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Kinetic characterization of a novel acid ectophosphatase from *Enterobacter asburiae*

Vanessa Sayuri Sato, Renato F. Galdiano Júnior, Gisele Regina Rodrigues, Eliana G. M. Lemos, and João Martins Pizauro Junior*

Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Departamento de Tecnologia, 14884 900, Jaboticabal, São Paulo, Brasil

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Expression of acid ectophosphatase by *Enterobacter asburiae*, isolated from *Cattleya walkeriana* (*Orchidaceae*) roots and identified by the 16S rRNA gene sequencing analysis, was strictly regulated by phosphorus ions, with its optimal activity being observed at an inorganic phosphate concentration of 7 mM. At the optimum pH 3.5, intact cells released *p*-nitrophenol at a rate of 350.76 ± 13.53 nmol of *p*-nitrophenolate (*p*NP)/min/10^8 cells. The membrane-bound enzyme was obtained by centrifugation at 100,000 × *g* for 1 h at 4°C. *p*-Nitrophenylphosphate (*p*NPP) hydrolysis by the enzyme follows "Michaelis-Menten" kinetics with V = 61.2 U/mg and $K_{0.5}$ = 60 μM, while ATP hydrolysis showed V = 19.7 U/mg, $K_{0.5}$ = 110 μM, and n_H = 1.6 and pyrophosphate hydrolysis showed V = 29.7 U/mg, $K_{0.5}$ = 84 μM, and n_H = 2.3. Arsenate and phosphate were competitive inhibitors with *Ki* = 0.6 mM and K_i = 1.8 mM, respectively. *p*-Nitrophenyl phosphatase (*p*NPPase) activity was inhibited by vanadate, while *p*-hydroxymercuribenzoate, EDTA, calcium, copper, and cobalt had no inhibitory effects. Magnesium ions were stimulatory ($K_{0.5}$ = 2.2 mM and n_H = 0.5). Production of an acid ectophosphatase can be a mechanism for the solubilization of mineral phosphates by microorganisms such as *Enterobacter asburiae* that are versatile in the solubilization of insoluble minerals, which, in turn, increases the availability of nutrients for plants, particularly in soils that are poor in phosphorus.

***Keywords*:** acid ectophosphatase, *Enterobacter asburiae*, inhibition, *p*-nitrophenylphosphate, ATPase, pyrophosphatase

Introduction

Absorption of phosphates by plants depends on microorganisms that solubilize insoluble minerals through the production and secretion of organic acids or specific enzymes such as phytases and phosphatases, which increase the availability of soluble phosphates, especially in soils where phosphates are unavailable or present in limited quantities (Sashidhar and Podile, 2010). Thus, microorganisms may play an essential role in phosphorus nutrition and in the growth of most plants in soils that are poor in phosphates.

The ability of endophytic bacteria to solubilize inorganic phosphate (Pi) is well documented, and it is of great interest owing to the beneficial effects of phosphate-solubilizing bacteria on plant growth (Richardson, 2001; Rodriguez *et al*., 2006; Park *et al*., 2011).

Although it is known that the presence of a phosphate repressible acid phosphatase is widespread in nature, it is not universally present in all enterobacterial species. Knowledge regarding the occurrence and role of ectophosphatases in these microorganisms, as well as their possible physiological roles in nutrition, protection, cell differentiation, proliferation, adhesion, virulence, and infection is limited. These phosphatases also find applications in the fields of bioremediation and environmental microbiology (Macaskie, 1990; Macaskie *et al*., 1992; Rossolini *et al*., 1998).

In this study, we identified, induced, expressed, and characterized a novel ectophosphatase produced by *Enterobacter asburiae*. Since the physiological roles of this enzyme from *Enterobacter* sp., remain to be elucidated, the kinetic characterization of this ectophosphatase may provide new information on the mechanisms of induction and secretion, its physiological roles in the adhesion and survival of *Enterobacter asburiae,* and the use of this species as a possible biofertilizer.

