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Fungal extracellular phosphatases: their role in P cycling under different pH and P sources availability

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Abstract

Aims: The aim of this work is to analyse the effect of pH, fungal identity and P chemical nature on microbial development and phosphatase release, discussing solubilization and mineralization processes in P cycling.
Methods and Results: P solubilizing fungi (*Talaromyces flavus*, *T. helicus L*, *T. helicus N*, *T. diversus* and *Penicillium purpurogenum*) were grown under three pH conditions (6, 6·5 and 8·5) and with different inorganic (calcium, iron, aluminium and rock) and organic (lecithin and phytate) P sources. P solubilization, mineralization, growth and phosphatase production were recorded. Acid and neutral environments maximized fungal development and P recycling. P chemical nature changed the phosphatases release pattern depending on the fungal identity. Acid phosphatase activity was higher than alkaline phosphatases, regardless of pH or sample times. Alkaline phosphatases were affected by a combination of those factors.
Conclusions: P chemical nature and pH modify fungal growth, P mineralization and solubilization processes. The underlying fungal identity-dependent metabolism governs the capacity and efficiency of P solubilization and mineralization. P solubilization and mineralization processes are interrelated and simultaneously present in soil fungi.
Significance and Impact of the study: This study constitutes a reference work to improve the selection of fungal bioinoculants in different environmental conditions, highlighting their role in P cycling.

Introduction

Phosphorus (P) is an essential nutrient that has low bioavailability on soils due to its high reactivity with soil elements like calcium, iron or aluminium (Deubel and Merbach 2005). Approximately 95–99% of the total soil P is usually found in insoluble organic and inorganic forms that plants cannot directly use for their development (Richardson *et al.* 2011). This low bioavailability limits plant growth and yield, compromising the global food supply. Cycling of P is very important in natural ecosystems and also in fertilized grasslands (Richardson *et al.* 2011). The correct and efficient management of organic and inorganic P resources is needed for sustainable agriculture. Therefore, P cycling, particularly P solubilization and mineralization achieved by soil micro-organisms, is a very important and interesting topic to study.

Soil micro-organisms play an important role in increasing the available P through inorganic P solubilization and organic P mineralization processes (Kumar Adhya *et al.* 2015). P solubilization is mainly due to different mechanisms, and combinations thereof, such as pH decrease, organic acid release, proton extrusion

related to ammonia assimilation process (Illmer and Schinner 1995; Jones 1998; Whitelaw *et al.* 1999; Scervino *et al.* 2010; Stefanoni Rubio *et al.* 2016). However, the principal mechanism for P mineralization is the production and release of acid or alkaline phosphatases depending on their optimum pH activity (Rodriguez and Fraga 1999; Richardson *et al.* 2000; Deubel and Merbach 2005). Alkaline and acid phosphatases use organic P as substrate to convert it into soluble inorganic forms of P (Beech *et al.* 2001). While acid phosphatases can be released by plants and micro-organisms (Yadav and Tarafdar 2001), alkaline ones are probably mostly of microbial origin (Tarafdar and Claassen 1988). Several studies have shown that an increase in soil phosphatases improves not only the available P in soils but also plant growth (Yadav and Tarafdar 2003; Garg and Bahl 2008; Ma *et al.* 2009; Garcia-Lopez *et al.* 2015). Tarafdar *et al.* (2001) showed that microbial acid phosphatases were found to be more efficient in organic P hydrolysis than those released by plants. The microbial production and the activity of these enzymes are modified by a large list of factors like species, strains, pH conditions, presence and nature of P sources, cations, among others (Bünemann 2008; Stefanoni Rubio *et al.* 2016), and assessing their impact under controlled conditions is important.

Previous studies were mostly focused on the isolation, identification or characterization of microbial strains and limited to evaluate their ability to produce phosphatases (El-Tarabily *et al.* 2008; Aseri *et al.* 2009; Pawar and Thaker 2009; Kapri and Tewari 2010) or to promote plant growth (Sindhu *et al.* 2014; Martínez *et al.* 2015; Manzoor *et al.* 2016). Nevertheless, these studies did not discuss the factors affecting P solubilization and mineralization processes and the relationship between them. To understand soil P cycling, it is important to study the interconnection between these processes, assessing the role of soil fungi. In this work, fungal P solubilization and mineralization were studied, and the effect of P source nature and pH on the production and activity of phosphatase enzymes is discussed.

Material and methods

Effect of pH on fungal P solubilization and phosphatase production

To evaluate the effect of pH on P solubilization and phosphatase production, five fungal strains (*Talaromyces flavus* BAFC 3125, *T. helicus N* BAFC 3126, *T. helicus L* BAFC 3127, *T. diversus* BAFC 3128 and *Penicillium purpurogenum* BAFC 3303), previously selected for their ability to solubilize P (Scervino *et al.* 2010), were characterized in growth and phosphatase production under three different pH conditions: (i) without buffer at pH 6 (WB); (ii) with buffer Tris–HCl at pH 8·5 (Tris–HCl) and (iii) with buffer MES at 6·5 (MES).

The medium used in the experiments was the National Botanical Research Institute's Phosphate (NBRIP) (Nautiyal 1999). This medium contains (l^{-1}): glucose 10 g, $MgSO_4$ 0·12 g, KCl 0·2 g, $MgCl_2$, $6H_2O$ 5 g, $(NH_4)_2SO_4$ 0·1 g and $Ca_3(PO_4)_2$ (PC, Sigma-Aldrich 21218, St. Louis, MO, USA) 5 g as an insoluble P source. Erlenmeyer flasks (100 ml) were filled with 20-ml NBRIP and placed in an orbital shaker at 250 rev min^{-1} and 25°C. Each treatment consisted of three independent replicates and controls were performed without fungal inoculation.

