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Phosphorus Adsorption in Some Australian Soils and Influence of Bacteria on the Desorption of Phosphorus

M. F. Ahmed, I. R. Kennedy, A. T. M. A. Choudhury, M. L. Kecskés, and R. Deaker

SUNFix Center for Nitrogen Fixation, Food and Natural Resources, University of Sydney, Sydney, Australia

Abstract: Seven Australian soil samples were collected from different locations (Camden, Griffith, Narrabri, Rutherglen, Wagga Wagga, Wee Waa (Ivanhoe), and Yanco) to measure their phosphorus (P) adsorption rates. Soils were collected from the top 0–15 cm, and P was added at 0, 100, 200, 300, 400, and 500 μg P g^{-1} soil. Results indicated that P adsorption increased significantly with increasing levels of added P. In subsequent studies, soils from Griffith and Narrabri and two bacteria *Pantoea* spp. known as FA001 and FA010 were tested for P mobilization at 100 μg P g^{-1} soil concentration. The rate of P mobilization [P extracted by 0.01 M calcium chloride ($CaCl_2$)] in the Narrabri soil showed significant differences between treatments, but with and without bacteria, this was not the case for the Griffith soil. In Narrabri soil, the highest extractable P (0.492 μg g^{-1}) was obtained with the treatment containing the strain FA001 after bacterial lysis with trichloromethane ($CHCl_3$), and the lowest P (0.236 μg g^{-1}) was measured in the treatment without bacterial amendment and without $CHCl_3$ treatment, indicating the P-mobilizing ability of the strain FA001. It was found that the minimum P-adsorption capacities (revealed from the Langmuir and Temkin adsorption isotherms) of the Narrabri and the Griffith soils are 357 and 500 μg g^{-1}, respectively; the buffering capacities of the Narrabri and the Griffith soil are 71.7 and 93.7 μg g^{-1}, respectively. These findings indicate that soils with high P adsorption and buffering capacities are less likely to respond to the P-mobilizing bacteria. Therefore, the application of the Langmuir and Temkin

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Address correspondence to M. F. Ahmed, Faculty of Agriculture, Food and Natural Resources, Ross St. Building No. AO3, University of Sydney, NSW 2006, Australia. E-mail: faruqueahmed@optusnet.com.au or mohammad_faruque@yahoo.com.au

adsorption isotherms for estimating soil P-adsorption and buffering capacities can be used to predict the potential usefulness of biofertilizer application.

Keywords: Adsorption, bacteria, buffering capacity, desorption, mobilization, phosphorus, soil

INTRODUCTION

Information on the behavior of phosphorus (P) in soil is a fundamental understanding of plant nutrition and the soil biogeochemical cycle. Phosphorus deficiency is often reported in well-weathered Oxisols and Ultisols because of their strong acidic reactions and abundance of aluminum (Al^{3+}) and iron (Fe^{3+}) ions that complex P (Tisdale, Nelson, and Beaton 1985). In the case of relatively young soils, such as Inceptisols, it has been found that a greater portion of the total P is in the available form, compared to mature soils where acidity is often stronger (Tisdale, Nelson, and Beaton 1985).

Phosphorus deficiency in wheat soils is very common in Australia. Farmers need to apply phosphatic fertilizers to maximize wheat yields. For maintenance dressings of P fertilizer to maximize yield varied with crops and soils, for example, wheat needs 15–25 kg P ha^{-1} (Martin, Leonard, and Stamp 1976; Anonymous 2006). A soil receiving P for each crop may show lower maximum adsorption capacity than soil receiving no P in the field (Abedin and Salaque 1998).

Phosphorus usually has a high affinity for soil, resulting in slow downward movement through the soil matrix (Eghball, Sander, and Skopp 1990; Sims, Simard, and Joern 1998) or laterally through interflow. Significant amounts of P may move by preferential flow paths (Jensen et al. 1998; Simard, Beauchemin, and Haygarth 2000) with little adsorption to the soil matrix (Jensen et al. 1998). However, P is likely to be adsorbed with soil Al and Fe in acidic soils (Maguire et al. 2001). The high P content of soil and consequential loss of P from soil to water cause eutrophication. To determine eutrophication risk there is a need to assess the environmental utility of current tests for P in soil. Sims, Simard, and Joern (1998) reported that soil tests clearly do not characterize site hydrology or nutrient-management practices and cannot identify the risk of direct P loss in runoff from fertilizers and organic wastes applied to the soil surface, thus making risk-management decisions based solely on agronomic soil test P is a flawed approach. To estimate eutrophication risk, it may be necessary to substitute current soil test methods with a new approach for assessing the capacity of a soil to retain P against leaching (Edwards and Withers 1998; Sims et al. 2000).

Adsorption and desorption reactions are considered as key aspects of the chemical behavior of P in soil. Adsorption describes the removal of phosphate

ions from solution to soil components (Abedin and Salaque 1998); desorption describes the reverse process, of removal of bound soil P to the solution (Kuo, Hellum, and Pan 1988). As an equilibrium process, the amount of P adsorbed is determined largely by the solution P concentration (Syers and Lu 1990).

Phosphorus adsorption in soils has been studied extensively (Barrow 1978, 1983; Beck, Robarge, and Buol 1999; Maguire et al. 2001; Novak and Watts 2004). These investigations examined the effect of soil properties such as pH, temperature, flooding, and redox potential on P sorption. As recovery of applied P by crops is typically only 15–20%, it is likely that the major portion of the applied P fertilizer is adsorbed to soils at their various adsorption sites. Thus, continuous application of P fertilizer should mitigate soil P sorption affinity (Abedin and Salaque 1998). On the other hand, growing crops continuously without P applications may expose more sites for P sorption as a result of desorption. Desorption isotherms characterize the release of adsorbed P, but the process is extremely slow and usually not completed in a period of hours or days. The rate of desorption decreases with time, increases the period of contact between soil and P fertilizer, and reduces the amount of desorbed P in the solution (Tisdale, Nelson, and Beaton 1985). Generally, desorption appears to become very slow after about 2 days (Tisdale, Nelson, and Beaton 1985).

Desorption is dependent on the nature of the adsorption complex. For example, variscite ($AlPO_4 \cdot 2H_2O$) and strengite ($FePO_4 \cdot 2H_2O$) are more stable than calcium compounds of P [eg, $(Ca_3(PO_4)_2]$ and expected to be prominent in acid soils, desorbing P at a slow rate (Kelly and Midgley 1943; Haseman, Brown, and Whitt 1950; Tan 1993; Zhang et al. 2001), and they (variscite and strengite) are not dependent on the maximum phosphate buffering capacity of the soils (Kuo, Hellum, and Pan 1988). However, P desorption is also dependent on the P adsorption capacity of soils as estimated by the classical Langmuir equation (Kuo, Hellum, and Pan 1988).

