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Effect of citrate on Aspergillus niger phytase adsorption and catalytic activity in soil

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Current developments in cropping systems that promote mobilisation of phytate in agricultural soils, by exploiting plant-root exudation of phytase and organic acids, offer potential for developments in sustainable phosphorus use. However, phytase adsorption to soil particles and phytate complexion has been shown to inhibit phytate dephosphorylation, thereby inhibiting plant P uptake, increasing the risk of this pool contributing to diffuse pollution and reducing the potential benefits of biotechnologies and management strategies aimed to utilise this abundant reserve of 'legacy' phosphorus. Citrate has been seen to increase phytase catalytic efficiency towards complexed forms of phytate, but the mechanisms by which citrate promotes phytase remains poorly understood. In this study, we evaluated phytase (from Aspergillus niger) inactivation, and change in catalytic properties upon addition to soil and the effect citrate had on adsorption of phytase and hydrolysis towards free, precipitated and adsorbed phytate.

A Langmuir model was fitted to phytase adsorption isotherms showing a maximum adsorption of 0.23 nKat g-1 (19 mg protein g-1) and affinity constant of 435 nKat $g^{-}1$ (8.5 mg protein g-1), demonstrating that phytase from A.niger showed a relatively low affinity for our test soil (Tayport). Phytases were partially inhibited upon adsorption and the specific activity was of 40.44 nKat $mg^{-}1$ protein for the free enzyme and 25.35 nKat $mg^{-}1$ protein when immobilised. The kinetics of adsorption detailed that most of the adsorption occurred within the first 20 min upon addition to soil. Citrate had no effect on the rate or total amount of phytase adsorption or loss of activity, within the studied citrate concentrations (0-4mM).

Free phytases in soil solution and phytase immobilised on soil particles showed optimum activity (>80%) at pH 4.5-5.5. Immobilised phytase showed greater loss of activity at pH levels over 5.5 and lower activities at the secondary peak at pH 2.5 when compared to the free enzymes or in soil solution. The effect of ionic strength on enzyme activity was studied by increasing NaCl concentration on the activity buffer. A significant loss of activity was seen at ionic strengths over 0.6 M but enzymes in soil solution showed increased loss of activity on initial increase in ionic strength. No significant effect of citrate on phytase catalytic efficiency was observed towards free, adsorbed and precipitated (Al, Fe, Ca) phytate, except for the free phytase towards adsorbed phytase which showed a $\sim$160% increase in P release with the addition of citric acid.

This data suggest that citrate addition has no impact on the adsorption or catalytic activity of phytase in soil solution or that immobilised on soil particles, suggesting that its impact is associated with the availability of the substrate rather than effects on the enzyme per se. The ionic strength of soil solution does, however, have an impact on phytase activity suggesting that both wetting/drying cycles and fertilisation will have discrete impacts on the activity of phytases once released to soil and thus their ability to make organic P available for uptake by plants and microbes.