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ORIGINAL PAPER

A novel xylan degrading β-D-xylosidase: purification and biochemical characterization

Michele Michelin · Simone C. Peixoto-Nogueira · Tony M. Silva · João A. Jorge · Héctor F. Terenzi · José A. Teixeira · Maria de Lourdes T. M. Polizeli

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Abstract *Aspergillus ochraceus*, a thermotolerant fungus isolated in Brazil from decomposing materials, produced an extracellular β-xylosidase that was purified using DEAE-cellulose ion exchange chromatography, Sephadex G-100 and Biogel P-60 gel filtration. β-xylosidase is a glycoprotein (39 % carbohydrate content) and has a molecular mass of 137 kDa by SDS-PAGE, with optimal temperature and pH at 70 °C and 3.0–5.5, respectively. β-xylosidase was stable in acidic pH (3.0–6.0) and 70 °C for 1 h. The enzyme was activated by 5 mM $MnCl_2$ (28 %) and $MgCl_2$ (20 %) salts. The β-xylosidase produced by *A. ochraceus* preferentially hydrolyzed *p*-nitrophenyl-β-D-xylopyranoside, exhibiting apparent K_m and V_{max} values of 0.66 mM and 39 U (mg protein)$^{-1}$ respectively, and to a lesser extent *p*-nitrophenyl-β-D-glucopyranoside. The enzyme was able to hydrolyze xylan from different sources, suggesting a novel β-D-xylosidase that degrades xylan. HPLC analysis revealed xylans of different compositions which allowed explaining the differences in specificity observed by β-xylosidase. TLC confirmed the capacity of the enzyme in hydrolyzing xylan and larger xylo-oligosaccharides, as xylopentaose.

Keywords *Aspergillus ochraceus* · β-xylosidase · Purification · Xylan

M. Michelin · T. M. Silva · J. A. Jorge · H. F. Terenzi · M. de Lourdes T. M. Polizeli (✉)
Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, Ribeirão Preto, SP 14040-901, Brazil
e-mail: polizeli@ffclrp.usp.br

M. Michelin · J. A. Teixeira
Departamento de Engenharia Biológica, Universidade do Minho, Campus Gualtar, 4710-057 Braga, Portugal

S. C. Peixoto-Nogueira · M. de Lourdes T. M. Polizeli
Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, Ribeirão Preto, SP 14040-901, Brazil

Introduction

β-1,4-xylan is the major hemicellulose component of the lignocellulosic biomass (Eriksson and Wood 1985; Polizeli et al. 2005; Polizeli 2009). It is formed by a backbone of β-1,4-linked-D-xylopyranosyl residues, and different substitute groups in the side chains. Several enzymes are required for the hydrolysis of xylan to xylo-oligosaccharides, including β-D-xylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) and β-xylosidase (1,4-β-D-xylan xylohydrolase, EC 3.2.1.37). Xylanases cleave internal xylosidic linkages producing xylo-oligosaccharides, while β-xylosidases hydrolyze xylo-oligosaccharides and xylobiose releasing xylose (Wong et al. 1988; Sunna and Antranikian 1997).

According to Biely (1985, 1993), β-D-xylosidases can be recognized and classified as xylobiases and exo-1,4-β-xylanases in relation to their relative affinities for xylobiose and larger xylo-oligosaccharides, respectively. However, recent works suggest that β-xylosidases can be defined as enzymes that catalyze the cleavage of xylobiose and attack the non-reducing ends of short xylo-oligosaccharides to release xylose (Rasmussem et al. 2006; Knob et al. 2010). Besides, most of the purified β-xylosidases are not able to degrade xylans (Knob et al. 2010).

β-xylosidases are essential enzymes of the microbial xylanolytic system, and contribute to decrease the inhibition of xylanases by the end-product of xylan hydrolysis

(Poutanen and Puls 1988; Guerfali et al. 2008). This enzyme is cell-associated in most bacteria and yeast, but it is freely found in the culture media of some fungi (Guerfali et al. 2008; Saha and Bothast 1999).