There are several references available for estimation of P in soil microbial biomass (Brookes, Powlson, and Jenkension 1982, 1984; Gijsman et al. 1997; He et al. 1997; Bliss, Comeford, and Muchovej 2004); the increase in P availability to plants through the inoculation of phosphate-solubilizing bacteria in pot experiments and under field conditions (Banik and Dey 1981; Chabot, Hani, and Cescas 1996; deFreitas, Banerjee, and Germida 1997; Zaidi, Khan, and Amil 2003); and of P mobilization in liquid media and on agar plates (Sperber 1958; Whitelaw, Harden, and Helyar 1999). But there are few if any research studies where specific bacteria have been used for quantifying desorption of soil P at field capacity of soil.

The aim of this study was to quantify the capacity of P-mobilizing bacteria in a field environment. In the first part of this study, seven soils were used for measuring P adsorption capacity using different adsorption equation, and in the second part of this work, two soils and two bacterial

isolates were used to estimate their P-desorbing ability. It was hypothesized that the steady-state level of mobile P would be increased under the action of biofertilizer P strains. The following investigation aimed to evaluate the P-adsorption behavior of different agricultural soils and to determine this respective desorption kinetics of adsorbed P when inoculated with bacterial strains that have P-solubilizing activities.

MATERIAL AND METHODS

Part 1: Phosphorus Adsorption in Some Australian Soils

Soil Sampling, Preparation, and Determination of Physicochemical Properties

Soil samples 2–3 kg (0–15 cm deep) were collected from seven sites, six in New South Wales (NSW) and one in Victoria (VIC). The samples were as follows: (1) Camden, NSW, Cobbity (University of Sydney Farm): This soil is from fallow land depleted of almost all nutrients and high in organic matter. (2) Narrabri, NSW, Narrabri: This soil is a Vertisol from Narrabri cotton field No. 5; cropping patterns have been cotton–wheat–fellow. (3) Griffith, NSW: This is a soil from a rice farm with a cropping pattern of rice–fallow–rice. (4) Rutherglen, VIC: This is a wheat rhizospheric soil, collected from Rutherglen Agricultural Research Station, with cropping pattern wheat–fallow–wheat. (5) Wagga Wagga, NSW: This is a wheat rhizospheric soil with a wheat–fallow–wheat cropping pattern. (6) Wee Waa, NSW, Ivanhoe: This is a natural grassland rhizospheric soil collected from 12 km east of Wee Waa. (7) Yanco, NSW: This is a soil from rice field, with a rice–fallow–rice cropping pattern.

Methods

The soil samples were air dried, passed through a 2-mm sieve, and stored in plastic bags. Soils were analyzed for organic matter; total P and total nitrogen (N) were determined by Wollongbar Agricultural Institute, Wollongbar, NSW. The pH, cation exchange capacity (CEC), available P, and texture were determined at the Faculty of Agriculture, Food, and Natural Resources, the University of Sydney. Organic matter was determined by the potassium dichromate and sulfuric acid (H_2SO_4) digestion method (Walkley and Black 1934). Total P was determined following inductively coupled plasma (ICP) (acid digest) method. Total N was determined by combustion (LECO-3336). Soil pH was measured in 1:5 soil–water ratio by the glass electrode (Peech 1965). Available P was determined following (Colwell 1963). The Olsen method (Olsen et al. 1954) was modified by Colwell (1963) who increased the soil/solution ratio from 1:20 to 1:50

and the reaction time from 30 min to 16 h to decrease the influence of the soil P buffering on P extractability. The Colwell test extracts a higher proportion of available soil P than does the Olsen test (Adepoju, Pratt, and Mattigods 1982). The cation exchange capacity was determined using atomic absorption spectrometry, the soils being extracted by ammonium chloride at pH 7.00 (Tucker 1974). Soil texture was determined following the hydrometer method.

Determination of Phosphorus Adsorption Capacity in Soil Samples

Two-g samples of each soil were weighed into 100-mL centrifuge tubes with three replications. A range of P solutions (0, 10, 20, 30, 40, and 50 mg P L^{-1}) was prepared by dissolving potassium dihydrogen phosphate (KH_2PO_4) in 0.01 M calcium chloride ($CaCl_2 \cdot 2H_2O$) solution in Millipore water, and 20-mL aliquots of the solutions were added to the centrifuge tubes to give 0, 100, 200, 300, 400, and 500 μg of added P g^{-1} of soil. The samples were than shaken for 24 h at room temperature (24°C). After 24 h of shaking, the tubes were centrifuged at 12,000 rpm (Sorvall RC 5C; Heraeus; Model-930437) for 12 min and then filtered through Whatman No. 42 filter paper (Agbenin 2003). The filtrates were analyzed for available P following the method of Murphy and Riley (1962). The amount of P adsorbed per gram of soil was calculated from the difference in the P added to the soils and the P present in the solution. The P in the control (no P) treatment was taken into account. The adsorbed P was calculated as μg g^{-1} soil.

Adsorption isotherms describe solute adsorption by solids from an aqueous solution at constant temperature and pressure and show the amount of adsorbate (solute) sorbed as a function of equilibrium concentration in solution. The P adsorption data for the soils used in this study were fitted into the following adsorption equations.

Langmuir Adsorption Equation

$$\frac{c}{(x/m)} = \frac{1}{kb} + \frac{c}{b}. \tag{1}$$

Here c is the equilibrium solution P concentration (μg P mL^{-1}), x/m is the mass of P adsorbed per unit mass of soil (μg g^{-1}), k is a constant related to bonding energy of P to the soil, and b is the maximum P adsorption capacity (μg g^{-1}). A plot of c/(x/m) versus c gives a straight line if the adsorption process fits this model. The values of b and k are obtained from the slope (1/b) and the intercept (1/kb), respectively.

Freundlich Adsorption Equation

$$\frac{x}{m} = ac^{b} \tag{2}$$

By rearranging,

$$\log \frac{x}{m} = \log a + b \log c \tag{3}$$

where x/m is the mass of P adsorbed per unit mass of soils ($\mu g\ g^{-1}$), and c is the equilibrium solution P concentration ($\mu g\ mL^{-1}$), and a and b are constants. A plot of log (x/m) versus log c gives a straight line if the adsorption process fits the model. The values of a and b are obtained from the intercept (log a) and the slope (b), respectively.

Temkin Adsorption Equation

$$\frac{x}{m} = a + b \ln C \tag{4}$$

where x/m is the mass of P adsorbed per unit mass of soil ($\mu g\ g^{-1}$), c is equilibrium solution P concentration ($\mu g\ mL^{-1}$), and a and b are constants. A plot of x/m against ln c gives a straight line if the adsorption process fits the model. The values of a and b are obtained from the intercept (a) and the slope (b), respectively. The b value of Temkin equation is considered as the P-buffering capacity (retention capacity of adsorbed P) of soil ($\mu g\ g^{-1}$).

