PENICILLIUM RADICUM: STUDIES ON THE MECHANISMS OF GROWTH PROMOTION IN WHEAT

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DECLARATION

I declare that the work does not contain material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

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December 2004. Signed,

Simon Anstis

ABBREVIATIONS

l.s.d	least significant difference
Ca-P	dibasic calcium phosphate, monetite
DRBC	dichloran rose bengal chloramphenicol agar
EtOH	ethanol
Fe-P	crystalline iron phosphate, $FePO_4$
IAA	indole-3-acetic acid
Ggt	*Gaeumannomyces graminis* var. *tritici* isolate #8
MEA	malt extract agar
MEB	malt extract broth
MeOH	methanol
RP	rock phosphate
s.e	standard error
P_o	phytate, inositol hexaphosphate
PDA	potato dextrose agar
R_f	retention factor
SCP	4% sucrose citrate-phosphate buffer, pH 5
Tris	Tris[hydroxymethyl]aminomethane
TRP	tryptophan
WS	water soluble

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SUMMARY AND SCOPE OF THESIS

The aims of this study were to investigate the P solubilising activity of *Penicillium radicum* and identify other possible mechanisms of plant growth promotion that were not related to P solubilisation. The plant chosen for the studies was wheat, the largest cereal commodity produced by Australian agriculture. Given the large area that is planted annually to wheat, this crop represents a large potential market for *P. radicum* - based inoculants. However, it is unlikely that *P. radicum* will be effective in all wheat growing regions and all environmental conditions seen in these areas. Research on the modes of action may help to identify the conditions where *P. radicum* has a good chance of being effective.

The P solubilising activity of *P. radicum* was assessed by examining the amount of P released from rock phosphate (RP) *in-vitro*. The effectiveness of *P. radicum* to solubilise RP was compared to that of another P solubilising fungus, the isolate *P. bilaiae* RS7B-SD1. Both fungi were cultured in a liquid medium that contained either NO_3^- or NH_4^+ as the sole source of N. Changes in culture pH, soluble P and gluconic acid concentration were determined for both fungi in a 168 hour incubation in both buffered (100 mM Tris-HCl pH 7.8) and non-buffered medium. For *P. radicum,* the maximum concentration of soluble P was 6.8 fold higher in the presence of NH_4^+ compared to NO_3^-. In contrast, for *P. bilaiae* RS7B-SD1 the highest concentration of soluble P measured in the fungal culture was not significantly affected by N-source. In buffered medium, *P. radicum* did not appear to solubilise RP and levels of soluble P were generally <1 mg L^{-1}. In contrast, the RP solubilising activity of *P. bilaiae* RS7B-SD1 was not affected by buffering. Increased RP solubilisation with NH_4^+ as the N

source and lack of RP solubilisation in buffered medium suggested that acidification was the main mechanism of P solubilisation by *P. radicum.* RP solubilisation by *P. bilaiae* RS7B-SD1 was similar over the range of culture conditions tested and mechanisms of RP solubilisation are likely to be a combination of mechanisms that relate to both acidification and the production of organic anions.

The effect of inoculation with *P. radicum* on plant growth and P nutrition was studied under glasshouse conditions using a sand culture assay that supplied defined sources of P. The plant growth and P response to *P. radicum* inoculation were determined in two separate experiments. In Experiment 1, plants were grown to pre-heading stage and supplied with either dibasic calcium phosphate (Ca-P), crystalline iron phosphate (Fe-P), rock phosphate (RP) or phytate (P_o) as the source of P. In Experiment 2, plants were harvested after 8 weeks and supplied with either NO_3^- or NH_4^+ as the sole source of N and the P sources were either Ca-P or RP. In Experiment 1, the plant P response (defined as higher shoot P concentration and P uptake) to inoculation was dependent on the P source. The greatest plant P response to inoculation was observed for Ca-P and no significant P response was measured in plants that were supplied with Fe-P, RP or P_o. In pots that supplied Fe-P as the P source, there was an increase in shoot dry matter in response to *P. radicum* inoculation but this occurred without a concomitant plant P response. In Experiment 2, the plant P response to inoculation was dependent on the N source. In the presence of NH_4^+, *P. radicum* significantly increased the availability of P sources RP and Ca-P. While there was no significant plant P response under NO_3^- supply, there was a significant increase in dry matter production due to *P. radicum* inoculation. When the data of Experiments 1 & 2 are taken together, results suggest that *P. radicum* possesses at least two mechanisms of plant growth promotion, (1) P

solubilisation and (2) general growth promotion that is independent of P solubilisation. In agreement with P solubilisation in solution cultures, the P solubilisation mechanism of *P. radicum* in sand culture required NH_4^+. The ability of *P. radicum* to increase plant growth independently of a plant P response gave further evidence of general growth promoting abilities of the fungus. While sand culture is a useful tool to elucidate the fungal mechanisms of plant growth promotion, this approach cannot fully reflect the complexity of the rhizosphere in non-sterile soil. Hence, a subsequent experiment was done to determine the effect of *P. radicum* on plant growth and P nutrition in a number of field soils.