Growing interest on the bioconversion of hemicellulose has recently arisen, as a consequence of its potential application in several agro-industrial processes. These include the conversion of hemicellulose materials to fuels and chemicals, paper-pulp delignification, improvement of beer consistency, improved digestibility of animal feedstocks and clarification of juices (Zamost et al. 1991; Viikari et al. 1994; Kulkarni et al. 1999; Saha 2003a; Zanoelo et al. 2004).

The present study reports the purification and biochemical characterization of a β-xylosidase produced by an isolate of *Aspergillus ochraceus*. This fungus is a good producer of xylanolytic enzymes and relevant studies related to the β-D-xylanase production have been published by the group (Betini et al. 2009; Michelin et al. 2010). Besides, this strain has the advantageous characteristics as the secretion of high levels of thermostable β-xylosidase in the presence of xylan as carbon source, which is able to hydrolyze xylans of various origins and, therefore, with differences of structures and solubility.

Materials and methods

Microorganism and culture conditions

Aspergillus ochraceus was isolated from decomposing fruit and leafs, in the Ribeirão Preto region—São Paulo, Brazil. This strain was identified and deposited as *A. ochraceus* at the certified Mycology Culture Collection URM at Federal University of Pernambuco (Recife, Brazil, number 604). Stock culture was propagated at 30 °C on slants of solid 4 % (w/v) PDA (Biolife®) medium and stored at 4 °C.

Growth and enzyme production were followed by a two-stage procedure. Conidia from 7 day-old cultures, with a concentration of 2×10^7 spores ml^{-1}, were inoculated into 250 ml Erlenmeyer flasks containing 50 ml of the liquid medium described by Rizzatti et al. (2001) with 1 % (w/v) glucose as carbon source. The cultures were incubated at 30 °C, without agitation, for 72 h. The mycelial mass was transferred to 250 ml Erlenmeyer flasks containing 50 ml of Czapeck medium (Wiseman 1975) with 1 % (w/v) oat spelt xylan (Sigma®) as carbon source and incubated at 30 °C, under 100 rpm agitation, for 72 h.

Enzymatic assays and protein determination

β-Xylosidase activity was determined by incubating 200 µl of enzyme sample with 50 µl of 0.92 mM *p*-nitrophenyl-β-D-xylopyranoside (PNP-xyl) substrate, and 150 µl of 0.15 M citrate–phosphate buffer (pH 4.5) at 70 °C for 10 min. The reaction was stopped by adding 1 ml of sodium tetraborate saturated solution, and the *p*-nitrophenol released from the substrate was measured by spectrophotometer at 405 nm. One unit of β-D-xylosidase activity was defined as the amount of enzyme that releases 1 µmol of *p*-nitrophenol per minute. Specific activity was defined as enzyme units per milligram protein. Other aryl-β-glycosidase activities were assayed with the appropriate substrates (*p*-nitrophenyl-β-D-glucopyranoside; *p*-nitrophenyl-β-D-arabinopyranoside; *p*-nitrophenyl-β-D-galactopyranoside; *p*-nitrophenyl-β-D-cellobiopyranoside; 4-O-methyl-D-glucurono-D-xylan and 4-nitrophenyl-acetate) under the same conditions described for PNP-xyl.

Regarding β-xylosidase activity against natural substrates (xylobiose and some xylans), the assays were performed by incubating 200 µl of the pure enzyme sample with 200 µl of 1 mg/ml substrate in 0.15 M citrate–phosphate buffer (pH 4.5) at 70 °C for 20 min. Releasing sugars were estimated with 3′,5′dinitrosalicylic acid (DNS) and measured by spectrophotometer at 540 nm (Miller 1959). One unit of β-xylosidase activity was defined as the amount of enzyme that releases 1 µmol reducing sugar equivalent to xylose per minute. Protein concentration was estimated as described by Lowry et al. (1951) using bovine serum albumin as standard.