Statistical Data Analysis

Statistical analyses of the data to determine the goodness of fit to these equations were done using the GenStat (7th edn) statistical program (Payne et al. 2003).

Part 2: Enhancement of Phosphorus Desorption by Bacterial Isolates

Soils

In this investigation, two of the soils (Narrabri and Griffith) were selected. The highest ($500\ \mu g\ g^{-1}$) and the second highest ($357\ \mu g\ g^{-1}$) maximum P-adsorption capacities had been attained with the Griffith and Narrabri soils, respectively, shown in the first part of this investigation. It is notable that the Narrabri and the Griffith soils had the highest and the second highest clay contents respectively (Table 1).

Table 1. Selected properties of seven soils

Properties	Soils						
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
Total P ($\mu g\ g^{-1}$)	150.0	240.0	280.0	370.0	150.0	300.0	380.0
Total N ($\mu g\ g^{-1}$)	1900.0	1600.0	1300.0	2600.0	890.0	1500.0	1200.0
OM (%)	5.1	4.6	2.5	7.6	3	4.6	3.2
Available P ($\mu g\ g^{-1}$)	3.9	11.3	11.1	30.3	17	3.8	18.1
pH	5.4	4.1	7.4	5.4	4.8	5.7	6.4
CEC (cmolc kg^{-1})	5.6	6.5	32.9	4.4	4.5	3.3	12.6
Sand (%)	59.5	56.0	33.1	55.5	63.0	83.0	63.3
Silt (%)	26.2	9.8	17.3	34.6	17.3	7.3	7.4
Clay (%)	14.3	34.2	49.5	9.9	19.7	9.7	29.4
Soil texture	Sandy loam	Sandy clay loam	Clay	Sandy loam	Sandy loam	Loamy sand	Sandy clay loam

Bacteria

Seven bacteria with P-mobilizing ability have been isolated and identified from three soil samples (Ahmed 2006). Two strains, FA001 and FA010, with capability for mobilizing P in liquid culture were selected for investigation of their P-mobilizing capacity in soil.

Preparation of Bacterial Samples for Inoculation of Soils

Bacterial strains FA001 and FA010 were cultured on nutrient broth plates (NB, Difco) for 24 h. Sterile deionized water was used to prepare bacterial suspensions. The bacteria were suspended in different amounts of water for the two different soils to obtain the field capacity condition based on soil clay content (Anonymous 2005). Live bacterial cells were added from suspensions of 10×10^7 and 8×10^7 colony-forming units (CFU) per 2 g of soil for strains FA001 and FA010, respectively.

Treatments

The treatments were designed to show (1) the contribution of bacteria to P desorption by increasing soluble P and (2) the contribution of chloroform and electrolyte solution/equilibrium solution (0.01 M $CaCl_2$) to enhancing desorption or absorption of P from the soil. Therefore, the following treatments were used for each of the soils:

T_1 = soil and bacteria (FA001) lysed with $CHCl_3$,
T_2 = soil and bacteria (FA001) not lysed,
T_3 = soil and bacteria (FA010) lysed with $CHCl_3$,
T_4 = soil and bacteria (FA010) not lysed,
T_5 = soil without bacterial amendment lysed with $CHCl_3$, and
T_6 = soil without bacterial amendment not lysed.

Experimental Procedure for Phosphorus Desorption by Bacteria

Two g of air-dried soil was taken in screw-capped polypropylene centrifuge tubes (acid washed in 2 M HCl overnight). After P adsorption using KH_2PO_4 solution in 0.01 M $CaCl_2$ at 100 μg P g^{-1} soil (soil–solution ratio 1:50) for 24 h, the samples were dried at 40°C overnight. To achieve field capacity depending on the clay content (Anonymous 2005), 40% and 28% bacterial suspensions were added in the Narrabri and Griffith soils, respectively. In the case of uninoculated control treatments (T_5 and T_6), the same amount of sterile water was added. Aiming to simulate natural conditions of the photosphere soil, where organic substances are excreted from plant roots, glucose was added at 2 g kg^{-1} soil in all treatments as powder at the time of inoculation, then vortexed for a min. After inoculating, the samples

were capped and incubated in the oven at 28°C for 3 days to obtain maximum desorption and/or bacterial influence to desorb P. Two extracting solutions were used in succession, 0.01 M $CaCl_2$ (pH 5.76) followed by 0.5 M $NaHCO_3$ at pH 8.5. The basic objectives of this part of study were to show whether bacterial inoculation affecting the mobilization of soil adsorbed P.

Chloroform Extraction Procedure

Chloroform was used to lyse bacteria/microbial cells so that microbial P could be made available in the extraction of 0.01 M $CaCl_2$ and 0.5 M $NaHCO_3$. This approach has been used to estimate soil microbial P (Brookes, Powlson, and Jenkension 1982, 1984) or microbial P after growth in the liquid medium (Hedley and Stewart 1982). However, in this experiment, the approach differs from the previous research where bacteria were added to the soil containing adsorbed P, and then the bacterial cells were grown and subsequently disrupted using chloroform.

After 3 days of incubation, during which bacterial numbers would be expected to increase, the specified samples (T_1, T_3, and T_5) were treated with 3 mL of chloroform. The tubes were mixed by vortexing for three 10 s interval over a period of 30 min at room temperature (24°C). The tubes remained capped for about 4 h to allow microbes to be lysed. The caps were then removed to allow evaporation of the $CHCl_3$ at 24°C over 16 h. Then 0.01 M $CaCl_2$ solution was added to the samples at 10 mL g^{-1} soil in $CHCl_3$-treated and untreated tubes. The tubes were than shaken by an end-to-end shaker (50 rpm) overnight (16 h) at room temperature (24°C). The soil suspension was centrifuged at 12000 rpm (5–10°C; Sorvall RC 5C; Heraeus, Model-930437) for 12 min and filtered through Whatman No. 42 filter paper (Agbenin 2003) using vacuum filtration to obtain the $CaCl_2$ extraction in the supernatant. Then the moist soil pellets were used for the second extraction with 0.5 M $NaHCO_3$ (pH 8.5) at 1:50 ratio (100 mL for 2 g soil) (Colwell 1963). Shaking time and rate, temperature, centrifugation, and filtration were similar as with the previous extraction with 0.01 M $CaCl_2$.

Phosphorus Determination

Phosphorus extraction was carried out according to the methods of Colwell (1963) using 0.01 M $CaCl_2$ and 0.5 M $NaHCO_3$ extracting solution, and the inorganic P was determined from the aliquots following the methods of Murphy and Riley (1962).

Statistical Data Analysis

Data were analyzed using the statistical software GenStat (ver. 7.0) using two-way-analysis of variance (Payne et al. 2003).

