

6.1 General discussion

The hypothesis advanced in this thesis was that it would be possible to isolate P-mobilising bacteria from soils, to validate their P mobilisation ability *in vitro*, and thus predict their effectiveness for promoting plant growth as a result of the interaction between poorly soluble soil-P and inoculated bacteria.

Some *Enterobacter* sp., *Pantoea* sp. and *Klebsiella* sp. bacteria have been identified as P-mobilising bacteria in liquid medium from different sources of insoluble P in laboratory experiments (Chung *et al.*, 2005). *Pantoea agglomerans* has been identified as a P-solubilising bacterium able to mobilise P from different sources of insoluble P in an *in vitro* experiment (Son *et al.*, 2006). These reports do not contain unequivocal evidence for the mobilisation of soluble P that can be used by plants. While P-mobilisation by microorganisms is commonly observed under laboratory conditions, results confirming these observations as yield increases in the field have been highly variable (Gyaneshwar *et al.*, 2002). This variability has hampered large scale application of phosphate solubilising microorganisms in agriculture. One factor that can potentially explain this variability in field response is 'poor understanding of interactions between physical and chemical characteristics of soil and how these interact with biological P availability' (Richardson, 2001). This factor has been addressed in studies described in this thesis and the results presented should contribute to successful biofertiliser development.

The bacterial strains (Chapter 2) isolated as the most effective P-mobilisers on agar plates and identified as *Pantoea* spp. were used in the experiments described in Chapters 3, 4 and 5. The strains FA001 and FA010 were the most successful in the experiments described in all these Chapters. Isolation of P-mobilising bacteria based on their ability to create a 'halo' zone around their colonies is a useful simple method as the clear zone indicates P- mobilising ability. It has been reported, however, that in the case of some P-mobilising bacteria, the cleared zone is not apparent in successive cultures (Kucey, 1983). Thus there is a possibility using this method of isolation, of the selection of some weakly solubilising bacteria, as observed with the isolates FA002, FA003, FA004, FA005 and FA009.

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The use of Bromocresol purple as an indicator which changes colour in the isolating media from purple to yellow when the pH is below 5.3, could be useful for the identification of bacteria that can mobilise insoluble P by changing pH. It has also been reported that using Bromocresol purple, the colour of the medium could change to yellow, and in some cases revert back to purple (Kucey, *et al.*, 1989). For P mobilisation made by other processes such as chelation (Kucey, 1983; Kirk *et al.*, 1999), selection of P mobilising bacteria based on a 'halo' zone may be an appropriate method.

In Chapter 3 of this thesis it was shown that *Pantoea* spp. (FA001 and FA010) can mobilise more insoluble P than other bacteria (*Burkholderia* sp.) from all the different types of insoluble P suspended in liquid culture. They mobilised the greatest amount of P from all types of insoluble P in media containing $(\text{NH}_4)_2\text{SO}_4$ and NH_4NO_3 as sources of N. Using $(\text{NH}_4)_2\text{SO}_4$ as a source of N acidifies the medium and more P can be solubilised from insoluble forms (Parks *et al.*, 1992; Wenzel *et al.*, 1994). It is of interest that the bacterial strains FA002, FA003, FA004 and FA009, mobilised more P in the NH_4NO_3 -containing medium than the $(\text{NH}_4)_2\text{SO}_4$ -containing medium suggesting a different means of mobilisation than a fall in pH value.

The results obtained on the most effective strains are consistent with reports that *Pantoea* spp. can solubilise insoluble P in liquid media (Chung *et al.*, 2005; Son *et al.*, 2006). In the present study bacteria were isolated from soils with different properties. The best strain FA001 (*Pantoea ananatis*) was isolated from soil from Wee Waa (Inceptisol, pH 6, clay 10 per cent) and the strain FA010 (*Pantoea agglomerans*) was isolated from Wagga Wagga soil (Red Kandosol, pH 5, clay 20 per cent). It may be more appropriate to isolate P-mobilising bacteria from specific soil types to develop a biofertiliser for that specific soil. For example, Nguyen *et al.* (2003), isolated P-mobilising bacteria from rice rhizosphere which grow effectively in rice fields and thus contribute to increase rice yields. Nautiyal *et al.* (2000) isolated P-mobilising bacteria from the rhizosphere of chickpea grown in alkaline soils. Purnomo *et al.* (2004) isolated P-mobilising bacteria from rice soil. Citrate has been reported to be a good chelator of metal ions (Cunningham and Kuiack, 1992) and in the studies reported in Chapter 3 it was found that strains FA001 and FA010 were the only

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strains that secreted citrate. The organic anion α -ketogluconate has been recognised as a very good chelator (Kucey, 1988; Hwangbo *et al.*, 2003) that makes complexes with inorganic cations (aluminium and iron), but in this study α -ketogluconate was not measured. In the study of organic anion production described in Chapter 3, only $\text{Ca}_3(\text{PO}_4)_2$ and $(\text{NH}_4)_2\text{SO}_4$ were used as sources of P and N, respectively.