Purification of extracellular β-xylosidase

DEAE-cellulose chromatography column

All procedures of purification were performed at 4 °C. The enzyme extract (160 ml) was dialyzed against 0.15 M citrate–phosphate buffer in semi-permeable cellulose membrane tubing in order to remove salts and smaller molecules. After that, the dialyzed sample was applied on a DEAE-cellulose column (1.9 × 9 cm) pre-equilibrated with a 10 mM Tris–HCl buffer (pH 7.5). The column was extensively washed with this buffer and β-xylosidase was eluted with 200 ml of a gradient of 0–1.5 M NaCl in same buffer. Fractions of 2.5 ml were collected and the peak containing β-xylosidase activity was pooled, dialyzed overnight against distilled water, lyophilized and suspended in 2 ml of 100 mM sodium acetate buffer, pH 5.5, plus 150 mM sodium chloride.

Sephadex G-100 chromatography column

The concentrated active β-xylosidase fractions obtained from the ion exchanger column (2 ml) were applied to a Sephadex G-100 gel filtration column (1.5 × 57 cm) at 4 °C. The column was equilibrated and eluted with the same buffer used to suspend the sample (100 mM sodium

acetate buffer, pH 5.5, plus 150 mM sodium chloride). Fractions of 1.2 ml were collected and the active fractions were pooled, dialyzed against distilled water, lyophilized and suspended in 1 ml of 100 mM sodium acetate buffer, pH 5.5.

Biogel P-60 chromatography column

Concentrated enzyme (1 ml) was applied to a Biogel P-60 gel filtration column (1.2 × 45 cm) equilibrated and eluted with 100 mM sodium acetate buffer, pH 5.5, and the active fractions were pooled and used for enzyme characterization. The proteins were quantified by measuring the absorbance at 280 nm. The procedure was carried out at 4 °C and fractions of 1.0 ml were collected.

Polyacrylamide gel electrophoresis and carbohydrate content

Electrophoresis under non-denaturing conditions was carried out by the method of Davis (1964), using 6 % acrylamide. SDS-PAGE (7 %) was carried out according to the method of Laemmli (1970), using myosin (205 kDa); phosphorylase b (97.4 kDa); bovine serum albumin (66 kDa); egg albumin (45 kDa); carbonic anhydrase (29 kDa) as molecular mass standards (Sigma). After the runs, the gels were stained with Comassie Brilliant Blue.

Total neutral carbohydrate was estimated by the method of Dubois et al. (1956), using D-mannose as standard.

Kinetic parameters

Kinetic parameters (K_m and V_{max}) were determined by incubating the enzyme under optimal conditions of temperature and pH, with different concentrations of *p*-NP-xyl (0.092–9.2 mM). The values of Michaelis constant were calculated from Lineweaver–Burk plots (1934).

Chromatography of hydrolysis products

Thin layer chromatography (TLC silica gel plates, DC-Alufolien Kieselgel 60, Merck) was performed using the methodology described by Fontana et al. (1988). A volume of 5 µl of the compounds formed by the action of 50 µl pure β-D-xylosidase on 50 µl of substrate in 0.15 M citrate–phosphate buffer (pH 4.5) at 70 °C were applied on silica gel plates, and subjected to two sequential ascending chromatography runs using butanol:ethanol:water (5:3:2 by vol) as solvent system. After air-drying the plate, the spots were detected by spraying with concentrated H_2SO_4 and methanol (1:9 v/v) containing 0.2 % (w/v) orcinol, followed by heating at 100 °C.

The substrates used were 1 % (w/v) birchwood xylan and xylo-oligosaccharides obtained by the action of a purified endo-xylanase from *A. ochraceus* on 1 % (w/v) birchwood xylan after 30 min of reaction.

