FA009 (Table 3.11) contained amounts of soluble P that were lower or not significantly different from the control. It is possible that the reason for the reduced levels of soluble P in this medium is that P mobilised by these strains from $AlPO_4$ was less than the P requirement for the growth and development of these strains. Similar results were observed for the cultures containing $FePO_4$ (Table 3.14). Bacterial P was not included in the soluble P measured in the medium in these experiments (Tables 3.11 and 3.14). The lower soluble P values after incubation are in comparison with initial P concentrations.

Five days after inoculation it was found that in all five cultures the strains FA001 and FA010 mobilised greater amounts of P from the calcium substrates than from aluminium and iron phosphates (Table 3.17). For cultures containing the strain FA001, P mobilised from aluminium and iron phosphates was about five per cent of that mobilised from calcium salts. For cultures containing the strain FA010, P mobilised from $AlPO_4$ was about three per cent of that from calcium salts and there was a net loss of soluble P in the culture containing $FePO_4$ (Table 3.17). Although the soluble P in cultures containing the bacterial strains FA001 and FA010 was substantially greater in cultures containing calcium sources rather than aluminium and iron phosphate, there were differences between these strains. One day after inoculation, P mobilisation in the cultures containing the strain FA010 was about 50 per cent of that in the cultures containing the strain FA001. Five days after inoculation, P mobilisation in cultures containing the strain FA010 was about 80 per cent of that in the cultures containing the strain FA001. This may indicate that the FA001 strain grows more rapidly in these media in the early part of the five day incubation period.

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 (Table 3.18). The differences in –ΔpH between cultures containing the strains FA001 and FA010, and the other strains was not as great as the difference between the P mobilised. There were some differences between the –ΔpH in cultures containing the strains FA001 and FA010. The –ΔpH 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 –ΔpH 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 –ΔpH values for six cultures containing bacterial strains and a control culture, and the relationship between mean soluble P and mean –ΔpH values were examined for the media containing $Ca_3(PO_4)_2/(NH_4)_2SO_4$, $Ca_3(PO_4)_2/NH_4NO_3$, and RockP/NH_4NO_3. A significant relationship between the soluble P and –ΔpH was found in both individual and mean data (Figures 3.2, 3.3, 3.11, 3.12, 3.19 and 3.20). These relationships demonstrate the influence of the reduction in pH on bacterial P-mobilisation 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 –ΔpH values for six cultures containing bacterial strains and a control culture was significant for $AlPO_4$, (Figure 3.27) and for $FePO_4$, (Figure 3.29). The relationships between the mean soluble P and mean –ΔpH values were insignificant for $AlPO_4$, (Figure 3.28) and $FePO_4$, (Figure 3.30). These relationships for the aluminium and iron P-containing media suggest that –ΔpH is less important for P-mobilisation 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-mobilisation 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 recognised (Salih *et al.*, 1989; Halder *et al.*, 1990; Nautiyal *et*

al., 2000). Ammonium (NH_4^+) assimilation associated with proton (H^+) exchange and subsequent acidification is a common phenomenon (Dighton and Boddy, 1989; 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 (Table 3.18) might be because of ammonium assimilation by proton (H^+) exchange and subsequent acidification of the medium. Higher P mobilisation 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 mobilised in the media containing NH_4NO_3 rather than $(NH_4)_2SO_4$. These results suggest that there may be different mechanisms for P solubilisation, or different growth rates for these bacterial strains with different N sources. It has been reported that different strains of *Rhizobium* were able to solubilise hydroxyapatite in liquid culture without NH_4^+ and it was concluded that different mechanisms, other than acid production, were involved in P mobilisation (Halder and Chakrabartty, 1993). In this experiment (Tables 3.1 and 3.5) the utilisation of NH_4NO_3 meant supplying fewer NH_4^+ ions then when $(NH_4)_2SO_4$ was used. The increased mobilisation of P with these four strains was consistent with the finding of Halder and Chakrabratty (1993).

