Interaction of enzymes with soil colloids: adsorption and ectomycorrhizal phosphatase activity on tropical soils.

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Abstract
Ectomycorrhizal fungi phosphatases may play a significant role in phosphorus nutrition by the solubilisation of soil organic phosphorus, and this may be of particular importance for highly weathered tropical soils. The expression of phosphatase catalytical activity is highly pH dependent and may be modified to various degrees when in contact with soil colloids. This modification is expected to depend on the nature of the organo-mineral surfaces. The adsorption and modification of acid phosphatase activity, of both intra and extracellular enzymes obtained from three ectomycorrhizal fungi strains in contact with three tropical soils, were studied. The effect of physical fractionation (clay-sized fraction and sieved soil) and chemical cleaning (treatment with H_2O_2 or dithionite-citrate-bicarbonate to remove organic matter or iron oxides) of the organo-mineral surfaces has been studied. Measurements of catalytic activity were made in the range of pH 3 – 7. The extent of adsorption and the resulting change in catalytic activity varied between fungal strains, and even for a given strain between extra and intracellular enzymes. The difference between soils was rather small, and neither physical nor chemical treatments had marked effects on the interaction with enzymes.

Key words
Intracellular, extracellular, organic matter, iron oxides; coatings, catalytic activity.

Introduction

Ectomycorrhizal fungi contribute to phosphorus nutrition by synthetising and secreting phosphatases that solubilise soil organic phosphorus, which is not directly assimilated by plants (Quiquampoix and Moussain 2005). The action of these enzymes may be important for highly weathered tropical soils which are depleted in mineral phosphorus. In contact with soil phosphatases are adsorbed and their catalytic activity is modified to varying degrees (Leprince and Quiquampoix 1996). Activity depends strongly on pH, nature of mineral surfaces and organic adsorbents. Several studies have investigated the adsorption and activity of enzymes on pure clay minerals (Quiquampoix and Leprince 1996), clay-organic matter complexes (Gianfreda 1991; Kelleher 2004), and oxides (Shindo 2002) but few studies have compared natural soils. We have investigated the behavior of phosphatases produced by ectomycorrhizal fungi, in contact with contrasting tropical soils in order to understand better the role of orano-mineral coatings in soil.

Materials and methods

Preparation of phosphatases
Phosphatases used from three strains of ectomycorrhizal fungi: *Suillus collinitus* , denoted ,and two strains of *Hebeloma cylindrosporum* , denoted H_1 and H_2. Fungi were cultivated *in vitro* at 25 ° C in the dark for 30 days (Leprince 1995). Composition of the nutrient solution was: 0.1 mM NaCl ; 4 mM KNO_3 ; 1 mM KCl ; 2 mM NH_4Cl ; 1 mM Mg SO_4 ; 1 mM $CaCl_2$; 0.3 µM thiamine-HCl ; 125 mg/l ferric citrate; 10 g/l glucose ; trace elements as recommended by Morizet and Mingeau, (1976) . At the end of culture, the nutrient solution containing the extracellular phosphatase was filtered and frozen until required. Thalli were crushed in a mortar with 10% polyvinylpolypyrrolidone (PVPP) and sand. The mixture was taken up in a solution of acetate buffer (pH 5.5) containing 5 mM dithiotrietol. The suspension was centrifuged and the supernatant frozen until required.

Soils colloids
Soil samples from three tropical regions were selected for this study, all had clayey texture and were sampled from the A horizon 0-10 cm. A vertisol (V) was taken from Martinique, French West Indies and two ferralsols were taken from Niari, South Congo (F_1) and Paraná, South Brazil (F_2). The air dried soils were crushed and sieved to 200 µm. The clay fraction of the soils (≤ 2µm) was separated by sedimentation. Subsamples of the sieved soils also underwent chemical treatments with either H_2O_2 or citrate-bicarbonate-dithionite (Mehra & Jackson, 1960). After chemical treatments, soil residues were rinsed with water and $CaCl_2$ solution to remove excess chemical reagents.