Materials and Methods

Isolation of the bacterium and 16 rRNA gene sequence analysis

Bacteria was isolated from the roots of *Cattleya walkeriana*, an orchid species, according to the procedure described by Tsavkelova *et al*. (2007), at the Laboratory of Biochemistry of Plants and Microorganisms (Department of Technology), Faculdade de Ciências Agrárias e Veterinárias, Câmpus de Jaboticabal-SP, Brasil. Partial sequencing of the 16S rRNA gene, using specific primers 8F and 907R, identified the bacteria (Nercessian *et al*., 2005).

Partial nucleotide sequences of the 16S rRNA were obtained using DNA BigDye® Terminator v3.1 Cycle Sequencing Kit's, according to the product specifications. The capillary sequencer, model ABI 3100 (Applied Biosystems), was used to sequence the amplicons.

The sequences were subjected to nucleotide similarity analysis with the GenBank database for their identification and classification. These sequences showed 92–98% similarity

*For correspondence. E-mail: jpizauro@fcav.unesp.br; Tel.: +55-16-3209-2675

when compared to sequences from the database. The FASTA sequence was analyzed by comparison using a local tool BLASTN (Altschul *et al*., 1997) from NCBI (National Center of Biotechnology Information) and was classified by RDP (Ribosomal Database Project).

For the phylogenetic relationship analysis, the program Clustal X v.1.81 (Thompson *et al*., 1994) was used to align the sequences, and Mega 6.0 (Tamura *et al*., 2011) was used to generate a phylogenetic tree, using the neighbor-joining algorithm (Saitou and Nei, 1987) with the nucleotide substitution model made by Jukes Cantor (Jukes and Cantor, 1969). The 16S rDNA sequence obtained was registered in the International Gene Bank (GenBank), with the following accession number: JQ982500JN.

Growth conditions of the bacterium and enzyme preparation

Enterobacter asburiae was grown in modified liquid National Botanical Research Institute's Phosphate (NBRIP) growth medium (Nautiyal, 1999) containing glucose, 20 g/L; $NaNO_2$, 2.0 g/L; $MgSO_4 \cdot 7H_2O$, 0.5g/L; KCl, 0.5g/L; $FeSO_4$, 0.01g/L, and different concentrations of potassium phosphate. An inoculum of 10^6 cells/ml was transferred to 250 ml Erlenmeyer flasks containing modified NBRIP medium (pH 6.0) that was previously autoclaved at 121°C, 1.5 atm, for 30 min. The cultures were maintained at 30°C with shaking at 140 rpm for 72 h. Actively growing cells were harvested by centrifugation at 5,000 × *g* for 5 min, and the obtained pellet of intact bacteria was washed, centrifuged twice with 8 ml of 100 mM glycine buffer (pH 3.5), and gently homogenized using a "potter system" to avoid cell disruption. The bacteria were kept in the same buffer until use in the assays.

To obtain a crude enzyme extract from *Enterobacter asburiae*, the cells were disrupted by sonication, as described by Rombola *et al*. (2014). The integral cells were removed by centrifugation at 5,000 × *g* for 15 min. The supernatant was subjected to differential centrifugation, first at 15,000 × *g* for 15 min and subsequently at 100,000 × *g* for 1 h, resulting in the deposition of the enriched membrane-bound enzyme. This fraction was suspended in 8 ml of the same buffer by using a "potter system" and centrifuged again, under the same conditions. Finally, aliquots (1.0 ml) were frozen in liquid nitrogen and stored at -85°C, without appreciable loss of activity when stored for less than 2 months. The supernatant containing a mixture of soluble proteins was also kept in the same buffer, under the same conditions, until use in the assays.

Acid phosphatase assay and determination of protein content

p-Nitrophenyl phosphatase (*p*NPPase) activity was assayed discontinuously at 37°C, in a Hitachi U-2000 spectrophotometer by evaluating the liberation of *p*-nitrophenolate (*p*NP) ions (ε = 17,600 M^{-1} cm^{-1}, pH 13) at 410 nm. Standard assessment conditions were as follows: 100 mM glycine buffer (pH 3.5), containing 1 mM *p*NPP in a final volume of 1.0 ml. Reactions were initiated by the addition of intact cells or enriched fractions of membrane bound-ectophosphatase and terminated by the addition of 1 ml of 1 mol/L NaOH. Controls in which cells or enriched fractions of membrane-bound ectophosphatase were added after termination of the reaction were used as the blank. Before determining the concentration of released *p*-nitrophenol, the tubes were centrifuged at 1,500 × *g* for 20 min, and the supernatant was measured spectrophotometrically at 410 nm. For ATPase and pyrophosphatase, the activity was assayed discontinuously by measuring the amount of Pi released, according to the procedure described by Pizauro *et al*. (1995). One enzyme unit (U) was defined as the amount of enzyme releasing 1.0 nmol of product per min at 37°C. Protein concentration was determined by the procedure described by Hartree (1972), using bovine serum albumin as the standard.