RESULTS AND DISCUSSION

Part 1: Phosphorus Adsorption in Some Australian Soils

Phosphorus Concentration in the Solution

Interaction effects of soil and added P rate was significant. Phosphorus concentration in the equilibrium solution increased gradually with increasing rate of added P up to 500 $\mu g\ g^{-1}$ in all soils (Table 2). The Griffith soil did not show any significant increase up to 300 $\mu g\ P\ g^{-1}$ soil, indicating its high P adsorption capability. The Yanco soil showed significant increase in P concentration from 200 $\mu g\ P\ g^{-1}$ soil, whereas the other five soils showed significant increase P concentration from 100 $\mu g\ P\ g^{-1}$ soil. In the treatment with no P addition, only the equilibrium solution P concentration was highest in the Ivanhoe (Wee Waa) soil, probably indicating a soil textural effect (Table 2). In 100 $\mu g\ P\ g^{-1}$ soil treatment, the highest equilibrium P concentration was obtained with Wee Waa soil, whereas the lowest equilibrium P concentration was obtained in the Griffith soil (Table 2). The highest and the lowest P concentrations were obtained with the Rutherglen and the Griffith soils, respectively, with all other treatments (200, 300, 400, 500 $\mu g\ P\ g^{-1}$ soil).

Regression analysis indicated that the increase in P concentration in the equilibrium solution due to addition of P was linear in all seven soils (Table 3). However, the rate of increase was the highest in Rutherglen soil followed by Wee Waa, Wagga Wagga, Camden, Narrabri, Yanco, and Griffith soils, respectively (Table 3). The difference between equilibrium

Table 2. Effect of added P concentration on equilibrium solution P concentration in different soils

Added P ($\mu g\ g^{-1}$ soil)	P remaining in the equilibrium solution ($\mu g\ mL^{-1}$)						
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
0	0.00 f B	0.00 c B	0.08 f B[a]	0.41 f B	0.30 f B	1.16 f A	0.08 e B
100	1.99 e B	0.08 c D	1.09 e C	5.32 e A	5.17 e A	5.70 e A	0.45 e D
200	7.42 d C	0.26 c F	4.39 d D	12.78 d A	11.51 d B	11.96 d B	3.34 d E
300	13.76 c C	0.79 c F	8.77 c D	20.95 c A	18.29 c B	20.84 c A	6.26 c E
400	22.00 b D	1.99 b G	14.54 b E	30.55 b A	27.25 b C	29.91 b B	11.66 b F
500	30.40 a C	3.34 a F	19.42 a D	38.64 a A	36.24 a B	36.92 a B	17.62 a E

Note. Soil and added P rate interaction was significant at F probability level of <0.001 with LSD (0.05) value of 0.7451.

[a]Means followed by a common small letter in a column and a common capital letter in a row are not significantly different by least significant difference (LSD) test at 5% level of probability.

Table 3. Regression equation and R^2 value relating added P and solution P concentration in the soils under study

Soil	Regression equation	R^2 value
Camden	$y = -3.004 + 0.0624x$	0.967^{***}
Griffith	$y = -0.564 + 0.0066x$	0.850^{*}
Narrabri	$y = -2.058 + 0.0404x$	0.961^{**}
Rutherglen	$y = -1.533 + 0.0786x$	0.992^{***}
Wagga Wagga	$y = -1.592 + 0.0722x$	0.987^{***}
Wee Waa	$y = -0.846 + 0.0744x$	0.989^{***}
Yanco	$y = -2.309 + 0.0355x$	0.926^{**}

*** = Significant at 0.01 level; ** = significant at 0.05 level; * = significant at 0.10 level.

solution P concentration and initial solution P concentration was attributed to the differences in P adsorption (Table 4). The trends for increase on equilibrium solution P concentration due to addition of P are in agreement with previous findings (Abedin and Saleque 1998; Akhter et al. 2003).

Phosphorus Adsorption

The interaction effect of added P level and soil was significant. The P adsorption increased significantly at all levels of added P in all the soils except Rutherglen where P adsorption was statistically similar at 300 and 400 $\mu g\ P\ g^{-1}$ soil of added P (Table 4). At 100 $\mu g\ g^{-1}$ added P, Griffith and Yanco soil were statistically similar, whereas at other levels of added P, the adsorption of P was significantly higher in Griffith soil compared to other soils. At all levels of added P, adsorption was the highest with the Griffith soil followed by the Yanco soil, while the lowest amount of adsorption occurred in the Rutherglen soil. However, the percentage of added phosphate adsorbed by the soil decreased with the increasing level of P addition (Table 5), indicating that the increase in the amount of P adsorbed in soils was not in proportion to the increasing level of P addition.

Many processes may interact in a complex matrix such as soils, humus, and soil component such as Fe and Al, affecting phosphate sorption. Phosphate sorption is associated with the occurrence of reactive surface sites in the mineral soil. Possibly when the sorption sites are filled by adsorbing phosphate ions, the percentage of added phosphate decreases with the increasing level of added P. Barrow (1978) reported that the affinity for adsorption decreases as the amount of adsorption increases. Other researchers also found that P adsorption increased significantly with increasing level of added P, and the percentage of added P decreases with the increasing level of added P (Abedin and Salaque 1998; Akhter et al. 2003), in agreement with the results of this experiment.

Table 4. Effect of P addition to P adsorption of different soil

Added P ($\mu g\ g^{-1}$ soil)	Adsorbed P ($\mu g\ g^{-1}$ soil)						
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
100	80.14 eC[a]	99.25 eA	89.89 eB	50.90 dD	51.28 eD	54.65 eD	96.25 eAB
200	127.29 dD	197.38 dA	156.52 dC	76.32 cF	87.94 dE	92.06 dE	167.26 dB
300	162.45 cD	292.13 cA	213.05 cC	94.61 bG	120.10 cE	103.24 cF	238.16 cB
400	179.99 bD	380.14 bA	255.33 bC	98.66 bG	130.52 bE	112.49 bF	279.19 bB
500	196.04 aD	466.65 aA	306.61 aC	117.70 aF	140.58 aE	142.44 aE	324.60 aB

Note. Soil and added P rate interaction was significant at F probability level of <0.001 with LSD (0.05) value of 8.273.

[a]Means followed by a common small letter in a column and a common capital letter in a row are not significantly different by least significant difference (LSD) test at 5% level of probability.