Phosphatases activity measurements

The enzyme activity was measured as function of pH using para-nitrophenyl phosphate (pNPP) as substrate. The pH of the solutions was adjusted in acetate or MOPS buffer (300mM). The catalytic reaction was stopped after 20 minutes with glycin buffer. The reaction product, para-nitrophenol was quantitatively measured in an UV-visible spectrophotometer at 405 nm. Three procedures for measuring phosphatases activity denoted A, B and C (Quiquampoix 1987a; Quiquampoix and Leprince 1996) were used to distinguish the contributions of adsorbed and solution phase phosphatase. Procedure A simply measured the catalytic activity of the enzyme in solution. Procedure B measured the activity in the presence of soil or soil clay, due to both adsorbed and non adsorbed enzyme. In procedure C, the activity in a supernatant solution after contact with soil was measured. A comparison of A and C allows the extent of adsorption to be calculated. Phosphatase activity for each procedure was expressed as the velocity of the catalytic reaction, V, in nKatal.g^{-1} fungus. We calculated the proportion of enzyme remaining free in solution, F as V_C/V_A and the relative activity of enzymes in the adsorbed state, R as $(V_B-V_C)/(V_A-V_C)$.

Results and discussion

Different interactions of phosphatases with soil colloids

Figure 1 shows the results of measurement procedures A, B and C of the intra and extracellular phosphatases with clay fraction of ferralsol F_1 at pH 5. For a better comparison of A, B and C, the histogram is represented as a percentage of maximum activity of each phosphatase. The observed behavior can be classified into four types: First, $V_A > V_B$ and $V_C = 0$, for H_{2i} (intracellular). Zero values of V_C indicate that the enzyme is completely adsorbed. Since $V_B < V_A$, some activity has been lost on adsorption. In a second case, observed for $V_A > V_B > V_C$ for H_{2e} (extracellular): some of the enzyme was adsorbed and adsorbed enzyme lost part of its activity. Thirdly, for H_{1e}, S_i and S_e, $V_A = V_B > V_C$ indicates that a part of the enzyme was adsorbed but that adsorbed enzyme retained its catalytic activity. Finally, $V_A = V_B$ and $V_C = 0$ for both intracellular enzymes of the *Hebeloma cylindrosporum* fungi, $\mathbf{H_{1i}}$, and $\mathbf{H_{2i}}$, indicating that despite complete adsorption, adsorbed enzyme completely retained catalytic activity. These results show that adsorption of enzymes is not complete as assumed by many studies. Only two of the enzymes studied (H_{2i} and H_{1i}) were completely adsorbed. Furthermore, phosphatases retain over 80% activity in contact with soil colloids in contrast to previous observations where adsorbed enzyme retained little activity (Quiquampoix and Leprince 1996).

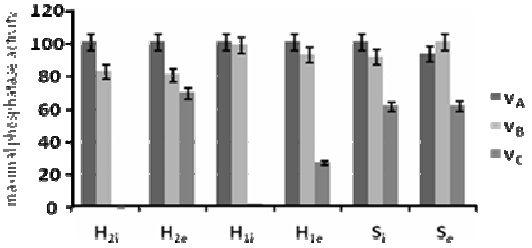

Figure 1: Effect of clay fraction of ferralsol (F_1) at pH5 on expression of phosphatase activity

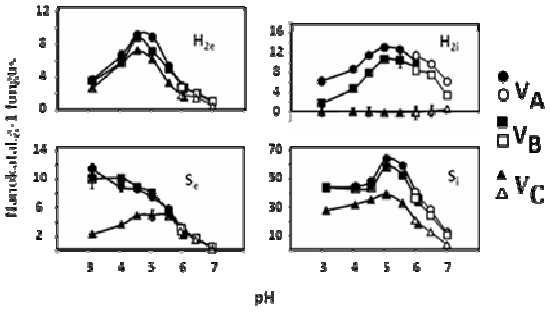

Figure2: Comparison of interactions between intra and extracellular enzymes of *Hebeloma cylindrosporum* H_2 and *Suillus collinitus* S with ferralsol F_1 (clay fraction). Closed symbols acetate buffer, open symbols MOPS buffer