Ectophosphatase assay

Influence of temperature: The enzyme was incubated in 100 mM glycine buffer (pH 3.5) at different temperatures in the range of 10–60°C for variable periods. After the desired period of incubation, aliquots containing the enzyme were removed and added to the ice bath to stop the inactivation process; the residual phosphatase activity was assayed under optimal test conditions, as described above.

Effect of different compounds: The effects of phosphate, EDTA, arsenate, magnesium, calcium, zinc, cobalt, levamisole, *p*-hydroxymercuribenzoate (PHMB), and vanadate on enzyme activity were studied using concentrations ranging from 1 µmol/L to 10 mmol/L. The reactions were carried out in 100 mM glycine buffer (pH 3.5), containing 5 mM *p*NPP and the mentioned compounds in a final volume of 1.0 ml. The remaining activity was assayed as described above.

Effect of $MgCl_2$ concentration: The enzyme was dialyzed in 100 mM glycine buffer (pH 3.5) for 6 h with two changes of buffer. The effect of $MgCl_2$ concentration on enzyme activity was determined in 100 mM glycine buffer (pH 3.5), containing 5 mM *p*NPP and different concentrations of the magnesium ions. The *p*NPPase activity was determined as described above.

Effect of pH on acid phosphatase activity: The effect of pH was assayed by evaluating enzyme activity in different buffers: 100 mM formic acid (pH 2.0 to 4.5), glycine (pH 2.0 to 4.5), maleic acid (pH 2.5 to 4.5), and acetate (pH 2.5 to 6.0); each reaction contained 5 mM *p*NPP. The phosphatase activity was determined as described above.

Estimation of kinetic parameters: V, v, $K_{0.5}$, and n_H obtained from substrate hydrolysis were determined using the software OriginPro 8.

Results and Discussion

Based on the results of the 16S rRNA gene sequencing analysis, it was possible to reliably determine that the sequence of the isolate EndW37 had similarity to those of *Enterobacter* species. The sequences of organisms within the same genus were downloaded from GenBank at NCBI and aligned to construct the phylogenetic tree, following which the sequences were compared. The results showed that the strain belonged to *Enterobacter asburiae* (Fig. 1) (GenBank accession no. JQ982500JN).

Synthesis of acid ectophosphatase depends on the concentration of Pi in the growth medium (Fig. 2). The concen-

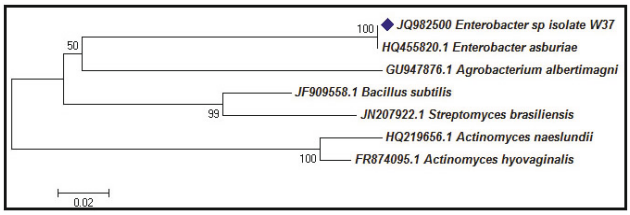

Fig. 1. Dendrogram of the partial sequences of 16S rDNA, proving the similarity of the isolated EndW37 with *Enterobacter asburiae*.

tration required to induce optimum synthesis of the enzyme was 7 mM, while lower or excess quantities of phosphate apparently reduced its expression; similar results have been reported for the expression of ectophosphatases in several cell types of bacterial, fungal and plant origins (Fernandes *et al.*, 1997; Kneipp *et al.*, 2004; Fonseca de Souza *et al.*, 2008), which is strictly induced by phosphate deprivation (Ullah and Cummins,1988; Olczak *et al.*, 2003; Kneipp *et al.*, 2004; Freitas-Mesquita and Meyer-Fernandes, 2014). In contrast, Braibant and Content (2001) demonstrated that membrane-bound phosphatase of *Mycobacterium bovis* BCG was not Pi-repressible.