Cultures were harvested at different times (6, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192 and/or 240 h) until stationary phase was reached by the fungal strains. The mycelial dry weight (DW), collection of supernatants, pH recording and P quantification were achieved following the protocols described in Della Mónica *et al.* (2014). The activities of acid (sodium acetate buffer 0·1 mol l^{-1}; pH 5·5) and alkaline (universal buffer 0·1 mol l^{-1} (citric : phosphoric : boric acid 0·03 mol l^{-1}); pH 9·0) phosphatases were measured spectrophotometrically (405 nm) using p-nitrophenylphosphate (2·3 mmol l^{-1}) as the substrate in aliquots from the supernatants of liquid cultures (Della Mónica *et al.* 2015). An enzymatic unit (EU) was defined as the amount of enzyme produced per gram of DW that hydrolyses 1 μmol p-nitrophenylphosphate per min in 1 ml of supernatant. P release per EU was expressed as μg P EU^{-1}.

Effect of the nature of P sources on fungal solubilization and mineralization

To evaluate the effect of the chemical nature of insoluble P sources on the P solubilization and mineralization processes and phosphatase production, fungal strains were incubated in 100 ml Erlenmeyers containing 20-ml NBRIP supplemented per litre with: Tennessee Brown Rock phosphate (P_2O_5 31·55%, CaO 44·06%, F 3·4%, SiO_2 10·1%; RP, NIST-SRM 56b) 2·5 g, $FePO_4$, $4H_2O$ (FP, Aldrich 436038) 7 g, $AlPO_4$ (AP, Aldrich 341452) 1 g or PC 5 g as inorganic P sources; and soy lecithin (LecP, Sigma-Aldrich 44924) 15 g or sodium phytate (FitP, Sigma P8810) 1 g as organic P sources. The initial broth pH was 7 and all treatments were done in triplicate. Cultures were incubated for 96 h and DW, soluble P, acid and alkaline phosphatases were measured as previously described. DW and soluble P were used to calculate the solubilization/mineralization capacity defined as the amount of released P per unit of DW, and solubilization/mineralization efficiency defined as the amount of

released P per unit of insoluble P added at the beginning of the experiment.

Data analysis

Data obtained were compared by two-way analysis of variance (ANOVA; factors: P source × fungal strains) and correlation tests using the statistical software Statistica10 (StatSoft, Tulsa, OK). All assumptions were tested before ANOVA analysis, and those that did not comply were treated with Box–Cox transformations. When significant differences ($P \leq 0{\cdot}05$) were found, *post hoc* comparisons were made to analyse the differences among treatments. Means were compared by Fisher's least significant differences test, and main effects were studied every time when no differences were found.

Results

Effect of pH on fungal P solubilization and phosphatase production

Most of the fungal strains tested developed their maximum DW in MES, except for *T. helicus N*, which reached the maximum DW in WB treatment with a pH around 5·5; and for *P. purpurogenum,* which produced its highest DW in alkaline pH (Figs 1–3). *Talaromyces diversus* and *T. flavus* did not grow in Tris–HCl (Fig. 3). Fungal growth was accompanied with a pH decrease in all treatments, but this was more remarkable in WB (Figs 1–3). However, P solubilization accompanied fungal growth in all treatments (Figs 1–3).

The highest P solubilization values were observed in WB (Fig. 1c). Here, *P. purpurogenum* reached the highest values (562 ± 57 mg P l^{-1}), followed by *T. flavus* (430 ± 29 mg P l^{-1}), *T. diversus* (360 ± 15 mg P l^{-1}), *T. helicus N* (244 ± 31 mg P l^{-1}) and *T. helicus L* (213 ± 30 mg P l^{-1}).

In MES treatments, all fungal strains could release P from insoluble P (Fig. 2c), with the maximum values given by *T. flavus* (271 ± 50 mg P l^{-1}), followed by *T. helicus L* (187 ± 9 mg P l^{-1}), *T. helicus N* (134 ± 3 mg P l^{-1}), *T. diversus* (99 ± 3 mg P l^{-1}) and *P. purpurogenum* (58 ± 5 mg P l^{-1}).

In Tris–HCl treatments, where pH is alkaline, only *T. helicus L*, *P. purpurogenum* and *T. helicus N* released P (Fig. 3c). As mentioned below, *T. diversus* and *T. flavus* did not grow or solubilize P. *Talaromyces helicus L* was the strain that could release more soluble P (99 ± 42 mg P l^{-1}), followed by *T. helicus N* (68 ± 6 mg P l^{-1}) and *P. purpurogenum* (60 ± 6 mg P l^{-1}). Controls did not present changes in pH or soluble P along the experiment (data not shown).