Table 5. Percentage of adsorbed P from the added P in the solution

P added ($\mu g\ g^{-1}$ soil)	P adsorbed as % available P in solution						
	Camden	Griffith	Narrabri	Rutherglen	Wagga	Wee Waa	Yanco
100	80.14	99.25	89.89	50.90	51.28	54.65	96.25
200	63.65	98.69	78.26	38.16	43.97	46.03	83.63
300	54.15	97.38	71.02	31.54	40.03	34.41	79.39
400	45.00	95.04	63.83	24.67	32.63	28.12	69.80
500	39.21	93.33	61.32	23.54	28.12	28.49	64.92

Regression analysis showed that the increase in P adsorption as a result of addition of P was linear in all the seven soils studied (Table 6). However, the rate of increase was the highest in the Griffith soil, followed by the Yanco and Narrabri, and the lowest rate was obtained with the Rutherglen soil. The R^2 values were significant at 1% level of probability for all the soils. The pH of Griffith soil was the lowest (pH = 4.07); the relative acidity might have a positive impact on P adsorption because of P retention with Fe^{2+}, Fe^{3+}, and Al^{3+} brought into solution at low pH. Some researchers also reported that the higher phosphate sorption obtained for the soil containing a higher total amount of extractable Al and Fe (Brady and Weil 2002; Agbenin 2003; Gielser, Anderson, and Persson 2005), which is in very similar agreement with the results of the Griffith soil.

Adsorption Isotherm

The P adsorption data for all soils fitted well to the Langmuir (Figure 1), the Freundlich (Figure 2), and the Temkin adsorption equations (Figure 3). Coefficients of determination (R^2 value) were significant at the 1% level of probability for all three adsorption equations in all seven soils, indicating they were

Table 6. Regression equation and R^2 value relating added P and adsorbed P in the soils under study

Soil	Regression equation	R^2 value
Camden	$y = 63.829 + 0.2845x$	0.945**
Griffith	$y = 11.841 + 0.9176x$	0.999**
Narrabri	$y = 44.603 + 0.5323x$	0.993**
Rutherglen	$y = 40.853 + 0.1559x$	0.953**
Wagga Wagga	$y = 39.730 + 0.2212x$	0.920**
Wee Waa	$y = 42.170 + 0.196x$	0.941**
Yanco	$y = 50.505 + 0.5686x$	0.984**

** = significant at 0.01 level.

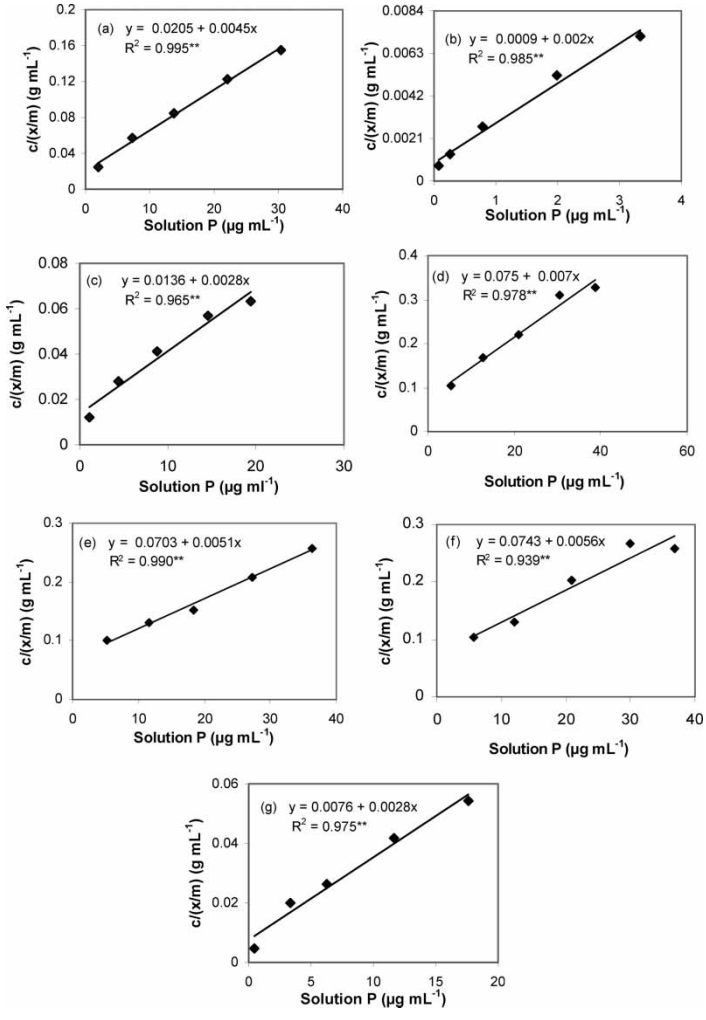

Figure 1. Langmuir adsorption isotherm for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils (** = significant at 0.01 level). Soil samples were treated with up to 500 µg P g^{-1} soil for 24 h at room temperature by mixing the soil samples with KH_2PO_4 in solution. The soil samples were separated by centrifugation and filtration and assayed for P content. c is the equilibrium solution P is concentration after treatment, and x/m is the mass of P adsorbed per unit mass of soil.

of similar utility in describing the sorption process. However, the Langmuir equation was best fitted for the Camden and the Wagga Wagga soils, whereas the Freundlich equation was best fitted for the Narrabri soil. The Temkin equation was best fitted for the Camden soil.