Although *Enterobacter asburiae* was used as a phosphate-solubilizing microorganism (Jeon *et al.*, 2003; Park *et al.*, 2011), the properties of the acid phosphatase produced by this microorganism was not fully established and the relevant data available in the literature are limited.

The benefits of using differential centrifugation to obtain membrane-bound acid phosphatase are that this method is easy to reproduce, fast, and highly reproducible for obtaining membrane-bound enzymes (Ciancaglini *et al.*, 2010), matrix vesicles (Ciancaglini *et al.*, 2006), vesicles made by sonication of *Burkholderia gladioli* (Rombola *et al.*, 2014), and for studying the proteins interacting with the membrane (Pizauro *et al.*, 1995). In addition, this method avoids the interaction of membrane-bound enzymes or vesicles with a surface-active gel that might result in a loss of bioactivity or quantity that may induce enzyme inactivation during chromatography. This method resulted in a separation of two fractions: the pellet (fraction I), which represents the membrane-bound enzyme with a specific activity of 61.2 U/mg for *p*NPP and 95% of the crude enzyme extract, and the supernatant (fraction II), composed of a complex mixture of soluble proteins, representing less than 5% of the crude enzyme extract.

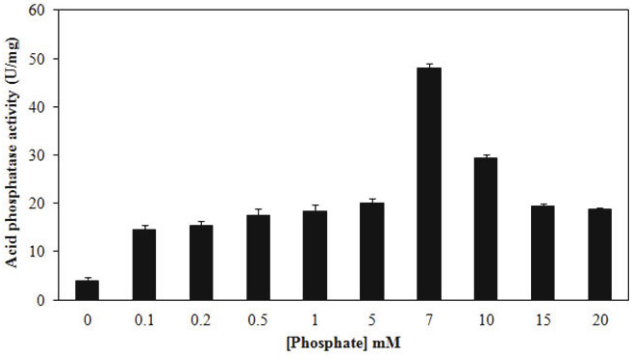

Fig. 2. Expression of *p*NPPase activity of membrane-bound acid phosphatase in *Enterobacter asburiae* grown in medium containing increasing concentrations of phosphate.

The expression of cell-surface phosphatases in *Enterobacter asburiae* was also evaluated in intact cells through the hydrolysis of *p*NPP at pH 3.5, at a rate of 350.76 ± 13.53 nmol of *p*NP/min/10^8 cells. This value is 1,500-fold higher than that described for the enzyme obtained from intact *Cryptococcus neoformans* cells (Collopy-Junior *et al.*, 2006). Hydrolysis of *p*NPP by *p*NPPase (ectophosphatase) from living *Enterobacter asburiae* cells indicates the presence of an active site facing the extracellular environment in *p*NPPase. The biological role of the membrane-bound acid ectophosphatase in *Enterobacter* has not yet been fully explored.

Determining the fact that cell-surface phosphatase has its active site facing the extracellular medium suggests that acid ectophosphatases plays a key role in signal transduction, interaction with different hosts, and plant nutrition. By the action of this ecto-enzyme, this bacterium may increase Pi liberation through mineral phosphate solubilization. Thus, a positive correlation exists between acid phosphatase production and Pi nutrition in plants (Ascencio, 1994; Baldwin *et al.*, 2001), particularly in soils that are poor in phosphates (Braz and Nahas, 2012; Rombola *et al.*, 2014).

High levels of acid phosphatase bound to the membrane of *Enterobacter asburiae* were obtained by centrifugation at 100,000 × *g* for 1 h at 4°C, with a *p*NPPase activity of 61.2 U/mg; this fraction was used for further studies.

The optimum pH for the hydrolysis of *p*NPP was approximately 3.5 (Fig. 3), and this pH was used for all measurements of the activity of acid ectophosphatase. This optimum pH value is in accordance with those determined for

