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Diversity of adsorption affinity and catalytic activity of fungal phosphatases adsorbed on some tropical soils

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ABSTRACT

Extracellular phosphatases from ectomycorrhizal fungi may contribute to plant nutrition, particularly in highly weathered tropical soils where available phosphorus is limited. However, the expression of their catalytic activity may be influenced by many factors including adsorption on organo-mineral surfaces of soils. We have investigated the pH-dependent adsorption and the resulting change in catalytic activity of trace amounts of extracellular fungal acid phosphatases in contact with various tropical soils. Different size fractions and the effect of chemical extraction of soil organo-mineral coatings were studied for acid phosphatases secreted by two *Hebeloma cylindrosporum* and one *Suillus collinitus* strains. The three enzymes differed in their average affinity for soil and the pH dependence. However the difference between enzymes was often greater than that between soils for a given enzyme. Only *Suillus* phosphatase had markedly different affinities for vertisol and ferralsol. In general, activity was largely conserved in the adsorbed state. Activity of *Suillus* phosphatases varied more than *Hebeloma* phosphatases between soils and soil physical and chemical fractionation. There was no relationship between the affinity of an enzyme for a surface and the resulting change in activity after adsorption. *Suillus* phosphatases showed more pH dependence of both affinity for soil and loss of activity after adsorption than the *Hebeloma* enzymes. These results highlight the variability of the interaction of fungal phosphatases with soil.

1. Introduction

Extracellular enzymes are known to play important roles in the biogeochemistry in soils of many essential elements, including phosphorus. Organic P may constitute a large proportion of soil P in many soils (30–65%, but up to >90%) and may thus play an important role in P nutrition, particularly in highly weathered tropical soils (Harrison, 1987; Vance et al., 2003; Turner and Engelbrecht, 2011). However it must be hydrolysed to inorganic P before it can be available to biological systems. The availability of soil organic P depends therefore not only on the size of stocks but also on the catalytic activity of phosphatases in soil. Phosphatases may be produced and released into soil by various micro-organisms, including ectomycorrhizal fungi (Quiquampoix and Mousain, 2005; Cairney, 2011). Catalytic activity of extracellular phosphatases is one of the mechanisms whereby ectomycorrhizal fungi contribute to the nutrition of higher plants (Cairney, 2011).

Extracellular enzymes in soil are likely to be adsorbed on organo-mineral surfaces. Adsorption may favour conformational changes that modify catalytic activity or may favour orientation of the active site towards the surface thereby restricting access to substrate or towards the solution thus enhancing substrate accessability (Leprince and Quiquampoix, 1996). Various mechanisms have been proposed to explain the extent of adsorption of enzymes on organo-mineral surfaces, including electrostatic forces and hydrophobic interactions, van der Waals forces, covalent bonds and intercalation of enzymes in interlayer spaces of swelling clays (George et al., 2007a; Huang et al., 2005; Quiquampoix, 2000; Quiquampoix et al., 2002; Violante et al., 1995). Some authors state that the extent of the interaction between enzymes and mineral surfaces depends strongly on the nature of the surface. For example, it has been reported that in general more catalytic activity is lost in contact with 2:1 type minerals than 1:1 minerals (Nannipieri et al., 1996) and that less activity is lost when organic matter coats surfaces than when enzymes are adsorbed on clays or aluminium oxides (Rao et al., 2000). A greater loss of activity of fungal phytases has been observed in contact with phyllosilicates than with iron oxides (Giaveno et al., 2010). Both adsorption and changes in activity depend strongly upon pH. A shift in optimal pH towards alkaline pH is often observed in the presence of mineral surfaces (Leprince and Quiquampoix, 1996; McLaren and Estermann, 1957; Quiquampoix,

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1987a). A strong pH dependence of native phosphatase activity is observed in soils, but the optimal pH is only loosely related to soil pH (Turner, 2010).

Many of the studies of the effect of mineral and organo-mineral surfaces on catalytic activity of enzymes have been carried out using pure minerals or synthetic complexes (Rao and Gianfreda, 2000; Rao et al., 1996; Rosas et al., 2008) chosen to simulate natural soil. Such model systems may be very different to the complex heterogeneous complexes in soils containing clay minerals, inorganic and organic particles and coatings (Rowell, 1994). In many cases the enzyme studied would not be found naturally in soil. Extracellular enzymes might be expected to perform better than intracellular enzymes in contact with organo-mineral surfaces. We have studied tropical soils since the role of extracellular phosphatases may be more crucial to phosphorus nutrition in these soils, than in less weathered or better fertilised temperate soils. We have used ectomycorrhizal fungi as sources of phosphatase despite the ongoing debate as to the importance of ectomycorrhizal fungi in tropical soils. While endomycorrhizal fungi are generally assumed to dominate in tropical forests, ectomycorrhizae are found in many tropical soils (Mayor and Henkel, 2006, and references within).

The objective of this study was to investigate the pH-dependent adsorption and the resulting change in catalytic activity of extracellular fungal acid phosphatases added to various fractions of contrasting natural tropical soils. In particular we have studied the effect of dominant mineralogy, particle size fractionation and the chemical removal of organic and mineral coatings.