Chemical composition of the xylans

The chemical composition of the xylans was performed following the procedure reported by Ruzene et al. (2008). Samples of 0.25 g of birchwood xylan, oat spelt xylan and eucalyptus xylan were treated with 2.5 ml of 72 % (w/w) H_2SO_4 under stirring at 30 °C for 1 h. The reaction was interrupted by adding 74.3 ml of distilled water and the mixtures were then transferred to 250 ml Erlenmeyer flasks. The flasks were autoclaved for 1 h at 121 °C for the complete hydrolysis of oligomers. The mixtures were filtered and the hydrolysates were analyzed by HPLC. A MetaCarb 87P (300 × 7.8 mm, Varian, USA) column was used to the analysis of the sugars—glucose, xylose, galactose, arabinose and mannose—under the following conditions: 80 °C, ultrapure water on mobile phase, flow rate 0.4 ml/min. The analysis of the acetic acid and galacturonic acid contents were performed using a Metacarb 87H column (300 × 7.8 mm, Varian, USA) at 60 °C, with 0.005 mol/l H_2SO_4 as mobile phase and flow rate 0.6 ml/min coupled at Jasco chromatograph with refraction-index and ultraviolet detectors. Sugars and acids concentrations were determined from calibration curves of pure compounds.

Results and discussion

Purification of extracellular β-D-xylosidase

β-D-Xylosidase was purified after three chromatography steps detailed in Materials and methods. Figure 1a illustrates the elution profile of the dialyzed culture filtrate from *A. ochraceus* on DEAE-cellulose column, in which a single peak containing β-xylosidase activity was retained by the resin and eluted at approximately 0.7 M of a NaCl linear gradient. The fractions containing β-xylosidase activity were applied to Sephadex G-100 column (Fig. 1b), where major contaminants were separated. After that, the fractions with β-xylosidase activity were applied to Biogel P-60 filtration column (Fig. 1c), resulting in a 22-fold purification and a recovery of 7.17 %. A summary of the purification procedures is presented in Table 1. In this table can also be observed the enzyme concentration used in the experiments of biochemical characterization. The purification protocol resulted in a pure enzyme that migrated as a single band on polyacrylamide gel electrophoresis (PAGE; Fig. 2—lane a).

