A KINETIC APPROACH TO SOIL PHOSPHORUS MOBILISATION BY INOCULANT BIOFERTILISER

Mohammad Faruque Ahmed

Faculty of Agriculture, Food and Natural Resources
The University of Sydney
New South Wales
Australia

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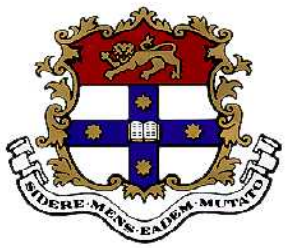

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STATEMENT OF ORIGINALITY

Unless otherwise stated, the results presented in this thesis are the original work of the author.

Mohammad Faruque Ahmed

FOR MY PARENTS

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ABBREVIATIONS

^{O}C	degree Celsius
cfu	colony forming unit
g	gram
g	gravational force
mg	milligram
µg	microgram
mL	millilitre
L	litre
cm	centimetre
mm	millimetre
µm	micrometre
nm	nanometre
v/v	volume per volume
w/w	weight per weight
%	per cent
CEC	cation exchange capacity
cmolc	centi mole charge
h	hour
min	minute
sec	second
M	molar
mM	millimolar
OD	optical density
spp.	species
DNA	deoxyribonucleic acid
rRNA	ribosomal ribonucleic acid
bp	base pair
kb	kilo base
Ltd	limited
Co.	company
PPB	phosphate peptone buffer
S	strain
FA	Faruque Ahmed
$Ca_3(PO_4)_2$	tricalcium phosphate
$CaHPO_4.2H_2O$	dicalcium phosphate
$AlPO_4$	aluminum phosphate
$FePO_4$	ferric phosphate
G	guanine
C	cytosine
T	thymine
A	adenosine
SP	super phosphate
Rock P	rock phosphate
Max	maximum
Diam	diameter

Website references: All website references have been included in the text giving the basic website address. The sites have been listed at the end of the references list with further details and date of use.

ABSTRACT

The research reported in this thesis examines on the feasibility of selecting soil bacteria able to convert insoluble forms of soil phosphorus (P) to mobile forms more available for plant growth. A kinetic approach was used in which rates of P-mobilisation were measured.

Bacteria were isolated from soils sampled from three locations, 1) a neutral to alkaline vertisol soil cultivated with cotton and wheat (Narrabri, NSW), 2), a neutral grassland rhizosphere soil (Wee Waa, NSW), and 3) an acid red soil cultivated with wheat (Wagga Wagga, NSW). Seven bacterial strains were successfully isolated using minimal medium containing $Ca_3(PO_4)_2$ as the sole source of P. The isolates were characterised using selective media and identified using 16S rDNA sequence analysis. The P-mobilising bacteria were identified by the clear 'halo' zones surrounding their colonies on agar containing insoluble P. The most effective P-mobilising bacterial isolates were obtained from the grassland and acid wheat soils and identified as *Pantoea ananatis* (FA001) and *Pantoea agglomerans* (FA010), respectively, belonging to the Enterobacteriaceae family. The other strains isolated were classified as *Enterobacter cloacae*, also belonging to the Enterobacteriaceae family, and several *Burkholderia* sp.; these were found to be less effective in P-mobilisation, indicated by smaller halos surrounding their colonies and solubilisation in liquid culture.

Several tests were conducted to validate the P-mobilising ability of these bacteria. In liquid minimal media containing 500 mg L^{-1} of different types of insoluble P, the strain (FA001) identified as *Pantoea ananatis* mobilised the highest P from $Ca_3(PO_4)_2$ at the highest kinetic rate (50.32 mg L^{-1} day^{-1}) followed by the strain FA010 identified as *Pantoea agglomerans* (45.77 mg L^{-1} day^{-1}), using $(NH_4)_2SO_4$ as source of N. With NH_4NO_3 as source of N, *P. ananatis* (FA001) also mobilised the highest amount of P (35.31 mg L^{-1} day^{-1}) from $Ca_3(PO_4)_2$ followed by *P. agglomerans* (FA010) (18.68 mg L^{-1} day^{-1}). But this was reversed when rock phosphate was the source of P. *P. agglomerans* (FA010) mobilised the highest P (27.27 mg L^{-1} day^{-1}), followed by *P. ananatis* (FA001) (24.53 mg L^{-1} day^{-1}) from rock phosphate-containing medium. Of these isolated strains, *P. ananatis* (FA001) mobilised small amounts of P from $AlPO_4$ and $FePO_4$. *P. agglomerans* (FA010) also mobilised small amounts of P from $AlPO_4$, but not from $FePO_4$.

There was very good correlation between acid production and P mobilisation, suggesting acid production as a major mechanism used for mobilising P by these isolated strains. There was some indication that chelation may also play a role, since both *P. ananatis* (FA001) and *P. agglomerans* (FA010) produced citric acid (2.55 mg L^{-1} day^{-1} and 2.89 g L^{-1} day^{-1}, respectively). The strong organic anion, citrate, can potentially chelate cations such as Ca^{2+} and Al^{3+}, reducing their chance of immobilising P.

To confirm that the results of these *in vitro* experiments were relevant for plant growth, glasshouse experiments were conducted in a P-depleted soil to examine the effect of the P mobilising bacteria on the grain yield of wheat. Two yield experiments were conducted, using $Ca_3(PO_4)_2$, $CaHPO_4.2H_2O$ and rock phosphate as sources of P. Consistent with the *in vitro* data results, for all the sources of P, *P. ananatis* (FA001) and *P. agglomerans* (FA010) resulted in significantly higher grain and straw yields in trials conducted in 2003 (Experiment 1) and in 2004 (Experiment 2). In Experiment 2 the percentage P content of the grain and straw was the same in treatments with bacteria and the controls. Another glasshouse experiment (Experiment 3) was conducted to evaluate the plant growth-promoting (PGPR) effects of these bacteria, using soluble phosphorus with Hoagland solution. In this experiment, *P. ananatis* (FA001) and *P. agglomerans* (FA010) were compared with some other recognised PGPR strains. The results suggest that both of these bacteria can increase grain yield of wheat by about 10 per cent compared to uninoculated controls, independently of the P-mobilising effects.

Since understanding the role of bacteria in P-mobilisation in relation to the chemical behaviour of P in soil was a principal objective of this thesis, *P. ananatis* (FA001) and *P. agglomerans* (FA010) were also used in an experiment to examine P adsorption to and desorption from soil. Two soils i) a brown sandy clay loam of pH 4.07 from Griffith, and ii) a heavy clay vertisol of pH 7.42 (Narrabri soil), were used for the desorption experiment. It was revealed that both these bacteria (*P. ananatis* (FA001) and *P. agglomerans* (FA010)) could significantly mobilise P from the vertisol with neutral pH, but not from the sandy clay soil with low pH. From Langmuir and Temkin adsorption isotherms which are indicative of P adsorption behaviour of these soils, it was concluded that for soils having high P-buffering capacity and high maximum P adsorption capacity these bacteria could not significantly mobilise phosphorus. Therefore it can be concluded that before applying P mobilising biofertiliser, maximum adsorption capacity and retention behaviour of soil should be estimated, so that the feasibility of effectively applying a particular biofertiliser can be predicted.

From the set of P-mobilising biofertiliser strains studied in this thesis, *P. ananatis* (FA001) was indicated as the best strain, meeting the criteria for mobilisation in the different tests. It is, therefore recommended for further testing as a biofertiliser strain in different soil types. However, whether this strain will be effective under field conditions remains to be tested.