It has been reported that lowering the pH in alkaline soil resulted in P-mobilisation (Bolan *et al.*, 1997) and that lowering the pH of liquid medium by bacterial acid production was responsible for P solubilisation (Nautiyal *et al.*, 2000; Lin *et al.*, 2006; Son *et al.*, 2006). Different degrees of acidity however, are necessary for solubilising P from different sources of P-containing mineral. It has been reported that a pH of from 3.4 to 4.6 was low enough for significant solubilisation of a Ca phosphate mineral (Stumm and Morgan, 1995). It was also reported that a pH of 2.7 was low enough for the solubilisation of colloidal Al-P but a pH of from 2.0 to 2.2 was not low enough for the solubilisation of crystalline $AlPO_4$, colloidal Fe-P or crystalline $FePO_4.4H_2O$ (Stumm and Morgan, 1995). The acidic conditions required for solubilisation of $AlPO_4$ and $FePO_4$ minerals (pH 2.0-2.2) are possibly too low for survival of P-mobilising bacteria.

It has been reported that organic anions that are secreted by bacteria may be responsible for the chelation of cations resulting in P solubilisation. The organic anions commonly secreted by bacteria include citrate, oxalate, lactate, gluconate and malate. Of the bacteria used in this experiment, only the strains FA001 and FA010 secreted citrate (Table 3.4). These strains also secreted oxalate (about 10 per cent of the amount of citrate) and succinate (about 20 per cent of the amount of citrate). Of the other strains examined, FA002 and FA003 secreted acetate, FA004 secreted oxalate and tartrate, and FA009 secreted oxalate and malate anions. Organic anions with one carboxyl group (lactate, formate and acetate) have very little metal-complexing ability and citrate has a high affinity for trivalent metals such as Al^{3+} and Fe^{3+} (Pohlman and McColl, 1986; Jones and Darrah, 1994; Jones and Kochian, 1996; Earl *et al.*, 1979). It has also been reported that citrate has the ability to dissolve Al-compounds (Fulton *et al.*, 1989). In this study (3.3.4) it was found that the bacterial strains FA001 and FA010 solubilised significantly more P from $AlPO_4$ than the other bacterial strains. Consistent with earlier results that citrate is a good chelating anion (Fulton *et al.*, 1989; Bolan *et al.*, 1997; Kirk *et al.*, 1999; Whitelaw *et al.*, 1999) these two strains secreted more than 5 µg mL^{-1} citric acid into the medium (Table 3.4). It has been reported that complexation of iron by malate, citrate and oxalate is highly dependent upon soil solution pH with little or no complexation at high soil pH (Parker *et al.*, 1995). The P mobilisation described in these experiments from aluminium and iron phosphates could be due to the combined effects of lowering the pH, and chelation by the citrate anion produced by the strains FA001 and FA010. Gluconic acid is known as a good chelator for mobilising insoluble P (Whitelaw *et al.* 1999), but in this study gluconate was not determined in the bacterial cultures. The strains FA001 and FA010 mobilised the highest amount of P from $AlPO_4$-containing medium. For the $FePO_4$-containing medium the strain FA001 mobilised the highest amount of P whereas in the culture containing the FA010 strain, the soluble P in the medium was significantly lower than in the control and lower than the zero time soluble P. Organic anions detected in cultures of FA001 and FA010 strains were citrate, succinate and oxalate, but it is possible that other potentially chelating anions could be present. In the $FePO_4$-containing medium, the strain FA001 mobilised the highest amount of P. It is possible that this strain produces sufficient organic anions such as 2-ketogluconate that might solubilise P from $FePO_4$-containing medium. It

has been reported that 2-ketoglutonate excreted by bacteria can mobilise P by chelation of cations (Banik and Dey, 1982; Kucey, 1988; Halder *et al.*, 1990). It is possible that the solubilisation of a small amount of $FePO_4$ in soil may give an advantage to the bacteria in both P and Fe acquisition over competing soil microorganisms and thus may enhance plant root colonisation and plant growth. In a study using colloidal $AlPO_4$ as a source of P it was found that more P was solubilised by gluconate than was solubilised by HCl at the same pH demonstrating the chelation effect of gluconate (Whitelaw *et al.*, 1999). The affinity of chelation by gluconate for aluminium and iron is higher than for calcium (Whitelaw *et al.*, 1999).

The Vietnamese strain 4P, known as a good P-mobiliser (Nguyen *et al.*, 2003), produced more than 7 $\mu g\ mL^{-1}$ tartaric acid (Table 3.4). Tartrate is not as effective as citrate for P mobilisation by chelation because citrate has three carboxyl groups and tartrate has only two carboxyl groups. It has been reported that 4P mobilised P by lowering the pH (Rose, 2007). The P-mobilising ability of this strain was not determined in this experiment.