Although liquid culture was used in experiment described in Chapter 3 as a technique to measure P-mobilisation from isolated bacterial strains, it should not be considered as a direct model for change in soil pH. Growth conditions for bacteria in soil are spatially and temporally highly variable, with the availability of carbon substrates dictating where growth is possible, as well as where pH can fall and P can be mobilised. As a consequence, acidification as a mechanism of mobilisation is expected to be localised near the root surface near where such substrates may be excreted from plants. Given the large pH buffering capacity of most soils, it would be unlikely that microbes significantly reduce the pH of the soil solution as a whole. However, the hypothesis of microbial P-biofertilisation requires only that spatially localised effects can be produced in proximity to the root surface where P-uptake as soluble forms of H_2PO_4^- and HPO_4^{2-} can occur.

Two glasshouse experiments with wheat were conducted in this study using the best selected P-mobilisers (Chapter 4). Only one soil was tested, a Camden soil low in available P (Table 5.1) (Chapter 4). The wheat plants treated with the strains FA001 and FA010 reproducibility gave greater grain and straw yield for growth in pots, indicating that these two strains may be useful as phosphatic biofertiliser if field trials confirm the pot trial results. Yield increases for different crops attributed to insoluble P solubilisation by inoculated bacteria have been shown for wheat (Harris, *et al.*, 2006); rice (Nugyen *et al.*, 2003); canola (Hall *et al.*, 1996); horticultural crops such as tomato, lettuce, (Suslow, 1982); and apple, citrus, beans and ornamental plants (Kloepper, 1994).

The overall hypothesis given above is consistent with the results obtained with wheat. As these glasshouse experiments were conducted with only one type of soil (Camden soil) it is not possible to predict the feasibility of the application of these two bacteria

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based on soil P-buffering and P-adsorbing capacities. Data in Table 5.7 show that the P-buffering and P-adsorbing capacities of the Camden soil are intermediate in the set of soils listed in the Table and lower in these properties than the Griffith and the Narrabri soils. Possibly for this reason, the plants inoculated with the strains FA001 and FA010 gave significantly greater yields than for other strains and control treatments in glasshouse experiments. As described in Chapter 3 in this thesis the strains FA001 and FA010 had mobilised significantly greater amounts of P in the liquid medium. The treatments inoculated with these two strains (Experiment 3, Chapter 4) in the presence of soluble P showed grain yield increases of about 10 per cent. Thus the increased grain and straw yields found in Experiment 1 and Experiment 2 could be the result of the combined effects of P-mobilisation and the plant PGPR effects of these two bacteria. The use of bacteria such as *Pseudomonas*, *Bacillus* and *Rhizobium* as inoculants has been associated with increased P uptake by plants and increased crop yields (Rodríguez and Fraga, 1999). It has also been reported that inoculation with *Pseudomonas putida* and *Pseudomonas fluorescens* increased root and shoot elongation in canola, lettuce and tomato (Hall *et al.*, 1996; Glick *et al.*, 1997). The roles of these bacteria in improved crop growth may include P-mobilisation, but all of these species have been reported as being PGPR effects.

The study on P-desorption described in Chapter 5 was conducted to see whether the results described as P-mobilisation in Chapter 3 are valid for the most promising bacterial strains (strains FA001 and FA010) taking into account soil properties related to P-adsorption. The amount of P mobilised by the two bacteria FA001 and FA010 in liquid culture, from Ca salts, was much more than from aluminium and iron salts. Although these two bacteria mobilised significantly greater amounts of P than other strains and mobilised more P from Ca salts than from the control treatment and AlPO_4 and FePO_4 , the amount of P released was small in comparison to plant requirements (Martin *et al.*, 1976). Experiments on bacteria-soil interaction in the laboratory to assess the possibility of variation in P-mobilising ability of bacteria in different soil have not been described. Here the two best bacteria FA001 and FA010 based on liquid cultures were used in laboratory experiments to investigate P-mobilisation from Ca salts (Chapter 5). It has been reported that P-desorption is highly influenced by the P-buffering capacity ; the higher the buffering capacity the lower the desorption of P

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will be (Barrow, 1967; Holford and Mattingly, 1976). By this approach it was found that such P-mobilising bacteria can not mobilise insoluble P from soil with high P-buffering and P-adsorbing capacities, as shown with the Griffith soil. By contrast, it was found (Chapter 5) that these bacteria were able to mobilise P from Narrabri soil with lower P-buffering and P-adsorbing capacities. Although small by measurement in CaCl_2 and NaHCO_3 , this may still be very significant in rhizosphere soil with high bacterial turnover. This result is consistent with some soils responding better than others to the presence of P-mobilising biofertiliser strains. Apparently more energy than that released from the microbial metabolism would be required to release P from its bonds with the Griffith soil.