Behavior of intra and extracellular phosphatases from the same fungus

Figure 2 shows the behavior of intra and extracellular phosphatases from **S** (*Suillius collinitus*) and $\mathbf{H_2}$ (*Hebeloma Cylindrosporum*) with clay fraction of ferralsol F_1. $\mathbf{S_e}$ and $\mathbf{S_i}$ have different behavior although from same fungus. While extracellular $\mathbf{S_e}$ was adsorbed weakly with little loss of activity, all intracellular $\mathbf{S_i}$ are adsorbed and part of their activity is lost. In contrast, $\mathbf{H_{2i}}$ and $\mathbf{H_2e}$ had similar behavior, partial adsorption and no loss of activity. This result shows that intra and extracellular phosphatases may differ both in intensity of enzymatic activity and in interaction with soil colloids. Phosphatases from different fungi may have markedly different behaviors. This study shows that the adsorption and modification of enzyme activity depend not only on nature of adsorbent surface but also on the enzyme.

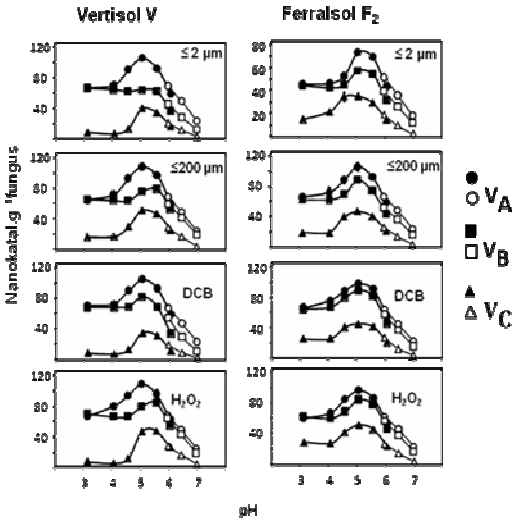

Figure 4: Comparison interactions of enzyme (S_i) with different fractions of soil V and F_2. Closed symbols acetate buffer, open symbols MOPS buffer

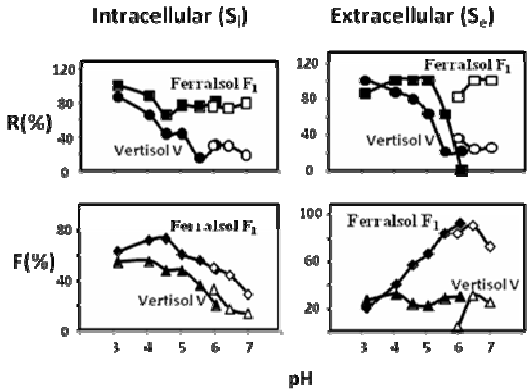

Figure 3: Relative activity (R) and free fraction of enzymes (F) of S_i (*Suillus collinitus*-intracellular) and Se (*Suillus collinitus*-extracellular) on two soils (V and F_1). Closed symbols acetate buffer, open symbols MOPS buffer

Effect of soil type

Figure 3 shows for S_i and S_e, effect of two soil types V (vertisol) and F_1 (ferralsol) on the proportion of enzyme remaining free in solution, F, and the relative activity of adsorbed enzyme, R as a function of pH. For this enzyme, both R and F are greater for the ferralsol F_1 than the vertisol V. This comparison suggests that stronger interaction with the vertisol leds to both greater adsorption and greater inactivitation of the enzyme.