Different numbers of live bacterial cells were added to the five different P-containing media. Throughout the five day experiments, in all media, the number of bacteria present in the cultures containing the strain FA010 was lower than for any other strains. Bacterial growth is dependent on the nutrient including soluble P, in a medium. Although different types of insoluble P were used in these experiments, there was some soluble P in all of these insoluble P sources (Table 3.19). In all the different P-containing media, the exponential growth phase of the bacteria was within one day to two days after inoculation (Table 3.19). It is likely that the amount of soluble P in all of the culture media was enough for initial bacterial growth and development.

It was difficult to separate the exponential growth and lag phase of inoculated cultures because the bacterial count was performed after 24 h. The greatest increase in bacterial number was obtained one day after inoculation in cultures containing the strains FA001, FA009 and FA010 and all insoluble sources of P, except $AlPO_4$. The highest growth in the medium containing $AlPO_4$ was obtained two days after

inoculation (Table 3.19). These three strains can be considered as fast-growing bacteria in comparison with strains FA003 and FA004 in which the highest bacterial levels were found two days after inoculation in cultures containing $Ca_3(PO_4)_2$. All bacterial strains reached their maximum bacterial number one day after inoculation in the culture containing rock phosphate. These cultures contained only about four per cent as much soluble P at the start of the experiment as in the $Ca_3(PO_4)_2$ cultures, suggesting that soluble P was not limiting in any of the cultures examined.

In the case of the $AlPO_4$-containing medium, the highest number of bacteria was obtained one day (FA003, FA004, FA009 and FA010) and two days (FA001 and FA002) after inoculation. Although Al^+ can be toxic, in this medium bacterial growth was substantial for two 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 *et al.*, 1986). Strong chelation of Al^{3+} prevented inhibition of bacterial growth, facilitating P mobilisation. 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 *et al.*, 1995; Jones and Kochian, 1996; Jones, 1998). It was suggested that organic anions containing triple carboxyl groups such as citrate mobilise P from $AlPO_4$ better than organic anions containing a single carboxyl group such as acetate (Lan *et al.*, 1995; Jones and Kochian, 1996). In the experiments described in this Chapter, the two bacterial strains that produced citrate (FA001 and FA010) did not grow better in $AlPO_4$-containing medium than the other strains. Strains FA003, FA004 and FA009 grew faster than FA001 (Table 3.19).

In $FePO_4$-containing medium, there was good bacterial growth of the strain FA001 after one day of incubation. The number of bacteria in cultures containing the strains FA001, 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 soluble P in these cultures was a 10-fold higher 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 two to three days after inoculation. This may be a consequence of the lower P values in the cultures from one day after inoculation. The decline in the total number of bacteria in the cultures over a five day incubation period may be caused by more acid conditions rather than specific toxic effects. In addition, lack of nutrition might cause a reduction in bacterial numbers in the medium over the five 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-mobilisers of the six strains examined in this experiment. The highest bacterial numbers were found with the strains FA002 in $Ca_3(PO_4)_2/(NH_4)_2SO_4$, FA003 in $Ca_3(PO_4)_2/NH_4NO_3$ and rockP/NH_4NO_3, FA001 in $AlPO_4/NH_4NO_3$, and FA009 in $FePO_4$ /NH_4NO_3 media. The highest amounts of soluble P were found with the strains FA001 and FA010 in all different P and N containing media. 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 mobilisation. The P-mobilisation 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.

3.6 Conclusion

The isolated bacterial strains could significantly mobilise insoluble P from $Ca_3(PO_4)_2$ and rock phosphate in minimal liquid medium. Strains FA001 and FA010 were the best P mobilisers. When $(NH_4)_2SO_4$ and NH_4NO_3 were used as sources of N with $Ca_3(PO_4)_2$ more P was mobilised using $(NH_4)_2SO_4$ as a source of N, possibly because the assimilation of ammonium alone caused a higher 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. On the other hand strains FA002, FA003, FA004 and FA009 showed increased P mobilising 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 $Ca_3(PO_4)_2$. Mobilisation 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 mobilising P from $AlPO_4$, and FA001 was identified as a minor P mobiliser 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-mobilisation.

The different rates of P-mobilisation with different bacteria, P sources and N sources need to be examined further. The reason more P was solubilised 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 Chapter. In the experiments described in this Chapter 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. From the results presented in this Chapter strains FA001 and FA010 show potential as P-mobilisers but experiments need to be conducted to measure their potential effectiveness at the field level.