The P solubilising activity of *P. radicum* was determined in four Australian field soils using isotopic dilution. Three soils were chosen on the basis of their chemistry of P retention: (1) Minnipa soil from South Australia was chosen due to P retention associated with the highly alkaline calcareous nature of this soil; (2) Innisfail Queensland, in this soil P retention was dominated by reaction with Fe oxides; and (3) Mt Schank South Australia, a volcanic soil where P retention was predominantly associated with Al oxides. The fourth soil, from Mingenew Western Australia, was chosen due to previous reports that *P. radicum* inoculation increased the yield of field grown wheat (Bio-Care Technology, unpublished data). The four field soils were each labelled with $KH_2{}^{32}PO_4$ and the specific activity (^{32}P) of the wheat seedling tissue was measured after four weeks growth. When the data was averaged across all four soil types, inoculation with *P. radicum* caused a significant 11.7% increase in the shoot dry weight of these seedlings. However, *P. radicum* did not cause any consistent significant difference in the specific activity (^{32}P) of plants when compared to uninoculated control plants. These results suggested that *P. radicum* did not have a strong ability to

solubilise P from the test soils, and mechanisms other than P solubilisation were in operation to stimulate plant growth.

The production of plant growth regulators (PGR) was considered as a mechanism of plant growth promotion not related to P solubilisation. To further explore the hypothesis that the production of PGR acts as a mechanism of plant growth promotion, the ability of *P. radicum* to produce the auxin, indole-3-acetic acid (IAA) was investigated. Examination with thin-layer chromatography and the *Avena* coleoptile straight growth assay indicated that fractions of *P. radicum* culture medium with chemical characteristics similar to IAA (i.e. similar reaction to the Salkowski reagent and R_f as IAA) also possessed auxin-like activity. Using competitive enzyme linked immunosorbent assay (ELISA) it was found that in liquid culture amended with the precursor tryptophan, *P. radicum* produced IAA at concentrations up to 0.406 μM. These studies show that *P. radicum* can produce IAA under laboratory culture conditions. To determine the significance of IAA as a mechanism of plant growth promotion, further studies need to link effects on plant growth and development to the production of IAA by *P. radicum.*

The ability of *P. radicum* to antagonise root pathogens and control root disease was considered as a further mechanism of growth promotion. Under *in-vitro* conditions, *P. radicum* produced hyphal growth patterns and enzymes (protease, β-1,3- and β-1,4-glucanase activity) that were indicative of hyperparasitism. Hyperparasitic growth was seen as hyphal coiling and branching of *P. radicum* against host hyphae of *Rhizoctonia solani*, *Fusarium pseudograminearum* and *Pythium irregulare* when these soilborne pathogens were studied in dual culture with *P. radicum.*

The effect of *P. radicum* on the fungal root disease severity of take-all was studied using a seedling bioassay under glasshouse conditions. The ability of *P. radicum* to suppress take-all disease appeared to be related to the timing of *P. radicum* infection of wheat seedling roots and placement of the *Ggt* inoculum in relation to the roots. Compared to soils where *Ggt* inoculum was only distributed at distances >1 cm below the seed, uniform mixing of the *Ggt* inoculum throughout the soil negated the beneficial effect of *P. radicum* on plant growth and its ability to reduce take-all root lesion size. Conversely, early infection of wheat roots by *P. radicum* gave wheat seedlings some protection against take-all disease. Where treatment with *P. radicum* was effective, increasing the inoculum dose significantly reduced take-all lesion size. While *P. radicum* exhibited antagonism towards *F. pseudograminearum, Py. irregulare, Bipolaris sorokiniana* and *R. solani* cereal root pathogens *in-vitro,* further studies under non-sterile soil conditions are needed to evaluate the potential for *P. radicum* to reduce root disease caused by these fungi.

In conclusion, it is unlikely that one single mechanism explains the beneficial effect of *P. radicum* on wheat growth. *In-vitro* studies showed that *P. radicum* has a number of attributes that could function as mechanisms of plant growth promotion. These attributes were, (1) P solubilisation, (2) production of IAA and (3) the ability to antagonise soilborne pathogens *in-vitro* and reduce the lesion size of the take-all disease in a seedling bioassay. Sand culture assays revealed that at least two plant growth mechanisms were in operation, (1) P solubilisation and (2) a general growth promotion mechanism that was independent of P solubilisation. In agreement with Whitelaw et al. (1999), the P solubilisation mechanism of *P. radicum* operates via an

acidification mechanism. The effectiveness of this mechanism may be limited by the availability of NH_4^+ in the rhizosphere. Since NH_4^+ appears to be required for P solubilisation there may exist an interaction between *P. radicum* and ammoniacal fertilisers. This will have implications for its effectiveness in the field.

In-vitro studies suggest that the general mechanism of growth promotion may be related to the production of PGRs such as IAA. In this aspect the known colonisation of the interior of wheat roots by *P. radicum* would ensure that IAA produced by the fungus is taken up by the root cell and less subject to chemical degradation and/or degradation by other soil microorganisms. Further studies are required to identify the effect of IAA production on plant growth and the effect of *P. radicum* inoculation on root disease severity in non-sterile soil.