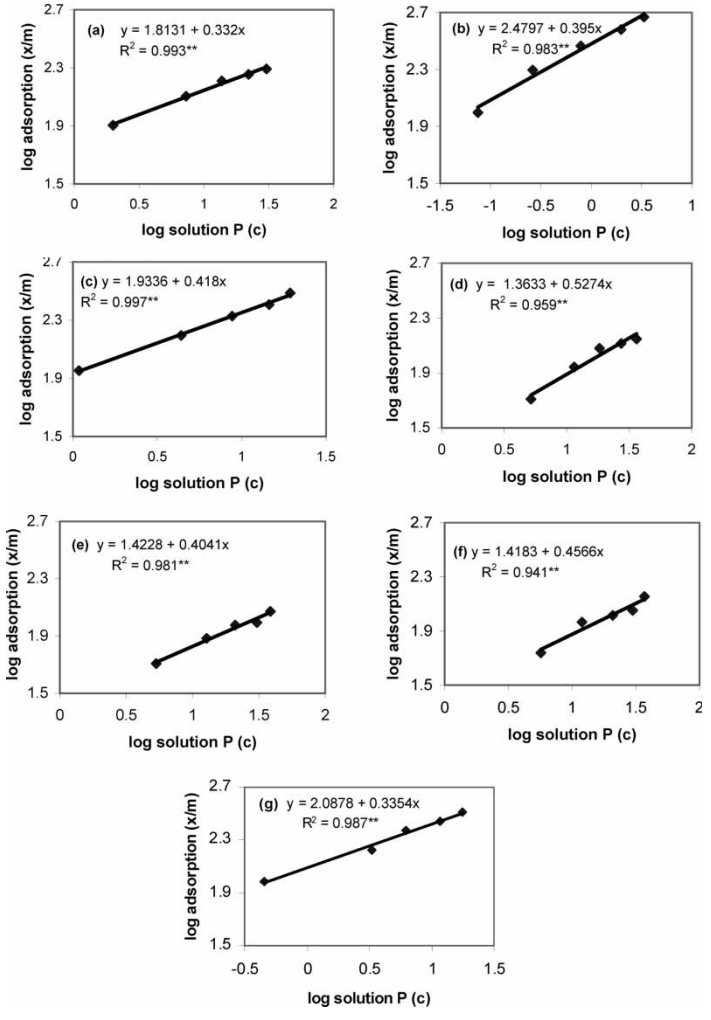

Figure 2. Freundlich adsorption isotherms for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils (**= significant at 0.01 level). Soil samples were treated with up to 500 μg P g^{-1} soil for 24 h at room temperature by mixing the soil samples with K_2HPO_4 in solution. The samples were separated by centrifugation and filtration and assayed for P is content. c is the equilibrium solution P concentration after treatment, and x/m is the mass of P adsorbed per unit mass of soil.

Maximum P adsorption, constant of energy adsorption, and buffering capacities of studied soil have been calculated from Langmuir and Temkin equations and are presented in Table 7.

The highest maximum P-adsorption capacity obtained from the b value of the Langmuir equation was 500 μg g^{-1} in the Griffith soil, whereas it was the

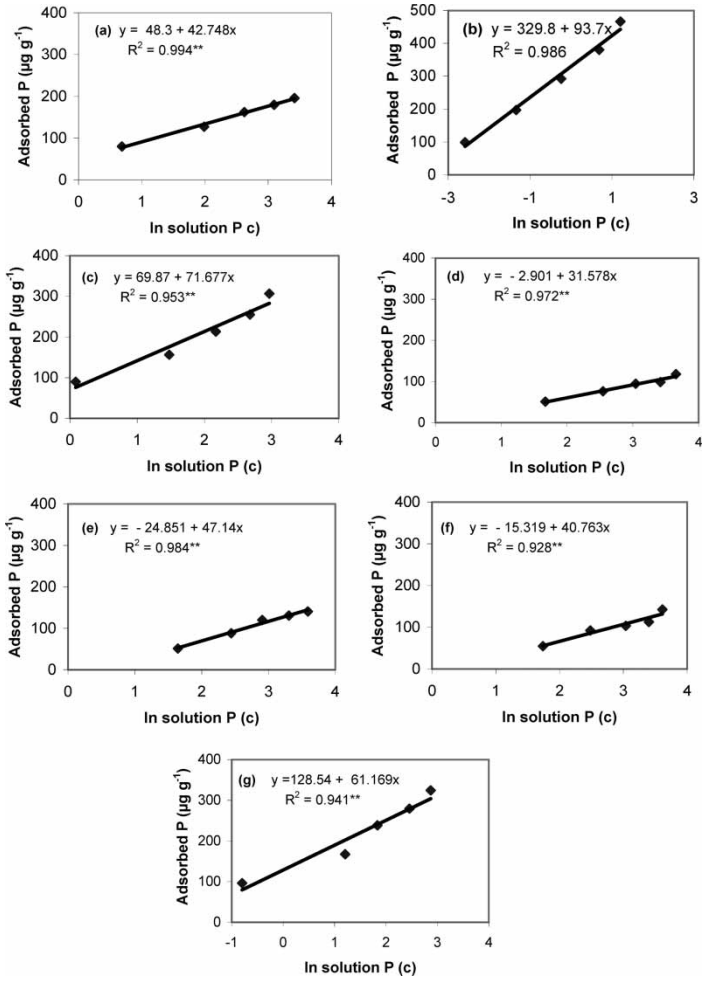

Figure 3. Temkin adsorption isotherms for P adsorption in (a) Camden, (b) Griffith, (c) Narrabri, (d) Rutherglen, (e) Wagga Wagga, (f) Wee Waa, and (g) Yanco soils (** = significant at 0.01 level). Soil samples were treated up to 500 µg P g^{-1} soil for 24 h at room temperature by mixing the soil samples with KH_2PO_4 in solution. The soil samples were separated by centrifugation and filtration and assayed for P content. After treatment the remaining solution P was converted into ln value, and then the value was correlated with adsorbed P (x/m) (µg g^{-1}).

lowest (142.9 µg g^{-1}) in the Rutherglen soil (Table 7). The constant k (obtained from the Langmuir equation), which is a measure of energy of adsorption, was the highest (0.368) in the Yanco soil followed by the Griffith soil (0.222), whereas it was the lowest in the Auscot soil (0.00279). Buffering capacity (µg g^{-1}) (obtained from the b value of the Temkin

Table 7. Maximum adsorption capacity, constant of energy of adsorption, and buffering capacity for P in the soils under study

Soil	Maximum P adsorption capacity[a] ($\mu g\ g^{-1}$)	Constant of energy of adsorption[b] (k value)	Buffering capacity[c] ($\mu g\ g^{-1}$)
Camden	222.2	0.2195	42.7
Griffith	500.0	0.222	93.8
Narrabri	357.1	0.00279	71.7
Rutherglen	142.9	0.093	31.6
Wee Waa	178.6	0.0753	40.8
Wagga Wagga	196.1	0.0725	47.1
Yanco	357.1	0.368	61.2

[a]Calculated b value from the Langmuir equation.
[b]Calculated k value from the Langmuir equation.
[c]Calculated b value from the Temkin equation.

equation) was the highest in the Griffith soil, followed by the Narrabri and the Yanco soils, respectively, whereas the lowest buffering capacity was obtained in the Rutherglen soil (Table 7).

In this study, it was found that these three adsorption isotherms (Langmuir, Freundlich, and Temkin) are applicable for P adsorption in the soils studied. Application of the adsorption isotherms for explaining P-sorption behavior in the soil matrix has been reported by other researchers (Barrow 1978; Akhter et al. 2003). The data from Table 7 show that the P-adsorption capacity is strongly correlated ($R^2 = 0.96$) to the buffering capacity of soil (Figure 4).

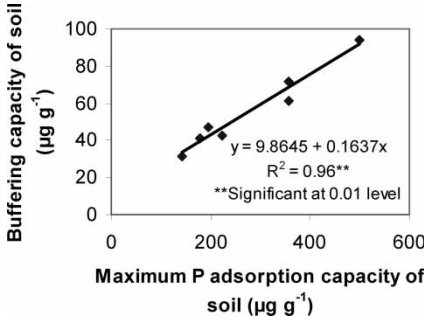

Figure 4. Correlation between maximum P adsorption capacity and buffering capacity of soil.