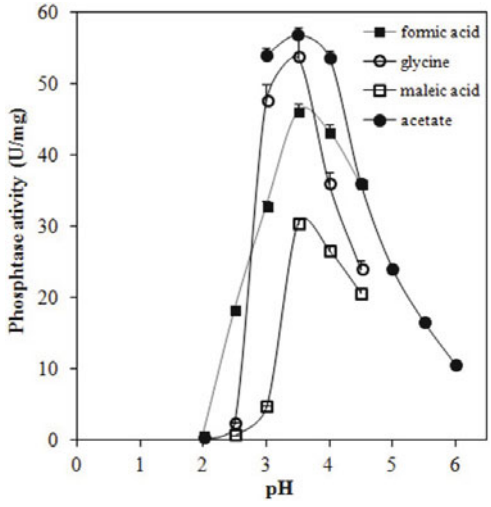

Fig. 3. pH sensitivity of the catalysis of membrane-bound acid phosphatase from *Enterobacter asburiae*. Enzymatic assays conditions containing 5 mM *p*NPP were buffered with 100 mM acetate for the pH range 2.5–6.0, 100 mM glycine for the pH range 2.0–4.5, maleic acid for the pH range 2.5–4.5 and 100 mM formic acid for the pH range 2.0–4.5. The pH before and after each determination did not differ by more than 0.05 units, and the reaction was initiated by the addition of membrane-bound enzyme.

Table 1. Kinetic parameters for the hydrolysis of *p*-nitrophenylphosphate (*p*NPP), ATP and pyrophosphate at pH 3.5 by membrane-bound acid phosphatase from *Enterobacter asburiae*

Substrate	Kinetic parameters		
	V (U/mg)	n_H	$K_{0.5}$ (μM)
ATP	19.7	1.6	110
*p*NPP	61.2	1.2	60
Pyrophosphate	29.7	2.3	84

Escherichia coli (Dassa and Boquet, 1981), *Aspergillus niger* (Wyss *et al*., 1998; Gargova *et al*., 2006), and *Aspergillus ficuum* (Ehrlich *et al*., 1994). This pH value differed from those reported for the optimum activity of a novel phosphatase encoded by the gene *phoI*, in the soil bacterium *Enterobacter* sp. 4, which was pH 5.5 for *p*-nitrophenylphosphate (Kang *et al*., 2006).

The optimum pH value was similar to those reported for ectophosphatases from *Herpetomonas* sp. (pH 4.0) and *Phytomonas mcgheei* and *P. francai* (pH 5.0) (Dutra *et al*., 2006) but differed from that for an ectophosphatase from *Cryptococcus neoformans* (pH 7.0) (Collopy-Junior *et al*., 2006).

This is the first study to report that the uptake of phosphate by *Enterobacter asburiae* from the environment depends on an ectophosphatase with an optimum pH between 3.0 and 4.0 because most studied microorganisms contain enzymes that act within a pH range of 5.0–6.5, e.g., the acid phosphatase from *Metarhizium anisopliae* (Cosentino-Gomes *et al*., 2013) and *Burkholderia gladioli* (Rombola *et al*., 2014) and other ectophosphatases (Freitas-Mesquita and Meyer-Fernandes, 2014).

Kinetic properties of the hydrolysis of various substrates by the membrane-bound enzyme are shown in Table 1. This enzyme showed broad substrate specificity, as it was able to hydrolyze different substrates at pH 3.5 (Fig. 4). The purified acid phosphatase from *Enterobacter asburiae* hydrolyzed *p*NPP with a specific activity of 61.2 U/mg. Interesting results were observed at lower pH values for V_{max}, which was 19.7 U/mg when ATP was used as the substrate, and 28.7 U/mg when pyrophosphate was used as the substrate, suggesting that the enzyme is a inespecific phosphomonohydrolase that also may utilize polyphosphate esters as substrates. While "Michaelian" kinetics behavior was observed for the hydrolysis of *p*NPP, for pyrophosphate and ATP, site-site interactions were observed (n_H about 1.6–2.3), and $K_{0.5}$ values were 60 μM, 110 μM, and 84 μM, respectively. The ability of the enzyme to dephosphorylate phosphoesters was similar to that observed for phosphatases from other sources (Rossolini *et al*., 1998; Kowluru, 2002). This behavior is similar to that observed for acid phosphatase secreted from *Entamoeba histolytica* (Anaya-Ruiz *et al*., 2003), which catalyzes the hydrolysis of *p*-nitrophenyl phosphate at acidic pH.