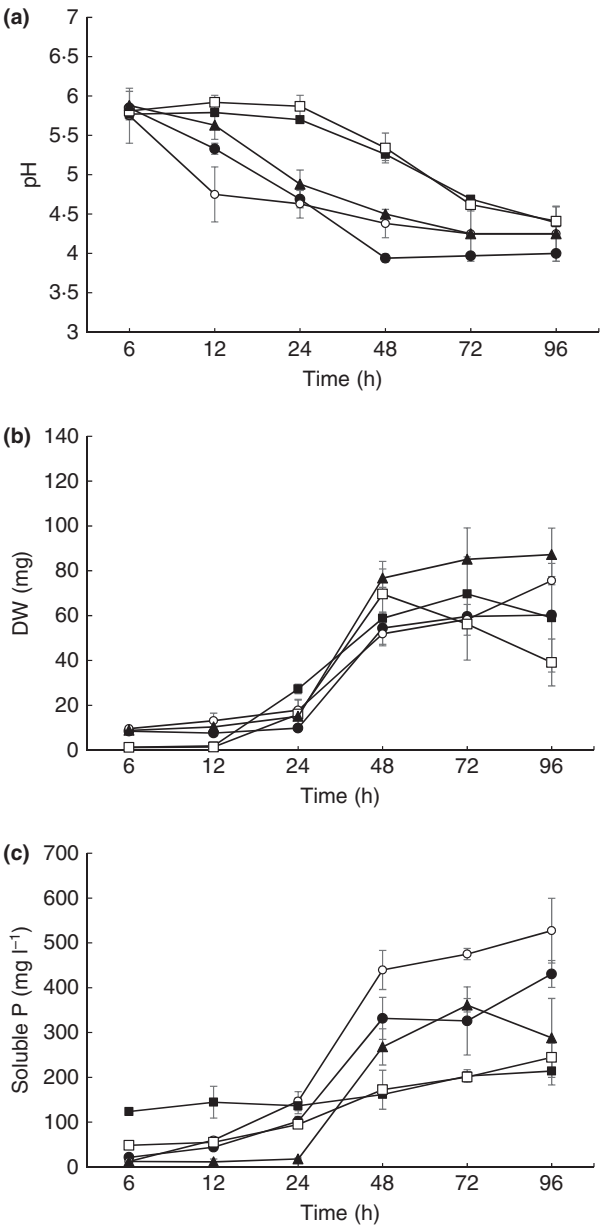

Figure 1 Fungal growth in NBRIP medium without buffer (WB) at pH 6 throughout experimental times. (a) Changes of pH values. (b) Fungal biomass in mg of dry weight (DW). (c) Soluble P released in mg l^{-1}. Fungal strains: (●) *Talaromyces flavus*, (○) *Penicillium purpurogenum*, (■) *Talaromyces helicus L*, (□) *Talaromyces helicus N*, (▲) *Talaromyces diversus*. Vertical bars are means ± SD.

Fungal extracellular phosphatase activities varied with the tested pH, fungal inoculation and sample times (Fig. 4a–f). No detectable enzymatic activity was found in controls without inoculation (data not shown). *Talaromyces flavus* and *T. diversus* in Tris–HCl treatments did not present changes in enzymatic activities at the tested times (data not shown). Acid phosphatase activity was higher

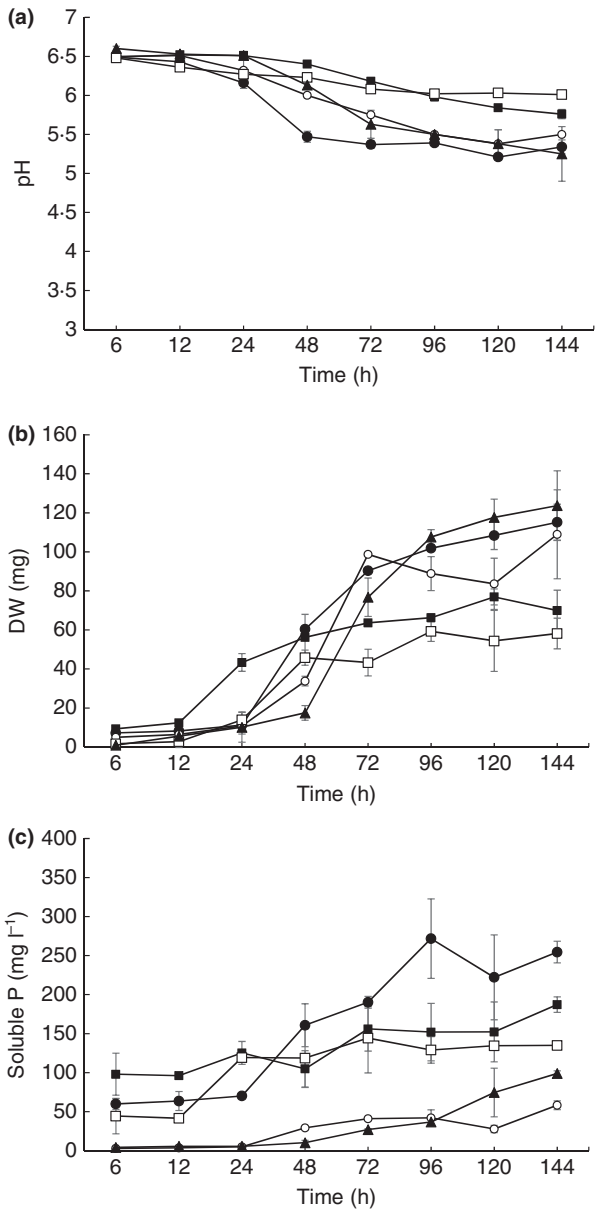

Figure 2 Fungal growth in NBRIP–MES buffered at pH 6·5 along experimental times. (a) Changes of pH values. (b) Fungal biomass in mg of dry weight (DW). (c) Soluble P released in mg l^{-1}. Fungal strains: (●) *Talaromyces flavus*, (○) *Penicillium purpurogenum*, (■) *Talaromyces helicus L*, (□) *Talaromyces helicus N*, (▲) *Talaromyces diversus*. Vertical bars are means ± SD.