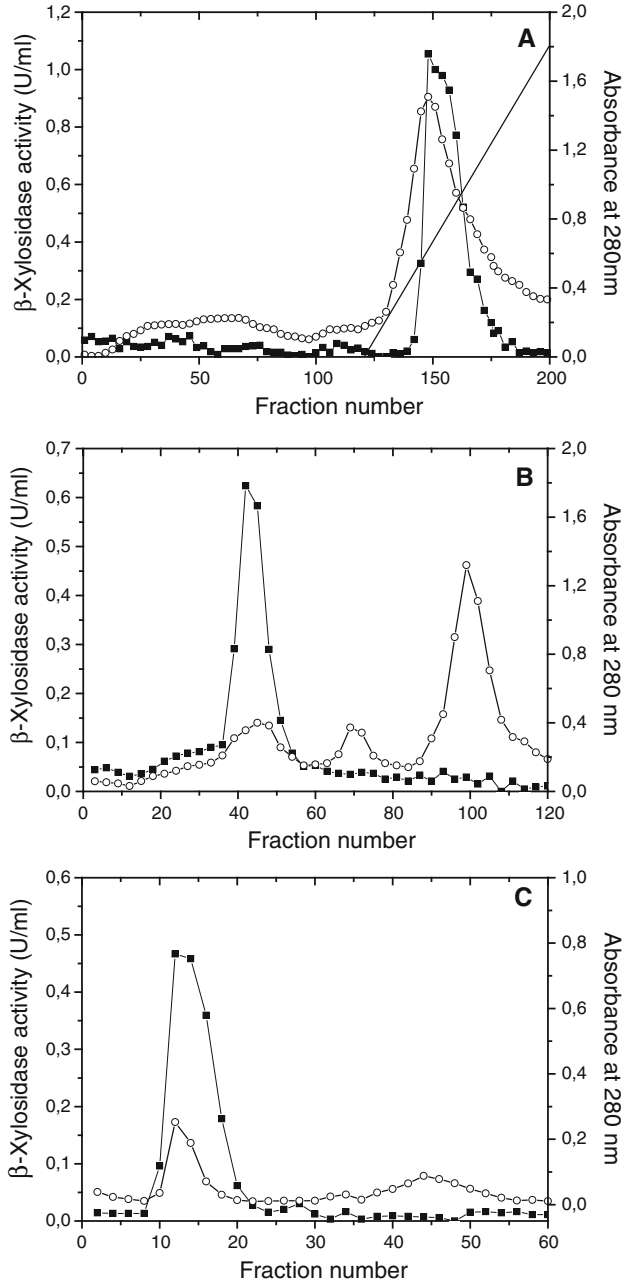

Fig. 1 Elution profile of the *β*-xylosidase activity from DEAE-cellulose (**a**), Sephadex G-100 (**b**) and Biogel P-60 (**c**) chromatography columns. Symbols: (*filled square*) *β*-xylosidase activity; (*open circle*) absorbance 280 nm; (*slashed line*) linear gradient of sodium chloride (0–1.5 M). The enzyme activity was determined at 70 °C and pH 4.5. More details in "Materials and methods" section

Table 1 Summary of the purification steps of the extracellular *β*-xylosidase by *A. ochraceus*

Purification steps	Protein (total mg)	Activity (total U)	Specific activity (U mg protein^{-1})	Yield (%)	Purification (-fold)
Crude extract	107.50	202.25	1.88	100.00	1.00
Filtrate dialyzed	75.00	233.00	3.11	115.20	1.65
DEAE-cellulose	26.25	109.75	4.18	54.26	2.22
Sephadex G-100	3.20	33.50	10.47	16.56	5.57
Biogel P-60	0.35	14.50	41.43	7.17	22.04

The enzyme activity was determined at 70 °C and pH 4.5

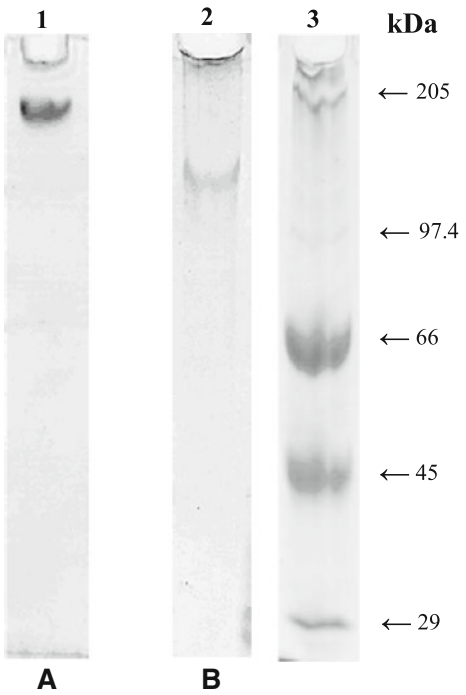

Fig. 2 PAGE (**a**) and SDS-PAGE (**b**) of the *A. ochraceus* purified *β*-D-xylosidase. *Lane 1* and *2*: purified *β*-xylosidase. *Lane 3*: molecular mass markers (myosin 205 kDA; phosphorylase b 97.4 kDa; bovine albumin 66 kDa; egg albumin 45 kDa and carbonic anhydrase 29 kDa)

Determination of M_r and carbohydrate content

The molecular mass of the purified *β*-xylosidase was estimated as 137 kDa by SDS-PAGE (Fig. 2—lane b). Most of the purified *β*-D-xylosidases exhibit molecular masses above 100 kDa (Sunna and Antranikian 1997). The *A. ochraceus* *β*-xylosidase is a glycoprotein that contains 39 % carbohydrate, which is in agreement with other fungal *β*-xylosidases described in the literature (Rizzatti et al. 2001; Herrmann et al. 1997; Wakiyama et al. 2008).