The study of the effects of various substances on acid phosphatase activity shows that its activity was inhibited by phosphate, arsenate, and vanadate, while EDTA, calcium, copper, cobalt, levamisole, and *p*-hydroxymercuribenzoate had no effects on *p*NPPase activity (Table 2).

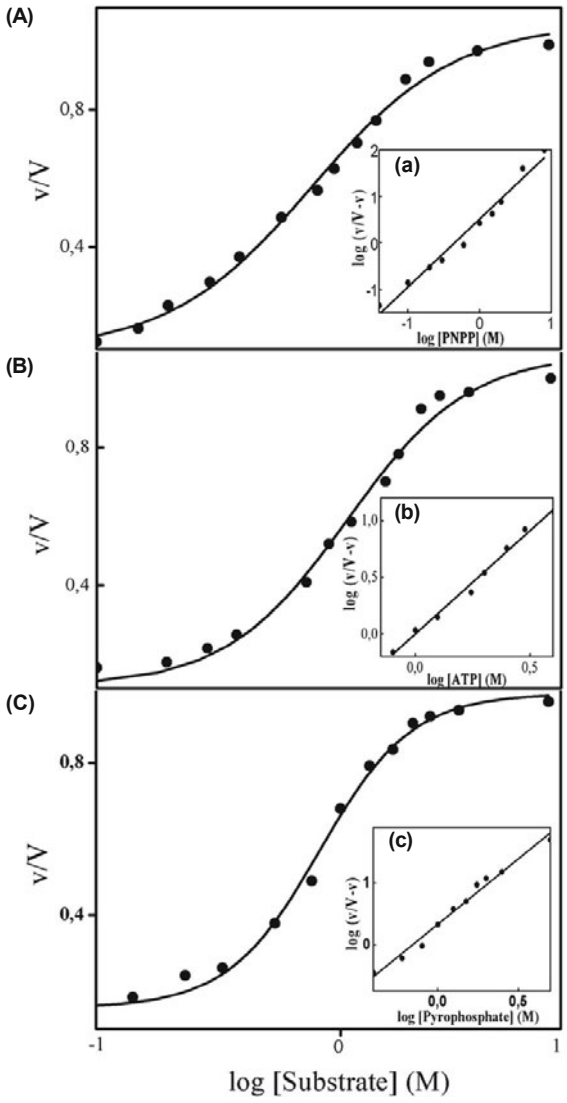

Fig. 4. Effect of substrate concentration on phosphohydrolytic activity of membrane-bound acid phosphatase from *Enterobacter asburiae*. The *p*NPPase (A), ATPase (B), and pyrophosphatase (C) activities were assayed discontinuously at 37°C. Standard assessment conditions were 100 mM glycine buffer, pH 3.5 a containing increasing concentrations of substrate from 0.1 mM to 10 mM. Representation of Hill for *p*NPPase (a), ATPase (b), and pyrophosphatase (c).

Table 2. Relative effectiveness of several reagents on the activity of membrane-bound acid phosphatase from *Enterobacter asburiae*. Specific activity of 100% corresponds to 61.2 U/mg.

Reagents	% Vm
Phosphate (10 mM)	58
EDTA (10 mM)	87
Arsenate (10 mM)	39
Magnesium (2 mM)	120
Calcium (1 mM)	94
Zinc (1 mM)	97
Copper (1 mM)	94
Cobalt (1 mM)	95
Levamisole (10 mM)	95
PHMB (1 mM)	81
Vanadate (0.5 mM)	63