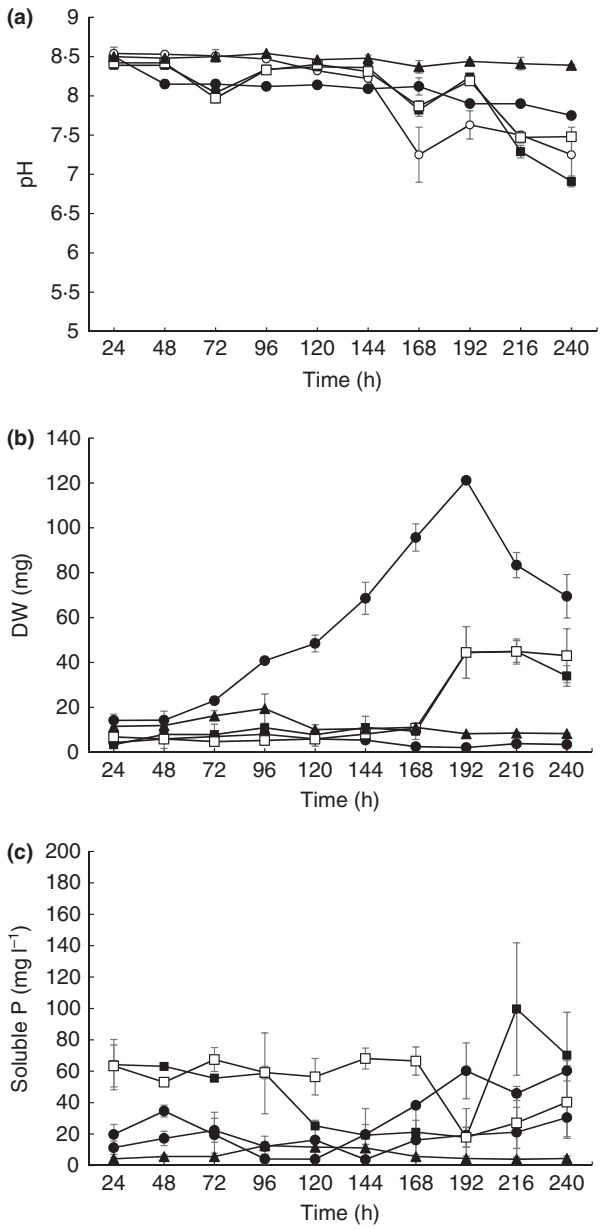

Figure 3 Fungal growth in NBRIP–Tris–HCl buffered at pH 8·5 along experimental times. (a) Changes of pH values. (b) Fungal biomass in mg of dry weight (DW). (c) Soluble P released in mg l^{-1}. Fungal strains: (●) *Talaromyces flavus*, (○) *Penicillium purpurogenum*, (■) *Talaromyces helicus L*, (□) *Talaromyces helicus N*, (▲) *Talaromyces diversus*. Vertical bars are means ± SD.

than alkaline phosphatases, regardless of pH, fungal strains or sample times (Fig. 4a–f). Acid phosphatase activity released by *T. helicus N* reached its maximum in WB medium (Fig. 4a), while *T. flavus*, *T. diversus*, *T. helicus L* and *P. purpurogenum* strains showed the highest values in MES treatments (Fig. 4c). The alkaline phosphatase activity observed in *P. purpurogenum* with Tris–HCl buffer was the highest, followed by *T. helicus N* and *T. helicus L* (Fig. 4f).

Effect of P sources on fungal P solubilization and mineralization

P Solubilization

The capacity of fungal solubilization was higher for calcium and iron phosphates than aluminium and rock phosphates (Table 1, SC). The highest value was observed