Marked differences in buffering capacities were noted among the soils. Although the buffering capacity of the Griffith soil was 93.8 $\mu g\ g^{-1}$, it was 31.6 $\mu g\ g^{-1}$ in the Rutherglen soil. Buffering capacity is recognized as the phosphate-retention characteristic in soil. The significance of buffering capacity in characterizing phosphate availability to plants has been demonstrated by many investigators (Olsen and Watanabe 1963; Mattingly 1965; Barrow 1967; Holford and Mattingly 1976b). Buffering capacity also has been related to the soils' fertilizer phosphate requirements (Ozanne and Shaw 1968). In the process of diffusion, P is desorbed from the soil of high concentration and transported to the unfertilized soil (Bhadoria et al. 1991). In conducting an experiment to find out soil phosphate diffusion coefficients, Bhadoria et al. (1991) found that P diffusion coefficient is dependent on (a) the buffer power, (b) whether P is being desorbed or adsorbed, and (c) on the time available for reaction. Therefore, as the buffering capacity was the lowest in the Rutherglen soil, it is expected that the diffusion of P could be faster in this soil compared to other six soils. As the Griffith soil has the highest buffering capacity, P diffusion would be slowest in this soil. For this reason, more P fertilizer may be needed in the Griffith soil to obtain equal plant growth.

Part 2: Enhancement of Phosphorus Desorption by Bacterial Isolates

Phosphorus Extraction by 0.01 M $CaCl_2$

The available P extracted by 0.01 M $CaCl_2$ for the two soils treated by two different bacteria, with and without $CHCl_3$, is presented in Table 8.

The rate of achieving equilibrium for obtaining desorption isotherms characterized by the release of adsorbed P using 0.01 M $CaCl_2$ is regarded as extremely slow (Tisdale, Nelson, and Beaton 1985; Maguire et al. 2001). However, 16 h was sufficient to obtained significant results in this experiment.

There were significant differences in extracted P among the treatments in the Narrabri soil, whereas there was no significant difference observed with the Griffith soil. The Narrabri soil released significantly higher amounts of P than the Griffith soil irrespective of treatments. In the Narrabri soil, the highest P desorption occurred in lysed soil inoculated with the strain FA001, followed by that with the strain FA010. The treatments T_2, T_4, and T_5 were not significantly different, whereas T_6 released a significantly lower amount of P compared to T_2 and T_4 (Table 8). Higher amounts of desorbed P in T_1 and T_2 might be due to the impact of bacteria and lysing. Other researchers reported that lysing by $CHCl_3$ could release approximately 40% of existing bacterial P into the solution (Brooks, Powlson, and Jenkension 1982; Bliss, Comeford, and Muchovej 2004), thus bacterial amendment and lysing might provide higher extractable P in these two treatments (T_1 and T_3).

Table 8. Phosphorus extracted from Narrabri and Griffith soils by 0.01 M $CaCl_2$ with and without $CHCl_3$ extraction

		P ($\mu g\ g^{-1}$ soil) using 0.01 M $CaCl_2$ solution	
Treatment no.	Description	Griffith	Narrabri
T_1	Soil + bacteria (FA001) lysed	0.006 a B[a]	0.492 a A
T_2	Soil + bacteria (FA001) not lysed	0.003 a B	0.270 c A
T_3	Soil + bacteria (FA010) lysed	0.003 a B	0.365 b A
T_4	Soil + bacteria (FA010) not lysed	0.001 a B	0.277 c A
T_5	Soil lysed	0.011 a B	0.259 cd A
T_6	Soil not lysed	0.006 a B	0.236 d A

Note. Interaction effect of soil and treatment was significant at F probability level of <0.001 with LSD (0.05) value of 0.029.

[a]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.

For interaction effects, the highest P extraction was obtained with T_1 followed by T_3. These two treatments were amended with bacterial strain FA001 and FA010, respectively, and lysed by $CHCl_3$. These results indicate that the bacteria (FA001 and FA010) effectively desorbed P from the Narrabri soil. The physicochemical properties of these two soils in Table 1 show that these two soils both contained similar amounts of available native P (11 μg P g^{-1} soil), but their pH and soil textural classes were different.

The initial pH values of the Narrabri and Griffith soils were 7.42 and 4.07, respectively, and their textural classes were clay and sandy clay loam. Because the pH of 0.01 M $CaCl_2$ solution was low (pH 5.76) with low ionic strength, it could not be expected to affect the soil pH. Thus, it is possible that the Griffith soil's acidic pH enhanced P fixation with free ions (Fe^{3+}, Al^{3+}) or Fe and Al oxides or complexed with dissolved organic compounds in soil solution (Brady and Weil 2002). It seems likely that the bacteria that were used in the Griffith soil failed to grow to a potential level at the acid pH or could not access any fixed P, possibly giving insignificant P desorption in 0.01 M $CaCl_2$. The constants of energy adsorption of the Narrabri and Griffith soils were 0.0028 and 0.222, respectively, and soil buffering capacities were 71.7 ($\mu g\ g^{-1}$ soil) and 93.8 ($\mu g\ g^{-1}$ soil), respectively (Table 7). Low buffering capacity probably diffuses more solute to the media, thus Narrabri soil gives more P in the solution than Griffith soil (Barrow 1967; Holford and Mattingly 1976a; Choudhury and Khanif 2000). Also, the recovery of microbial P varied inversely with P-sorption capacity (Hedley and Stewart 1982), which might affect the treatments.

Treatments inoculated with bacteria (T_1, T_2, T_3, and T_4) conferred higher amount of extractable P than uninoculated treatments (T_5 and T_6) into the

extracting (0.01 m $CaCl_2$) solution. It indicates a significant effect of bacteria mobilizing P from the soil. Many other researchers also reported that soil microorganisms are involved in a range of processes affecting P transformation, thus influencing subsequent availability of P to plant roots (Rodriguez and Fraga 1999; Richardson 2001). Of these inoculated treatments, the lysed treatments (T_1 and T_3) conferred a significantly higher amount of extractable P than unlysed treatments (T_2 and T_4), indicating that lysing enhanced microbial P into the extracting solution. Several other researchers (Brookes, Powlson, and Jenkension 1982; 1984; McLaughlian, Alston, and Martin 1986) also reported that lysing provided about 40% of microbial P into the extracting solution.

Most important, the maximum P-adsorption capacity and constant of energy for adsorption is relevant to desorption of P. The Griffith soil had the greater maximum P-adsorption capacity and constant of energy adsorption by comparison to the Narrabri soil. The constant of energy for adsorption gives the degree of strength of bonding in the ecosystem. Phosphorus mobilization by bacteria is not only due to pH changes, but also bacteria or any organisms might mobilize P, supplying to the crops in the microecosystem counting the ligand strength for desorbing P as solute from the soil complex.