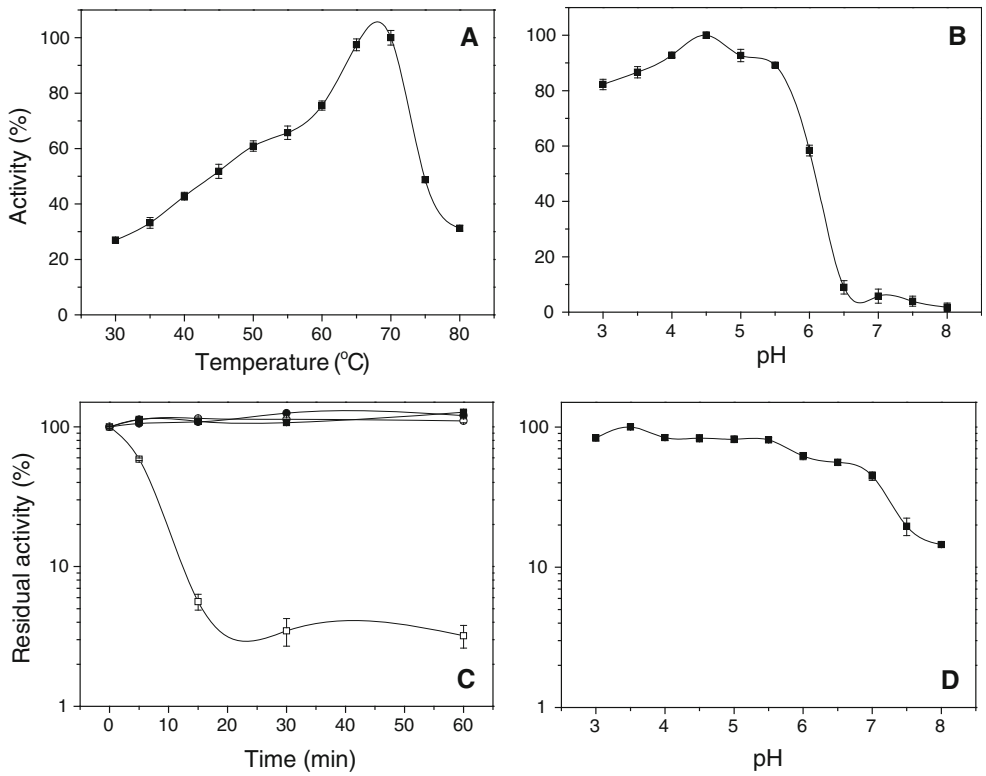

Fig. 3 Effect of temperature and pH on purified *β*-xylosidase activity. Temperature (**a**) and pH (**b**) optimal; thermostability at 60 °C (*filled circle*), 65 °C (*open circle*), 70 °C (*filled square*) and 75 °C (*open square*) (**c**); and pH stability for 1 h at 4 °C (**d**). The *β*-xylosidase activity was determined by discontinuous methods using 0.25 % (w/v) of the PNF-xylopyranoside synthetic substrate as described in "Materials and methods"

Effect of temperature and pH on enzyme activity and stability

Figure 3a and b illustrate the influence that temperature and pH had on *β*-xylosidase activity, respectively. The enzyme showed an optimum of activity at 70 °C and in a pH range of 3.0–5.5 (up to 80 % activity). Both, temperature and pH optima were similar to those reported for the *β*-xylosidase of *Aspergillus japonicus* (Wakiyama et al. 2008), *Trichoderma harzianum* (Ximenes et al. 1996), *Aspergillus awamori* K4 (Kurakabe et al. 1997) and *Aspergillus fumigatus* (Lenartovicz et al. 2003).