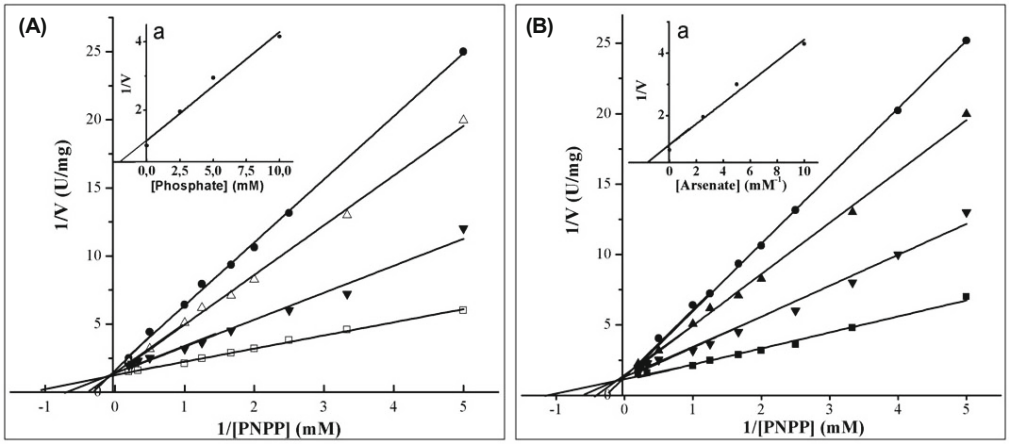

Fig. 5. Inhibition of acid phosphatase activity by arsenate (A) (■) none, (▼) 2.5 mM, (▲) 5.0 mM, (●) 10 mM and phosphate; (B) (□) none, (▼) 2.5 mM, (△) 5.0 mM, (●) 10 mM. (a) secondary plot for determination of *Ki*. *p*NPPase activity was assayed in 100 mM glycine buffer, pH 3.5, as described in 'Materials and Methods'.

Other phosphomonohydrolases and acid ectophosphatases are significantly inhibited by sodium vanadate (Fukami and Lipmann, 1982; Kneipp *et al*., 2003); however, enzyme activity was not inhibited by *p*-hydroxymercuribenzoate (Lau *et al*., 1987; Buzalaf *et al*., 1998), suggesting that this enzyme is not a tyrosine phosphatase. This assumption is supported by the fact that *p*-hydroxymercuribenzoate and orthovanadate are known to be potent inhibitors of protein tyrosine phosphatases (PTPases) in general (Fukami and Lipmann, 1982; Crans *et al*., 2014; Stanford *et al*., 2014). Similar results were observed for other phosphohydrolases and acid ectophosphatases that were also significantly inhibited by sodium orthovanadate (Fukami and Lipmann, 1982; Swarup *et al*., 1982; Kiffer-Moreira *et al*., 2007; Freitas-Mesquita and Meyer-Fernandes, 2014). It is suggested that vanadate binds at the active site, which is similar to that observed with the metastable intermediate that is formed during the hydrolysis of phosphate esters (Lopez *et al*., 1976; Rombola *et al*., 2014).

Arsenate and phosphate were competitive inhibitors with *Ki* = 0.6 mM and 1.8 mM, respectively (Fig. 5A and B). This behavior was expected, since vanadate is a phosphate analogue and a powerful competitive inhibitor of the phosphatase active site (Durmus *et al*., 1999). The acid phosphatase from *Enterobacter asburiae* is inhibited by classic inhibitors of ectophosphatases isolated from other sources (Dutra *et al*., 1998, 2006; Catta-Preta *et al*., 2013; Freitas-Mesquita and Meyer-Fernandes, 2014).

Although the acid ectophosphatase is not a metalloezyme, its active site faces the external environment; thus, the influence of different metal ions on enzyme activity should provide new insights on the enzyme's behavior. In fact, magnesium ions stimulated acid ectophosphatase activity by 50% and site-site interactions (n_H = 0.5) were observed, with $K_{0.5}$ = 2.2 mM (Fig. 6). This result suggests that this metal ion is essential for optimum activity of acid ectophosphatase from *Enterobacter asburiae*. These results do not differ significantly from those obtained for the enzymes from *Humicola grisea* (Buainain *et al*., 1998), *Scytalidium thermophilum* (Guimarães *et al*., 2006), and *Pseudallescheria boydii* (Kiffer-

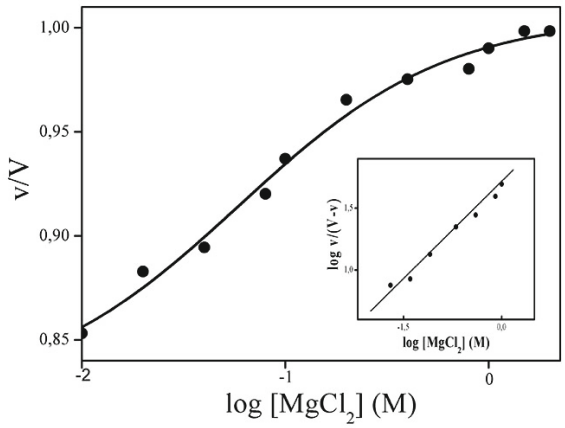

Fig. 6. Effect of the concentration of magnesium ions on the activity of membrane bound acid phosphatase from *Enterobacter asburiae*. The reaction was carried out in 100 mM glycine buffer, pH 3.5, containing different concentrations of magnesium ions. (b) Representation of Hill for the interaction of Mg^{2+} ions with acid phosphatase.