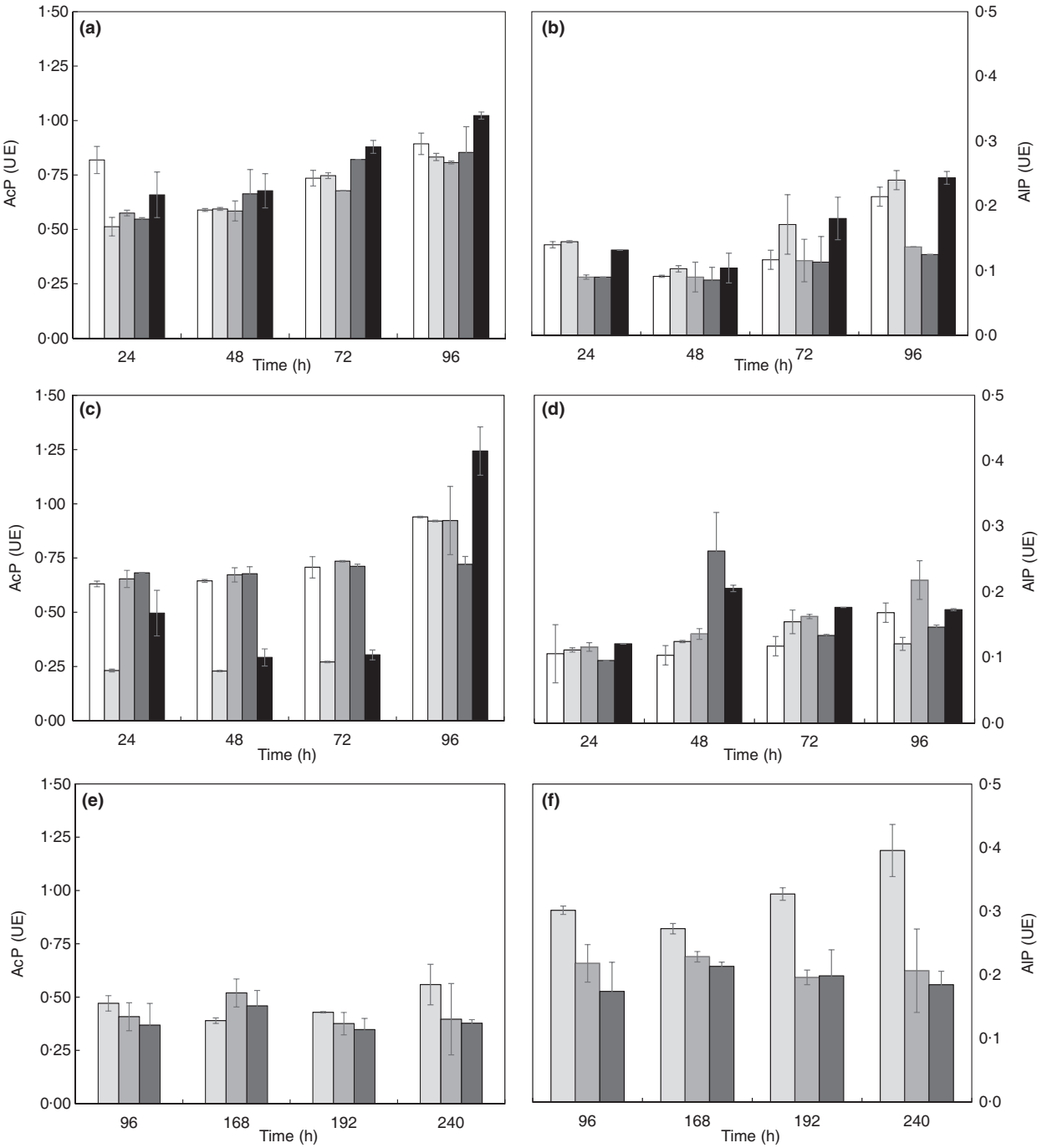

Figure 4 Extracellular acid (AcP) and alkaline (AlP) phosphatases produced by fungal strains through incubation times under different pH conditions. (a, b) Acid (a) and alkaline (b) phosphatases released in NBRIP without buffer, initially set at pH 6. (c, d) Acid (c) and alkaline (d) phosphatases produced in NBRIP buffered at pH 6·5 with MES. (e,f) Acid (e) and alkaline (f) phosphatases produced in NBRIP buffered at pH 8·5 with Tris–HCl. Different columns are fungal strains: (white) *Talaromyces flavus*; (grey) *Penicillium purpurogenum*; (dotted) *Talaromyces helicus L*; (lined) *Talaromyces helicus N*; (black) *Talaromyces diversus*. Columns are phosphatases values, vertical bars are SD.

in *P. purpurogenum*-PC followed by *T. flavus* in the same medium. However, the lowest value was found in *T. helicus L*-RP followed by *T. diversus*-AP. A clear interaction between P sources and fungal strains was found in this experiment showing that the solubilization capacity was maximized for some combinations (Table 1, SC).

When the solubilization efficiency was calculated, the highest value was observed in *T. flavus*-RP followed by *P. purpurogenum*-RP (Table 1, SE). In contrast, the combination *T. helicus L*-PC showed the lowest efficiency followed by *T. diversus*-AP. In general, the solubilization efficiency of FP was higher than AP, although *P. purpurogenum* and *T. flavus* showed the same efficiency for both sources. On the other hand, *T. helicus L* was more efficient in solubilizing FP and AP than PC (Table 1, SE).

We analysed the phosphatase production when fungal strains were grown in media with an inorganic P source. An interaction between fungal strains and P sources was observed (Table 1). Acid phosphatases were more influenced by the P chemical nature than the fungal strains (Table 1, AcP). Indeed, treatments with calcium phosphate (PC and RP) showed less activity than those with aluminium or iron phosphates. On the other hand, alkaline phosphatase production depended on the combination between fungal strain and P sources (Table 1, AlP). The highest activity was found in *T. helicus N*-RP and *T. helicus L*-RP, while the lowest was found in *P. purpurogenum*-PC and *T. flavus*-PC.

The correlation tests showed that soluble P and acid phosphatases were not correlated ($P = 0{\cdot}66$) as well as the DW and alkaline phosphatases ($P = 0{\cdot}11$). However, soluble P and alkaline phosphatases were negatively correlated ($P = 0{\cdot}0003$) as well as fungal biomass and acid phosphatases ($P = 0{\cdot}0002$).

P mineralization

When the mineralization capacity of FitP and LecP by the fungal strains was analysed, a statistical interaction between factors (P sources × Fungal strains) was found, although the values found in FitP were higher than LecP (Table 2, MC). *Talaromyces helicus L* showed the lowest mineralization capacity with LecP, followed by *T. helicus N*-LecP, while the highest value was observed in the *T. helicus N*-FitP combination (Table 2, MC).

However, the statistical analysis of the mineralization efficiency showed no interaction between factors (P sources × Fungal strains). The main effect study showed that the mineralization efficiency was higher in LecP treatments than FitP, independently of the fungal strains (Table 2, ME).

Acid phosphatase activity was higher than alkaline when organic P sources were used (Table 2). Particularly, the presence of LecP increased the enzymatic activity of all strains, except for *T. diversus*. The highest value was found in *T. flavus*-LecP, while the lowest was found in *T. helicus N*-FitP (Table 2, AcP). The alkaline phosphatases showed the maximum value in *T. helicus L*-LecP, while the lowest record was found in *T. helicus N*-FitP (Table 2, AlP).