Effect of granulometry, iron oxides and organic matter

Figure 4 shows effect of granulometry, iron oxides and organic matter of two soils (V and F_2) on Si catalytic activity. There are no marked differences between curves. Soil granulometry (<2µm and 200µm) and chemical treatments to remove iron oxides and organic matter have little effect on phosphatase activity. This is surprising, as previous studies have reported contrast in both adsorption and modification of catalytic activity when reference minerals are compared to synthetic organo-mineral complexes (Quiquampoix 1987b; Rao *et al*. 2000; Kelleher *et al.* 2004). The absence of a particle size effect, in contrast to the study of Huang *et al.* (2005)on a Ultisol is less surprising since the soils in the present study are clay textured and so the clay fraction may dominate the interactions with soils.

Conclusion

This study shows that the extent of adsorption and the activity of adsorbed fungal phosphatase varies considerable between enzymes. Behaviour may differ between fungal species and strains, and even for a given fungus between intracellular and extracellular enzymes. In contrast to observations for synthetic organo-mineral complexes, smaller differences were observed between contrasting soils and almost no effect of removal of organo-mineral coatings was detected. More work is required to understand the origin of the interactions of enzymes with natural soil surfaces in order to predict the expression of catalytic activity in complex systems.

References

Gianfreda L, Rao MA, Violante A (1991) Invertase (β-fructosidase): Effects of montmorillonite, Al-hydroxide and $Al(OH)_x$-montmorillonite complex on activity and kinetic properties. *Soil Biology and Biochemistry* **23**, 581–587.

Huang Q, Liang W, Cai P (2005) Adsorption, desorption and activities of acid phosphatase on various colloidal particles from an Ultisol. *Colloids and Surfaces B: Biointerfaces* **45**,209-214.

Kelleher BP, Simpson AJ, Willeford KO, Simpson MJ, Stout R, Rafferty A and Kingery WL (2004) Acid phosphatase interactions with organo-mineral complexes: influence on catalytic activity. *Biogeochemistry* **71**, 285-297.

Leprince F (1995) Mécanismes mis en œuvre par différents champignons ectomycorhiziens dans la mobilisation du phosphor*e. Thèse de Doctorat*, ENSA de Montpellier.

Leprince and Quiquampoix (1996) Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum. European Journal of soil Science* **47**, 511-522.

Mehra and Jackson (1960) Iron oxides removal from soils and clays by dithionite-citrate system buffered with sodium bicarbonate. *Clays and Clay Minerals* **7,** 317-327.

Morizet and Mingeau (1976) Influence des facteurs du milieu sur l'absorption hydrique (étude effectuée sur tomate décapitée en exsudation). Facteurs nutritionnels. *Annales Agronomiques* **27,** 183-205.

Quiquampoix H (1987a) A stepwise approach to the understanding of extracellular enzyme activity in soil. I. Effect of electrostatic interactions on the conformation of a β-D-glucosidase on different mineral surfaces. *Biochimie* **69**, 753-763.

Quiquampoix H (1987b) A stepwise approach to the understanding of extracellular enzyme activity in soil. II. Competitive effects on the adsorption of a β-D-glucosidase in mixed mineral or organo-mineral systems. *Biochimie* **69,** 765-771.

Quiquampoix and Moussain (2005) Enzymatic hydrolysis of organic phosphorus. In 'Organic Phosphorus in the Environment'. (Eds BL Turner, E Frossard, DS Baldwin). pp. 89-112. (CAB international).

Rao MA, Violante A, Gianfreda L (2000) Interaction of acid phosphatase with clays, organic molecules and organo-mineral complexes: kinetics and stability. *Soil Biology and Biochemistry* **32**, 1007-1014.

Shindo H, Watanabe D, Onaga T, Urakawa M, Nakahara O, Huang QY (2002) Adsorption, activity, and kinetics of acid phosphatase as influenced by selected oxides and clay minerals. *Soil Science and Plant Nutrition* **48,** 763-767.