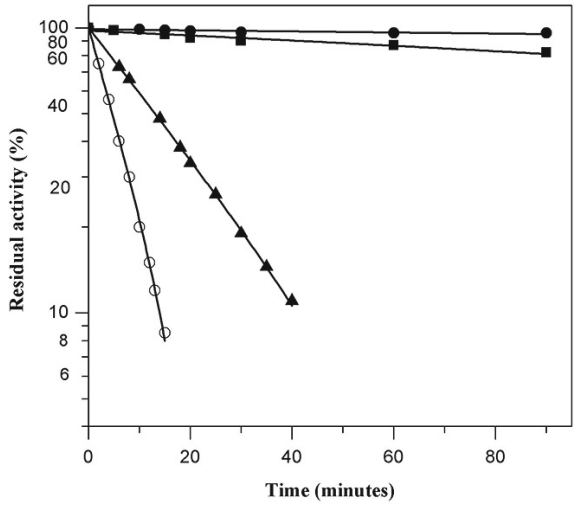

Fig. 7. Thermal inactivation of membrane-bound acid phosphatase by *Enterobacter asburie*. The residual activity was determined by the addition of 50 µl aliquots to 100 mM glycine buffer, pH 3.5, containing 5 mM *p*NPP, in a final volume of 1.0 ml. Values are the mean of duplicate determinations that differed by less than 5% (●) 45°C, (■) 50°C, (▲) 55°C, (○) 60°C.

Moreira *et al*., 2007). In addition, some reports state that ectophosphatases from different sources require magnesium ions for maximum activity (Catta-Preta *et al*., 2013; Freitas-Mesquita and Meyer-Fernandes, 2014). In contrast, zinc ions at millimolar concentrations had no effect on *p*NPPase activity in this study.

The enzyme was stable for up to 90 min at 45°C in 100 mM glycine buffer (pH 3.5) (Fig. 7). At higher temperatures, the enzyme was inactivated, showing a $t_{1/2}$ that varied from 18.25 min at 55°C to 5.48 min at 60°C.

First-order kinetics of thermal inactivation from 10°C to 60°C suggests that this enzyme is an ectoenzyme; however, it is not intrinsically associated with the membrane (Barton *et al*., 1985). While $t_{0.5}$ of ectophosphatase from *Enterobacter asburiae* was similar to that of ectophosphatase from *Burkholderia gladioli* (Rombola *et al*., 2014), it was lower than that of ectophosphatases from *Aspergillus niger* (Wang *et al*., 2004) and *Thermus thermophilus* HB27 (Wang *et al*., 2009).

The study of acid ectophosphatase is of great interest not only for the production of this enzyme but also for gathering knowledge regarding its kinetic and structural properties. Results obtained by Galdiano Junior *et al*. (2011) showed that the isolated *Enterobacter* sp. EndW37 solubilized phosphate, and the seedlings in which it was inoculated showed the highest mean values of leaf area, number and length of roots, and dry mass, proving the effectiveness of this bacterium for inducing plant growth. The bacteria found in the rhizosphere can promote plant growth through one or more mechanisms such as nitrogen fixation, induction of hormone production, and release of nutrients to the plant (Mikanova and Novakova, 2002; Shoebitz *et al*., 2009).

Conclusion

Evaluation of the activity of acid ectophosphatase from *Enterobacter asburiae* isolated from *Cattleya walkeriana* (*Orchidaceae*) roots is very interesting for understanding the role of the enzyme in processes such as cell differentiation and in the mechanisms underlying mineral phosphate solubilization in plant nutrition. Thus, this organism and its associated acid ectophosphatase may have great potential for application in soils with low phosphate concentrations to increase the phosphorus uptake of plants.

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