The correlation analysis showed no correlation between the phosphatase activity and the soluble P ($P = 0{\cdot}17$ acid, $P = 0{\cdot}56$ alkaline phosphatases), while a positive correlation was found in soluble P and fungal biomass ($P = 0{\cdot}0003$) as well as phosphatase activity and fungal biomass ($P = 0{\cdot}01$ acid, $P = 0{\cdot}005$ alkaline phosphatases).

Discussion

The fungal P solubilization and mineralization were studied in this work, assessing the effect of the nature of P source and the pH on the production and activity of extracellular fungal phosphatases. The P mineralization and solubilization processes are interrelated and act simultaneously in soil fungi depending on the nutrient availability and environmental conditions.

Fungal growth was accompanied with a pH decrease, independently of the initial culture pH. Although the final pH obtained was different in each treatment (3·8 in WB, 5·5 in MES and 7·25 in Tris), P solubilization was detected where fungal growth was observed. It is known that inorganic P solubilization is due to different mechanisms, such as organic acid release (Whitelaw 1999; Whitelaw *et al.* 1999; Scervino *et al.* 2010; Stefanoni Rubio *et al.* 2016), proton extrusion of the ammonium assimilation (Illmer and Schinner 1995), and/or the carbonic acid of the microbial respiration (Jurinak *et al.* 1986). Whitelaw *et al.* (1999) showed that lowering pH ≤5 produces a partial solubilization of PC because of the release of inorganic acids (e.g., HCl). Our results showed that even in pH above 5, there was soluble P released from PC. Previous studies showed that some fungal strains could present a reduced P solubilization ability in alkaline environments (Gyaneshwar *et al.* 1998). In the experiment buffered at pH 8·5, not only the fungal growth capacity was reduced in most species but also the PC solubilization. *Talaromyces helicus* and *P. purpurogenum* were the only fungal species that showed growth and solubilization ability in this alkaline medium. Although these species produced a slight lowering of pH, the amount of released P through inorganic PC solubilization was almost the same as the other strains at neutral or slightly acid pH. As the final pH was around 7, another mechanism like the organic acids production should be acting in this solubilization. Indeed, Scervino *et al.* (2010, 2011) showed that *P. purpurogenum* produced organic acids in alkaline medium and that their production pattern is dependent on the inorganic P source used. Stefanoni Rubio *et al.* (2016) also demonstrated that the P solubilization of *T. flavus* is mediated by organic acid production and modulated by the presence of different nutrients on the medium. The efficiencies and capacities on P solubilization appear to be

Table 1 Fungal P solubilization and extracellular phosphatase production in culture broth amended with inorganic insoluble P sources

Strain	P source	SC (units)	SE (%)	AcP (EU)	AlP (EU)
Talaromyces flavus	FP	0·26 ± 0·08 a	4·15 ± 0·63 c	0·26 ± 0·02 ef	0·074 ± 0·004 abcd
	AP	0·45 ± 0·15 d	4·45 ± 1·43 c	0·24 ± 0·03 cde	0·094 ± 0·004 def
	RP	0·40 ± 0·02 d	97·03 ± 1·82 h	0·08 ± 0·01 a	0·056 ± 0·003 abc
	PC	4·03 ± 0·06 g	43·06 ± 2·24 g	0·32 ± 0·03 f	0·045 ± 0·001a
Penicillium purpurogenum	FP	0·48 ± 0·01 d	5·09 ± 0·07 cd	0·21 ± 0·04 cde	0·092 ± 0·004 bcde
	AP	0·44 ± 0·07 d	5·14 ± 0·43 cd	0·25 ± 0·01 def	0·091 ± 0·004 bcde
	RP	0·50 ± 0·02 d	91·48 ± 0·91 h	0·06 ± 0·01 a	0·062 ± 0·003 abcd
	PC	5·13 ± 0·18 g	43·56 ± 3·12 g	0·07 ± 0·01 a	0·045 ± 0·009 a
Talaromyces helicus L	FP	1·05 ± 0·29 e	8·19 ± 2·70 e	0·22 ± 0·02 cde	0·087 ± 0·003 bcde
	AP	0·24 ± 0·06 c	3·52 ± 0·50 bc	0·17 ± 0·01 bc	0·077 ± 0·011 abcde
	RP	0·09 ± 0·01 a	20·82 ± 0·24 f	0·07 ± 0·01 a	0·113 ± 0·002 ef
	PC	0·15 ± 0·01 b	2·16 ± 0·25 a	0·07 ± 0·01 a	0·068 ± 0·013 abcd
Talaromyces helicus N	FP	1·08 ± 0·15 e	6·88 ± 0·03 de	0·18 ± 0·01 cd	0·061 ± 0·006 abcd
	AP	0·20 ± 0·01 bc	2·67 ± 0·03 ab	0·18 ± 0·01 bcd	0·085 ± 0·003 bcde
	RP	0·20 ± 0·06 bc	44·04 ± 14·1 g	0·11 ± 0·04 ab	0·129 ± 0·015 f
	PC	2·05 ± 0·30 f	26·15 ± 1·62 f	0·072 ± 0·01 a	0·076 ± 0·011 abcd
Talaromyces diversus	FP	0·27 ± 0·04 c	4·44 ± 0·71 c	0·20 ± 0·01 cde	0·055 ± 0·007 ab
	AP	0·10 ± 0·04 a	1·83 ± 0·73 a	0·18 ± 0·01 ab	0·077 ± 0·010 abcde
	RP	0·22 ± 0·01 bc	54·37 ± 2·31 g	0·10 ± 0·01 ab	0·092 ± 0·001 cdef
	PC	0·52 ± 0·03 d	6·84 ± 0·01 de	0·06 ± 0·01 a	0·078 ± 0·013 abcde

