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Behaviour of plant-derived extracellular phytase upon addition to soil

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Abstract

The behaviour of phytase after addition to three soil types with different sorption capacities was investigated. Phytase was collected from the roots of transgenic *Arabidopsis thaliana* that express a phytase gene from *Aspergillus niger*. Phytase activity in solution and on the solid phase of the soil was monitored over time. Phytase added to the solution phase of a soil suspension (1:20, w/v) was almost completely lost within 10 min in all soil types, while phytase in non-soil controls remained active in solution. Phytase activity lost from solution was recovered on the soil solid phase, suggesting rapid adsorption of the enzyme. Adsorption of phytase was less in soil taken from the rhizosphere of transgenic plants expressing *phyA*, indicating that the rhizosphere environment may help maintain phytase activity in solution. The activity of adsorbed phytase declined with time at a rate 2–4 times slower than that in the absence of soil. Adsorption of phytase in soils was highest at pH 4.5, which is below the reported isoelectric point (p*I*) of the *Aspergillus* phytase. As soil pH increased, adsorption decreased until, at pH 7.5, all phytase was in solution. Where phytase remained in solution, activity was maintained for at least 8 d. In contrast, the activity of adsorbed phytase was increasingly inhibited with time, particularly at low pH. By increasing the pH in soil suspensions, phytase that had remained active on the soil solid phase for 28 d was almost totally desorbed. Rapid immobilisation of phytase in soil may limit its capacity to interact with phytate, and this may compromise the ability of transgenic plants which exude phytase from their roots to acquire P from endogenous soil phytate.

Keywords: Alfisol; *Arabidopsis thaliana*; Enzyme adsorption; Organic phosphorus; Oxisol; pH; Phosphatase; Phytate; Rhizosphere; Spodosol

1. Introduction

Organic phosphorus (P) tends to accumulate in soil and can represent a large proportion of total-P. The predominant form of organic P in most soils is phytate (derivatives of inositol penta- and hexa*kis*phosphate) (Anderson, 1980; Turner et al., 2002, 2003), which accumulates in soil due to its interaction with soil constituents by either adsorption or precipitation reactions (sorption) (Anderson and Malcom, 1974; Shang et al., 1992). In addition, plants may not produce extracellular phytase, the enzyme which catalyses the hydrolysis of phytate (Hayes et al., 1999; Richardson et al., 2004).

In an effort to give plants access to P stored in soils as phytate, transgenic plants (*A. thaliana*, *Nicotiana tabacum* L., *Trifolium subterraneum* L. and *Solanum tuberosum* L.) which express phytase genes from a soil fungus (*Aspergillus* sp.) have been developed (Richardson et al., 2001; Mudge et al., 2003; George et al., 2004, 2005; Zimmermann et al., 2003). When grown in sterile, non-sorbing environments such as agar, transgenic plants accumulated up to five-fold more P than controls when supplied solely with phytate (Richardson et al., 2001; Mudge et al., 2003; George et al., 2004, 2005). However, the benefit of exuding phytase was compromised in soil environments. Transgenic *T. subterraneum* achieved only small (at best 20%) and inconsistent increases in P accumulation when grown in soil (George et al., 2004). Transgenic *N. tabacum* achieved more consistent improvement in P nutrition (up to 50%), but only in soils amended to improve phytate availability (George et al., 2005). Possible reasons for the relatively poor capacity of transgenic plants to acquire P from phytate in soil include: (i) poor phytate availability for mineralisation by phytase; (ii) the presence of phytase-exuding microorganisms, which may compensate for lack of phytase exuded by wild-type plants; and (iii) inhibitory effects of

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the soil environment on the activity of phytase exuded to the rhizosphere by the transgenic plants.

