Polyphosphates and microbial uptake of phosphorus: Studies with soil and solution culture

A thesis presented in fulfilment of the requirements for the Degree of Master of Agricultural Science, Faculty of Agricultural and Natural Resource Sciences, The University of Adelaide.

by

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Summary

Micro-organisms play a major role in phosphorus (P) cycling in most soils. They are involved in the concurrent processes of mineralization and immobilization of P in soil. Micro-organisms mineralize organic P in plant tissues and other soil organic matter to orthophosphate. This orthophosphate is then available for uptake by plants or micro-organisms or reaction with other components of the soil system. However, micro-organisms may also compete with plants for added P in fertilized soil.

Currently there is no measure of the potential P uptake of the soil biomass. The presence of polyphosphates was investigated as an indicator of the the phosphorus status and potential P uptake of some micro-organisms. Micro-organisms can grow without accumulating polyphosphates, but this accumulation is a result of adequate or luxury P uptake. Polyphosphate is a P- and energy-storage compound which accumulates in most micro-organisms when phosphorus does not limit microbial growth.

The aim of this project was to investigate microbial P uptake and polyphosphate content with a view to using microbial polyphosphate content as a measure of potential P uptake by micro-organisms. Three experiments were conducted with the following objectives:

i) to determine if the addition of wheat or annual medic root material to soil had differing effects on soil microbial C and P;

ii) to investigate the effect of previous P nutrition on P uptake of two bacteria and two fungi in single solution culture and relate this to the presence or absence of polyphosphates in these micro-organisms; and

iii) to investigate the presence of polyphosphates in field soil over a range of P status as estimated by extractable P concentration and to determine whether these polyphosphates are present in the microbial biomass.

Annual medic (*Medicago trunculata* cv Paraggio) and wheat (*Triticum aestivum* cv Molineux) plants were grown in a glasshouse and their finely chopped roots mixed with soil at similar rates of carbon addition. Soil was incubated at 20°C and 60% water holding capacity for 21 days and microbial carbon (C) and P measured at the time of peak carbon dioxide evolution (determined in a preliminary experiment). Soil microbial respiration was measured by addition of glucose solution measurement of the initial peak of CO_2 evolution. Glucose is an immediately metabolisable microbial substrate and the (immediate) maximum CO_2 evolution of soil treated with glucose solution is considered to be a measure of the size of the initial soil biomass. Microbial P was measured by fumigation with hexanol and extraction with 0.5 M sodium bicarbonate solution.

Medic roots mixed with soil at a rate of 43 mg C g^{-1} soil and 0.40 mg P g^{-1} soil stimulated peak soil respiration of 3.0 μg CO_2 g^{-1} soil h^{-1} and a soil microbial P increase of 8.3 μg P g^{-1} soil. Wheat roots mixed with soil at a rate of 37 mg C g^{-1} soil and 0.27 mg P g^{-1} soil stimulated peak soil respiration of 2.7 μg CO_2 g^{-1} soil h^{-1} and a soil microbial P increase of 0.6 μg P g^{-1} soil. Medic roots stimulated a larger relative increase in microbial P than wheat roots for a given rate of addition. This implied that, for a similar quantity of biomass, the P content of micro-organisms stimulated by medic roots was about three times that of micro-organisms stimulated by wheat roots. It was concluded that plant species affects the chemical composition of the soil biomass.

The effect of previous P nutrition on orthophosphate uptake and polyphosphate accumulation by two species of bacteria (*Aerobacter aerogenes* and *Enterobacter sp*) and two species of fungi (*Mucor racemosis* and *Thanatophorus cucumeris*) was measured.

Bacteria and fungi were grown in nutrient solution of low or high P concentration (0.15 and 0.70 mM P respectively for bacteria, and 0.40 and 0.94 mM P for fungi). P uptake from solution of intermediate P concentration was measured and subsamples of cultures were analysed for polyphosphates. Bacterial polyphosphate content was measured by extraction with sodium hypochlorite and hydrolysis for 10 minutes with 1N HCl. Fungal polyphosphate content was measured by extraction with ethanol and water and analysis by ^{31}P n.m.r. All micro-organisms pretreated with high P took up less orthophosphate from solution than those pretreated with low P. Polyphosphates were found in bacteria pretreated with high P (*A. aerogenes* 6.3 mg polyphosphate g^{-1} dry weight and *Enterobacter sp* 2.3 mg polyphosphate g^{-1} dry weight), but not in fungal hyphae.

Field soil of low, medium or high P status (2.8, 5.6, and 14.0 µg $NaHCO_3$-extractable P g^{-1} soil) from under wheat or medic plants was analyzed for polyphosphates. Samples were extracted with perchloric acid and activated charcoal. Extracts were hydrolyzed to determine polyphosphate content. Soil polyphosphate content ranged from 0 to 13.9 µg g^{-1} soil. Polyphosphates were present in all soil under medic, and in soil of medium and high P under wheat. Soil polyphosphate content increased with extractable P. Soil containing polyphosphates was sequentially fractionated with 0.5 M sodium bicarbonate, 0.1 M NaOH, ultrasonic dispersion, extraction again with 0.1 M NaOH and with 1 M HCl. Soil polyphosphates (up to 13.0 µg polyphosphates g^{-1} soil) were present in the microbial biomass. No polyphosphates were present in other soil extracts. Changes in soil effected by wheat growth appear to be less suitable for the synthesis and accumulation of soil polyphosphates than those effected by medic growth.

Micro-organisms containing polyphosphates took up less P from solution than those not containing polyphosphates. The polyphosphate concentration of field soil increased with extractable soil P. The presence, quantity and persistence of soil polyphosphates differered between soil under wheat and under medic plants.

All objectives of this project were achieved. Amendment of soil with medic or wheat roots had different effects on microbial C and P. Pretreatment of micro-organisms with low P solution increased subsequent P uptake. Pretreatment of micro-organisms with high P solution decreased subsequent P uptake. This was accompanied by the presence of polyphosphates in bacteria pretreated with high P, but not in fungi. Polyphosphates in soil were shown to be present in the soil microbial biomass, and to increase with extractable soil P.

Polyphosphates can be used as a measure of microbial P uptake in solution. It remains to be seen if polyphosphates can be used as a measure of P uptake by the microbial biomass.

Statement

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge and belief, this thesis contains no material previously published or written by another person, excepts where due reference is made in the text of the thesis.

I consent to this thesis being made available for photocopying and loan.

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