SC: P solubilization capacity, SE (%): P solubilization efficiency in percentage values, AcP: acid phosphatases activity in enzymatic units (EU), AlP: alkaline phosphatases activity in EU. Inorganic P sources: iron (FP), aluminium (AP), rock (RP) and calcium (PC) phosphate. Different letters within columns are significant differences among values as determined by Fisher's LSD test ($P \leq 0{\cdot}05$). Values are means ± SD.

strongly dependent on the fungal identity, according to our results. Furthermore, different strains of the same species presented differences in the efficiencies and capacities of P recycling (*T. helicus N* and *T. helicus L*). Scervino *et al.* (2010) showed that various strains of *T. helicus* produced different patterns of organic acids, depending on the inorganic P source used. These results reinforce the idea that the fungal metabolism underlying P solubilization governs the capacity and efficiency of P release from different inorganic P sources. It is clear that inorganic P solubilization processes depend on multiple variables such as medium pH, nutrient availability, insoluble P sources and fungal strains. Overall, our results support the hypothesis that there is a marked interaction of the known mechanisms involved in P solubilization and that this solubilization is mostly dependent on the microbial strain involved.

Another crucial pathway for P recycling by fungal strains is the release of acid and alkaline phosphatases to the soil. These enzymes degrade soil organic P compounds and therefore increase the P bioavailability for plants and micro-organisms. Despite their ecological and agronomic importance, the production of these enzymes has not been related to the P solubilization efficiency and capacity of microbial organisms. Our results showed that the enzymatic production was triggered by the fungal growth, even in those treatments where organic P sources were absent (PC, RP, AP, FP). Similar results have been reported previously in media with PC and different *Trichoderma* species (Kapri and Tewari 2010). Also, the enzymatic activity increased with the fungal biomass, suggesting that there is a direct relationship between the fungal growth and the extracellular phosphatase production and release. Furthermore, the alkaline and acid phosphatase activity increased along the culture, in agreement with previous reports (Gargova and Sariyska 2003; Aseri *et al.* 2009). The fungal growth under different pH showed that the enzymatic activity was modulated by this factor. It is known that acid phosphatases have their optimum activity in low pH environments (4–6), while alkaline have it in high pH media (8–10). Previous studies showed that pH generally affects the enzymatic activity, in coincidence with our findings (Nahas 2015). It appears that fungal growth in soils could benefit the acid phosphatase activity over alkaline ones, as they lower the pH even in buffered mediums, turning the environmental conditions more favourable for acid phosphatases, but further experiments should be performed to verify this hypothesis.

However, the phosphatase activity was modulated by the chemical nature of P sources and the fungal identity. Indeed, when iron and aluminium phosphate salts were added, the enzymatic activity increased compared with the other inorganic P sources added, in accordance with Oliveira *et al.* (2009). Furthermore, the strain *T.*

Table 2 Fungal P mineralization and extracellular phosphatase production in culture broth amended with organic insoluble P sources

Strain	P source	MC (units)	ME (%)	AcP (EU)	AlP (EU)
Talaromyces flavus	FitP	1·81 ± 0·16 b	21·51 ± 2·07 a	0·16 ± 0·01 bc	0·052 ± 0·018 a
	LecP	0·36 ± 0·07 a	89·95 ± 13·4 b	0·36 ± 0·20 a	0·095 ± 0·003 bc
Penicillium purpurogenum	FitP	1·72 ± 0·04 bc	20·78 ± 0·03 a	0·14 ± 0·01 cd	0·048 ± 0·002 a
	LecP	0·34 ± 0·06 a	70·55 ± 13·9 b	0·22 ± 0·02 ab	0·150 ± 0·010 cd
Talaromyces helicus L	FitP	1·88 ± 0·03 d	23·06 ± 0·72 a	0·10 ± 0·01 ef	0·062 ± 0·009 ab
	LecP	0·20 ± 0·04 a	51·19 ± 10·7 b	0·29 ± 0·03 a	0·222 ± 0·028 d
Talaromyces helicus N	FitP	2·07 ± 0·03 cd	25·70 ± 0·67 a	0·09 ± 0·01 f	0·045 ± 0·007 a
	LecP	0·24 ± 0·01 a	58·20 ± 3·50 b	0·15 ± 0·01 bc	0·096 ± 0·006 bc
Talaromyces diversus	FitP	1·48 ± 0·49 bc	21·71 ± 5·02 a	0·15 ± 0·01 bc	0·053 ± 0·005 a
	LecP	0·33 ± 0·01 a	88·87 ± 4·69 b	0·12 ± 0·01 de	0·051 ± 0·002 a

MC: P mineralization capacity, ME (%): P mineralization efficiency in percentage values, AcP: acid phosphatases activity in enzymatic units (EU), AlP: alkaline phosphatases activity in EU. Organic P sources: phytate (FitP) and lecithin (LecP). Different letters within columns are significant differences among values as determined by Fisher's LSD test ($P \leq 0{\cdot}05$). Values are means ± SD.