The action resonance theory (Kennedy 2001) explains nutrient mobilization as an increase in action and entropy, sustained by a field of energy quanta resonant exchange. The extra action of nutrients, sustained in such fields' energy, provides a matrix for optimization of the multiple factors maximizing yield in agriculture, effectively a multifactorial system providing optimal transport of nutrients (Nguyen et al. 2003; Kennedy, Choudhury, and Kecskés 2004). However, in the Griffith soil, it is apparent that the bacterial chemical and biological energy was significantly less than that needed to desorb the P molecule from the soil-adsorbed-P system, largely because the binding energy was greater. This viewpoint could be a starting point for obtaining mobilization, even with the Griffith soil.

Phosphorus Extraction by 0.5 M $NaHCO_3$

For the fraction of P available to 0.5 M $NaHCO_3$ extracting solution, the interaction effect of soils and treatments was significant (Table 9).

In the Narrabri soil, the highest amount of extractable P was found in T_3, which was statistically similar to T_1. Extractable P was significantly higher in these treatments compared to the rest of the treatments. The treatments T_2 and T_4 were statistically similar, and extractable P was significantly higher compared to T_5 and T_6. The treatments T_5 and T_6 remained at the lowest level for extractable P. The performance of T_2 ranked it in the second and third groups of this experiment in regard to extractable P in the solution. In the T_1 and T_3 treatments, the soil was amended with the FA001 and FA010 bacterial strains, respectively, and lysed by $CHCl_3$.

Table 9. Phosphorus extracted from Narrabri and Griffith soils by 0.5 M $NaHCO_3$ with and without $CHCl_3$ fumigation

		P ($\mu g\ g^{-1}$ soil) using 0.5 M $NaHCO_3$ solution	
Treatment no.	Description	Griffith	Narrabri
T_1	Soil + bacterial cell (FA001) lysed	57.42 abA[a]	57.42 aA
T_2	Soil + bacterial cell (FA001) not lysed	56.67 bA	50.68 bcB
T_3	Soil + bacterial cell (FA010) lysed	59.67 aA	59.67 aA
T_4	Soil + bacterial cell (FA010) not lysed	58.17 abA	52.92 bB
T_5	Soil lysed	58.17 abA	49.18 cB
T_6	Soil not lysed	51.42 cA	47.68 cB

Note: Interaction effect of soil and treatment was significant at F probability level of <0.001 with LSD (0.05) value of 2.92.

[a]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.

It is possible that bacteria mobilize P for their own biosynthesis, including that of phosphorylated nucleotides such as ATP, also producing organic acids; thus extractable P in those bacterial treatments were greater. In another experiment (Ahmed 2006), it was established by high performance liquid chromatography (HPLC) that these two bacteria are capable of producing citric, oxalic, and succinic acids. These organic acids are able to complex with different cations such as Al, Ca, Mn, and Fe known to be responsible for adsorbing P. Such complexes with these cations might make P available in the ecosystem. This is consistent with previous research for pot experiments and under also field conditions that show bacteria can mobilize soil P through chelation and reducing pH (Banik and Dey 1981; Chabot, Hani, and Cescas 1996; de Freitas, Banerjee, and Germida 1997; Zaidi, Khan, and Amil 2003).

In the Griffith soil, the highest and the lowest P was being desorbed by T_3 (59.67 $\mu g\ g^{-1}$ soil) and T_6 (51.42 $\mu g\ g^{-1}$ soil), respectively, using $NaHCO_3$ as extracting reagent (Table 9). There was no significant difference among T_1, T_2, T_4, and T_5. The treatments T_1, T_4, and T_5 remained in both the top and second group for P desorption. However, the treatment T_2 ranked second in term of P desorption. The T_5 treatment was confined for only native bacteria stimulated with glucose, but T_2 was amended by adding the strain FA001 in addition to the soil native microbes. The possible reason for T_2 being less effective for P desorption in comparison to T_5 is that T_2 immobilizes more P than it mobilizes in the solution, and the bacterial-immobilized P could not be accounted for as this treatment was not lysed. Conversely, T_5 was lysed, allowing microbial P to be added to the solution.

The reasons for lowest P dissociation in T_6 could be that (1) this treatment did not receive any inoculated bacteria (only the native microbes might

immobilize P) and (2) this treatment was not lysed using $CHCl_3$, thus biomass P remained in native microbes' cells. Some other researchers also reported that bacterial biomass P can be incorporated into the extracting solution lysing with $CHCl_3$ (Hedley and Stewart 1982; Brookes, Powlson, and Jenkinson 1982).

Possibly soil-buffering capacity did contribute to some extent to the soluble P in the extractant, even though there was a control (with and without bacteria). Bacteria did contribute in regard to P desorption into the extractant, and thus significantly different results were obtained in this experiment. The P mobilized by bacteria extracted by 0.01 M $CaCl_2$ was in a very small amount (Table 8) but it was significant with the Narrabri soil and insignificant with the Griffith soil. The small amount of P mobilized by bacteria possibly can partially fill the requirement of P for the plant. The difference in soil physicochemical properties of these two soils affected the effectiveness of bacteria to mobilize soil-adsorbed P. From this experiment, it seems likely that soil P adsorbing and buffering capacities can affect the effectiveness of the bacteria at mobilizing P (Table 7). If this experiment could be conducted using soils with the highest and lowest maximum adsorption and buffering capacities from the Table 7 (e.g., Griffith soil and Rutherglen soil), a more definite conclusion could be made. More quantitative research with different types of soil and different bacterial strains should enable us to understand the mechanism and feasibility of applying of strains for crop response. In addition, future research taking glucose and P as control, a study with soil autoclaving and without autoclaving could give some comprehensive results.

CONCLUSIONS

This study has shown that increasing levels of addition of P significantly increased P adsorption of the soils under study but that inoculation with P-mobilizing bacteria amended with glucose can desorb P. The buffering capacity ($\mu g\ g^{-1}$) (retention capacity of adsorbed P) and the maximum adsorption capacity ($\mu g\ g^{-1}$) of soil determine its P-adsorbing potential. While the results show that bacterial activity can be involved in P desorption, bacterial P-desorbing capability also seems to depend on P-retention capacity and the maximum adsorption capacity of the soil. The difference in response of the two soils examined also indicates that the capacity of different soils to respond to the presence of P-mobilizing strains of microorganisms will vary. Confirmation was obtained from this experiment that using 0.5 M $NaHCO_3$ (pH 8.5) in acidic soil for P determination facilitates determination of iron and aluminum-bound P.

It is concluded that by determining the buffering capacity ($\mu g\ g^{-1}$) and the maximum adsorption capacity ($\mu g\ g^{-1}$) of soil, the effectiveness of the application of phosphatic biofertilizer may be predicted.

More research is needed with a wider range of soils and an ample range of bacterial strains to confirm the feasibility to predict biofertilizer effectiveness. Although there are many unanswered questions related to the kinetics of P mobilization from different soil types, it is recommended that this approach be pursued further.

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