2. Materials and methods

2.1. Preparation of acid phosphatases

Three strains of ectomycorrhizal fungi were used to produce extracellular phosphatases: one strain of *Suillus collinitus*, (laboratory code J3.15.35) referred to as Sc and two strains of *Hebeloma cylindrosporum* (laboratory codes D3.25.2 and D2) referred to as Hc1 and Hc2. All strains were selected from the collection maintained by the Eco&Sols (Ecologie Fonctionnelle et Biogéochimie des Sols et des Agroécosystèmes, INRA-IRD-Cirad-SupAgro, Montpellier, France) unit and have been used in previous studies. In particular, they were found to secrete contrasting amounts of phosphatases and to contribute differently to phosphorus nutrition of pine seedlings (Matumoto-Pintro, 1996). They were grown on sterile agar gel (16 g/L) in Petri dishes at 25 °C in the dark for 25 days. Fungal plugs were taken and grown under sterile conditions in 25 mL liquid culture at 25 °C in the dark for 30 days. The sterilised (autoclaved at 120 °C for 20 min) nutrient solution contained NaCl (0.1 mM), KNO_3 (4 mM), KCl (1 mM), NH_4Cl (2 mM), $MgSO_4$ (1 mM), $CaCl_2$ (1 mM), thiamine–HCl (0.3 μM), ferric citrate (10 g/L), glucose (10 g/L), KH_2PO_4 (3.4 mM), and trace elements as recommended by Morizet and Mingeau (1976). At the end of the culture period the thalli were removed, the solutions filtered using a Vileda-Mop membrane, pooled, divided into smaller containers and frozen until required. Protein assays using the Bradford (1976) method indicated that the total protein concentrations were <15 mg/L (below the detection limit).

2.2. Soil samples

Five clay-textured tropical soils were chosen from the collection maintained by the Eco&Sols unit, all of which have been studied previously and had mineralogies typical of many tropical soils. All had been sampled from a depth of 0–10 cm. Some of their properties and references to publications are given in Table 1. The soils were gently crushed by hand using a ceramic mortar and pestle and sieved <200 μm. The clay-sized fraction, <2 μm was obtained by sedimentation, and sand-sized fraction, 50–200 μm by wet sieving after sonification. The clay-sized fraction was stored refrigerated as a suspension 10 g/L until required. The sand-sized fraction was dried before storage. Subsamples of the sieved soils were also chemically treated to remove mineral oxides (citrate-bicarbonate-dithionite, CBD, (Mehra and Jackson, 1960)) or organic matter (30% H_2O_2, (Lavkulich and Wiens, 1970)). Chemically treated samples were washed in $CaCl_2$ (1 M) to remove chemical residues and then rinsed in water to remove excess salt. The amount of Fe removed by the CBD treatment was measured by analysing the metal content of the extraction solution by Atomic Absorption Spectroscopy. The carbon content of the soils before and after H_2O_2 treatment was measured by dry combustion using Fisons-EA-1108 CHNS-O Element Analyzer®. The specific surface area of samples was measured using the BET method (Micromeritics-ASAP 2020®).

2.3. Measurement of enzymatic activity

Three procedures were used to distinguish activity in solution, extent of adsorption and catalytic activity in the adsorbed state, as described by Quiquampoix (1987a,b):

- Procedure A – enzymatic activity in solution, in the absence of soil material
- Procedure B – enzymatic activity in suspension containing 1 g/L soil material
- Procedure C – enzymatic activity in the supernatant solution of the above suspension.

The contact period between soil and enzyme in suspension prior to addition of substrate was 1 h. Soil and solution were separated

Table 1
Origin and some properties of the selected soils.

Soil	Sampling depth (cm)	0–2 μm (g kg^{-1})	2–50 μm (g kg^{-1})	50–200 μm (g kg^{-1})	Corg (after H_2O_2) (g kg^{-1})	Fe-CBD (g kg^{-1})	pH (H_2O)	Classification	Localisation	Reference
V	0–10	563	184	112	31.2 (23.5)	15.7	6.25	Smectitic Leptic Hapludert[a] (*EutricVertisol*)[b]	Martinique, French West Indies	(Chevallier et al., 2000)
*F*1	0–10	411	403	117	45.7 (14.3)	45.0	5.93	Andic Dystrustept[a] *Ferralsol*[c]	Madagascar	(Razafimbelo et al., 2006a)
*F*2	0–10	768	153	36	28.2 (11.3)	65.3	4.82	Typic Haplorthox[a] (*Orthic Ferralsol*)[b,c]	Congo	(Barthès et al., 2008; Kouakoua et al., 1997)
*F*3	0–10	495	339	140	29.5 (16.2)	71.8	5.33	Typic Hapludox[a] (*Orthic Ferralsol*)[b,c]	Brazil	(Razafimbelo et al., 2006b)
*F*4	0–10	800	190	10	34.6 (31.7)	81.2	7.1	Haplorthox[a] (*Rhodic Ferralsol*)[b,c]	Brazil	(Kouakoua et al., 1999)

Soil Survey Staff ([a]1999); FAO–UNESCO–IRIC ([b]1998). Ferralsol or oxisol using the USDA classification, Krasilnikov et al., 2009.