flavus produced the highest acid phosphatases with PC, AP and FP amendment, although the efficiency of P solubilization was different in each treatment. The high capacity and efficiency of this strain in releasing P from PC does not seem to affect enzyme production and activity, suggesting that this strain produces at least one kind of phosphatase not inhibited by soluble P. These results support the idea that a higher enzyme activity is not strictly dependent on the fungal development nor the soluble P from the P solubilization, but rather on the modulation of the extracellular phosphatases released. Indeed, previous studies showed that the presence of cations can modulate the activity of these enzymes (Dean 2002; Banik and Pandey 2009). Altogether, these results suggest that the strains evaluated in this report produce at least one kind of extracellular phosphatases that could be subjected to activation/inactivation by aluminium, calcium and/or iron cations, although further research should be done to confirm this hypothesis. Interestingly, our results showed that even in the absence of enzymatic substrate (organic P), a phosphatase activity was found in all treatments, showing that inorganic P solubilization and organic P mineralization are processes that can occur simultaneously in fungal organisms. Previous studies reported the ability of some fungal strains in P release from inorganic P salts and lecithin or phytate, and their effect on plant growth (Oliveira *et al.* 2009; Della Mónica *et al.* 2015). Probably, the constitutive and basal production of phosphatases could confer an adaptive advantage to colonization and growth in substrates with organic and inorganic P sources versus organisms that can use only one of both nutrients.

Several studies analysed the inorganic P solubilization by rhizospheric fungi (Kucey *et al.* 1989; Richardson 1994; Whitelaw *et al.* 1999; Goldstein 2007; Scervino *et al.* 2010, 2011; Stefanoni Rubio *et al.* 2016), but its relation with the organic P mineralization by fungi is not fully explained (Yadav and Tarafdar 2003; Aseri *et al.* 2009; Oliveira *et al.* 2009). The findings reported in this work showed that strains of *Penicillium* and *Talaromyces* had different capacity to mineralize FitP or LecP, depending on the chemical nature of the organic P source. The fungal capacity of FitP mineralization was the same for all the strains evaluated, suggesting that these fungi have the same kind of enzymes or other similar mechanisms to degrade this organic P compound. Indeed, a singular group of phosphatases known as phytases can degrade FitP. These enzymes are particularly important in P recycling and are produced primarily by micro-organisms (Richardson *et al.* 2011; Richardson and Simpson 2011). Contrasting, fungal LecP mineralization capacity varied among the strains tested. These observations could be due to the different chemical nature of these compounds. FitP has only one type of chemical bond and can be degraded mostly by phytases, while LecP can be degraded by several types of phosphatases. Regarding the production of alkaline phosphatases, the high activity observed in *T. helicus L* was not translated into a soluble P increase from LecP under experimental conditions (slightly acid pH), confirming previous reports showing that their highest activity is in alkaline mediums (pH ≥8). Interestingly, *T. flavus* presented the same capacity of LecP mineralization to *T. diversus*, but while *T. flavus* acid phosphatase production was the highest, *T. diversus* production was significantly lower. Similar results were observed for the other strains, suggesting that the amount, nature, substrate specificity and/or activity of the acid phosphatases involved in LecP mineralization depends on the fungal identity. However, when the efficiencies of mineralization were analysed, LecP was found to be much more efficiently used by the fungal strains

than FitP. The fungal efficiency of mineralization was defined as the amount of soluble P released from the insoluble P source. The observed results could be due to a greater release of phosphatase enzymes to the medium, coinciding with the results obtained in the quantification of acid phosphatase activity where the highest values were obtained in LecP. As in the organic P capacity of mineralization, the efficiency seems not to be affected by the production of alkaline phosphatases. Similar results were found previously (Tarafdar *et al.* 2003; Pandey *et al.* 2007), but the causes were not fully discussed. The major activity of alkaline phosphatases is around pH 8–10, while the medium pH used in the experiment was neutral acidic. The lack of activity of this kind of enzymes at experimental pH could be the main explanation for the results obtained. Interestingly, the enzymatic phosphatase release pattern was different among the strains used, although a tendency for augmented enzyme production in complex organic compounds (LecP) rather than pure ones (FitP) was observed.

When the relationship between the soluble P and the extracellular phosphatase activity was analysed, no correlation was found between these parameters. This lack of correlation could be due to various factors. The only P source available in the mineralization experiments was organic and, probably, the consumption of the released P could give rise to the lack of correlation between the enzymatic activity and the soluble P observed. Besides, as different kinds of phosphatases have different substrate specificities, the total mineralized P could be different depending on the composition of the phosphatases produced by each strain. On the other hand, a positive correlation was found between the soluble P and the fungal biomass produced, suggesting that the fungal growth is directly or indirectly mineralizing the organic P. The fungal biomass and the enzyme activity were positively correlated as well. Altogether, these results suggest that the production of phosphatases allows the fungal development throughout time due to the mineralization of organic P, and that this enzymatic activity depends on the fungal growth.

In conclusion, solubilization and mineralization processes are complex and depend on multiple factors, hindering the generalization and prediction of results for different strains and micro-organisms. Our results showed that pH influenced both P solubilization, by increasing the release of soluble P under acid conditions, and mineralization through acid phosphatases and phytases. Also, these mechanisms were modulated by the physicochemical nature of the P sources. Particularly, the presence of complex organic P sources enhanced P mineralization through phosphatases. Both processes were triggered and regulated by fungal growth and were simultaneously present in these organisms, highlighting the importance of microbiota in P biological mediated recycling in all stages from solubilization of phosphate rocks to mineralization of organic P.

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Conflict of Interest

No conflict of interest declared.

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