It is possible to conclude from the results presented in this thesis that there is potential for the development of phosphatic biofertilisers, subject to further study in laboratory experiments, greenhouse pot trials and field studies. The most promising bacterial strains isolated in these studies were *Pantoea* spp., but it is important to remember that bacterial populations in soil can be both beneficial and deleterious to plants. For example, strains of *Pantoea ananatis* have been reported to cause diseases such as centre rot of onion seed (Walcott *et al.*, 2002), and leaf blight and die-back of young shoots of Eucalyptus (Coutinho *et al.*, 2002). *Pantoea agglomerans* is reported to be associated with seed rot of cotton (Medrano and Bell, 2006) and a cause of septic arthritis in humans after palm tree thorn injury (Kratz *et al.*, 2003). Conversely, *Pantoea* sp, and *Pantoea agglomerans* also reported as P mobilisers without any comment regarding pathogenicity (Kim, *et al.*, 1997; Chung *et al.*, 2005). In contrast, Johansson *et al.* (2003) reported that *Pantoea* sp (probably *P. agglomerans*) suppressed some fungal disease as effectively as the fungicide guazatine and were effective against snow mould, caused by *Microdochium nivale* and seedling blight of wheat caused by members of the *Fusarium* complex. The P-mobilising strains of *Pantoea* sp. described in this thesis have not been examined for disease-causing capabilities. Before application of these strains as biofertiliser, it would be necessary to show that they are free of such pathogenic effects.

Even though the best strains were selected on the basis of P-mobilisation on agar plates and liquid culture, there is also the possibility of positive PGPR effects from

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the interaction indicated by the experimental results reported with yield of wheat. The promotion of root growth by phytohormones such as IAA (Zahir *et al.*, 2004) could also contribute both to a greater absorptive surface for P but also for the growth of more microorganisms by creating a greater clear zone for P-mobilisation. If so, it is not possible to separate these two effects since one may contribute to the other.

6.2 Future work

In future work, isolation of P-mobilising bacteria could be focused on a wider range of soils deliberately ranging across calcareous alkaline soil, neutral soil, or acid soil. Or for soil that has been used for production of wheat, rice or pastures. Alternative methods for preliminary isolation such as the use of pH indicators in agar plates could be used as well as the development of a 'halo' in the presence of insoluble P could be used.

The role of organic P compounds as sources of P for plant growth was not considered in this work although organic phosphates usually comprise about 50 per cent of total unavailable P in soil (Richardson, 2001). Phosphatases are recognised as microbial products able to dephosphorylate organic P into inorganic P, making it available to plants (Jones, 1998; Ehsanpour and Amini, 2003). The phosphatase activity of isolated bacteria could be measured so their potential for mobilising organic P could be assessed.

In studies of the mobilisation of P by bacteria in liquid suspensions different types of P- and N-sources should be used and the secretion of other organic anions including -ketogluconate should be determined. Determination of the P content of bacteria produced during the incubation would allow calculation of the total amount of P released from the insoluble P sources used.

Future pot studies could be carried out using the FA001 and FA010 bacterial strains and bacteria from other soils with different amounts of added soluble/insoluble P, and varying natural sources of P. It would be valuable to confirm the sources of P taken up by the wheat plants supplied with a range of insoluble P sources. Studies using

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isotopically labelled P sources could be used to monitor the mobilisation and plant uptake of soil P sources. It would also be interesting to examine the effects of autoclaving soils to destroy endogenous bacteria, and the use of variable amounts and sources of organic carbon.

The preliminary experiments described in Chapter 5 showing the effects of two bacterial strains (FA001 and FA010) on P-desorption from two soils could readily be extended. Considering the range of soils listed in Table 5.7 it would be useful to contrast the soil-P-bacterial interaction using Rutherglen soil (which has the lowest P-adsorbing (143 mg kg^{-1}) and P-buffering (32 mg kg^{-1}) capacities of the soils listed (Table 5.7)) with Griffith soil. Other soils with characteristics such as high Ca, high pH, acid soils, and soils used for specific crop production could also be examined to test the hypothesis that microorganisms adapted to particular soil types perform better in P mobilisation.

Experiments should be conducted using soils with a large range of P-buffering and P-adsorbing capacities. The development of a model based on physico-chemical soil properties including soil P-buffering and soil P-adsorbing capacities could be useful in determining soils likely to respond best to phosphatic biofertilisers.

Recommendations based on pot trials and laboratory experiments that indicate that specific bacteria may act as phosphatic biofertilisers need to be tested in the field. A potentially economically useful biofertiliser should be tested on a range of soils, with varying nutrient and P status, and with more than one crop.