The stability of extracellular enzymes in soil is affected by soil proteinase, microbial mediated degradation, deactivation by adsorption onto soil particles and interaction with metabolites (Nannipieri et al., 1996). Adsorption of enzymes to soil solid constituents is particularly common and proteins have been shown to have an affinity for the interface between the aqueous and solid phase of soil (Norde and Lyklema, 1991) as a result of both enthalpic (intermolecular) and entropic (intramolecular) forces (Quiquampoix, 2000; Quiquampoix et al.,. 2002). Adsorption of enzymes may reduce their affinity for substrates and thus reduce their effective activity. In some cases adsorption can inhibit activity irreversibly (Quiquampoix and Mousain, 2004). However, adsorption may also be necessary for the long-term persistence of enzymes in soils (Nannipieri, et al., 1996), by protecting them from degradation (Naidja et al., 2000). In fact, there are some instances of enzymes remaining active for thousands of years in geologically preserved soils (Skujins and McLaren, 1967).

In this paper, we investigate the behaviour of phytase, collected from the roots of transgenic *A. thaliana* which express the *phyA* gene from *Aspergillus niger*, after addition to various soil types. The partitioning of phytase activity between the soil solution and solid phase was assessed and the influence of the rhizosphere and soil pH on enzyme adsorption was investigated. The objective of the work was to determine whether the soil environment is detrimental to phytase activity and whether this can explain the compromised performance of transgenic plants that exude phytase to soils.

2. Materials and methods

2.1. Soil sampling, preparation and characterisation

Topsoils (0–10 cm depth) were collected from three sites, Tilba Tilba and Robertson in New South Wales (NSW) and Hall in the Australian Capital Territory (ACT), Australia (Table 1). Each soil was air-dried, mixed and passed through a 2-mm sieve to remove coarse material and vegetative matter and stored in this state at room temperature until required. Soils were either unamended, sterilised or exposed to a dense mat of

Table 1
Soil classification, mineralogy and chemical characteristics

Location	Tilba Tilba, NSW	Hall, ACT	Robertson, NSW
Latitude/longitude	36°19′S 150°30′E	35°3′S 149°40′ E	34°34′S 150 °35′E
Altitude (m O.D.)	156	608	130
Land-use	Pasture	Pasture	Pasture
Soil type (USDA)	Spodosol	Alfisol	Oxisol
pH (H_2O)	5.4	5.2	5.7
pH ($CaCl_2$)	4.3	4.6	4.8
Organic matter (g kg^{-1})	35.2	48.5	163.4
Resin P (mg kg^{-1})	1.6	2.6	1.5
Colwell P (mg kg^{-1})	4.3	12.9	32.3
Total P (mg kg^{-1})	60.0	316.5	2380.0
Organic P (%)	90.1	71.4	89.7
P sorption (% added)	24.5	56.7	91.8
NH_4 (mg kg^{-1})	5.8	19.2	130.1
NO_3 (mg kg^{-1})	0.4	13.9	37.7
Exchangeable cations ($mmol_c$ kg^{-1})			
Al	1.4	5.1	12.5
Mn	0.2	2.4	3.6
Mg	7.9	7.3	12.7
Ca	19.5	30.0	58.6
Na	1.3	0.6	1.2
K	1.5	8.3	4.1
All cations	31.8	53.7	92.7
Soil texture	Sand	Sandy loam	Clay loam
Mineralogy	Quartz[d]	Quartz[d]	Kaolinite[d]
	llite[tr]	Illite[tr]	Hematite[sd]
	Albite[tr]	Albite[tr]	Quartz[m]
	Orthoclase[tr]	Orthoclase[tr]	Calcite[m]
		Anatase[tr]	Smectite[tr]
			Anatase[tr]
			Gibbsite[tr]

The three soils were sampled (0–10 cm depth) from under pastures from across south eastern Australia and were chosen to represent typical low pH, low available P, organic P dominated soils. For mineralogy (% of mineral material) the key is d, dominant (>60%); sd, sub-dominant (20–60%); m, minor (5–20%); tr, trace (<5%).