The effect of temperature on the stability of the enzyme was also examined. The purified enzyme was completely stable for 1 h at 70 °C, and decayed with a half-life of approximately 6 min at 75 °C (Fig. 3c). Studies of the thermal stability indicated that the purified *A. ochraceus* *β*-xylosidase was more stable than the fungal *β*-xylosidase from *A. phoenicis* (Rizzatti et al. 2001) and *A. japonicus* (Wakiyama et al. 2008). Stability and activity at high temperatures are desirable properties in this type of enzyme, considering the fact that most industrial processes where xylanolytic enzymes can be useful are carried out at high temperatures (Wong and Saddler 1993; Knob et al. 2010). The enzyme was stable (in a range of pH of 3.0–6.0) at pH values between 3.0 and 6.0 for 1 h at 4 °C (Fig. 3d). This pH range was similar to *A. phoenicis* *β*-xylosidase (Rizzatti et al. 2001) but differed to the *β*-xylosidase of *Talaromyces thermophilus* (Guerfali et al. 2008) that was more stable when pH was higher than 5.0.

Influence of salts, EDTA and *β*-mercaptoethanol on the *β*-xylosidase activity

The effects of several chloride salts and other reagents on the *β*-xylosidase activity are shown in Table 2. The results demonstrated a slight stimulation by 5 mM $MnCl_2$ (28 %) and $MgCl_2$, (20 %) and an inhibitory effect by high concentrations (10 mM) of the same compounds. 2-mercaptoethanol is a reducing agent that can modify the tertiary structure of the enzyme and affect the enzymatic activity, and for *A. ochraceus* *β*-xylosidase, it was observed 14 % activation using 5 mM of these compound. The enzyme activity was drastically inhibited by sulfhydryl oxidant metal (Hg^{2+}), suggesting the existence of thiol groups at the active site of the enzyme (Sandrim et al. 2005). High inhibition was also observed in the presence of 10 mM $CoCl_2$, NaCl and NH_4Cl. EDTA did not significantly affect

Table 2 Influence of salts, EDTA and 2-mercaptoethanol on β-xylosidase activity

Reagents	Activity (%)		
	1 mM	5 mM	10 mM
None	100.00	100.00	100.00
NH_4Cl	84.06 (±0.15)	88.18 (±0.15)	37.24 (±0.11)
$HgCl_2$	1.42 (±0.10)	1.80 (±0.13)	10.70 (±0.10)
NaCl	77.21 (±0.11)	40.08 (±0.19)	35.19 (±0.17)
$CoCl_2{\cdot}6H_2O$	93.74 (±0.19)	105.01 (±0.20)	24.07 (±0.22)
$MnCl_2{\cdot}4H_2O$	102.81 (±0.20)	128.46 (±0.28)	62.76 (±0.25)
$MgCl_2{\cdot}6H_2O$	102.24 (±0.21)	120.04 (±0.25)	83.54 (±0.19)
$CaCl_2$	98.54 (±0.16)	94.16 (±0.17)	91.97 (±0.23)
KCl	98.54 (±0.22)	100.00 (±0.14)	97.08 (±0.15)
EDTA	104.38 (±0.12)	97.81 (±0.19)	99.27 (±0.18)
2-mercaptoethanol	91.54 (±0.25)	114.23 (±0.13)	104.12 (±0.15)

The assays were performed at 70 °C and pH 4.5

this activity, suggesting that metal ions were not required for enzyme activity.

Substrate hydrolysis

The purified enzyme hydrolyzed mainly PNP-xylopyranoside and PNP-glucopyranoside, and to some extent, also hydrolysed PNP-arabinopyranoside, PNP-galactopyranoside and 4-*O*-methyl-D-glucurono-D-xylan, but not PNP-cellobiopyranoside and 4-nitrophenyl-acetate (Table 3). These results are in contrast to the usual characteristics of β-D-xylosidase, which are very specific for xylopyranosides, while the more unspecific β-D-glucosidases act both on PNP-glu and PNP-xyl (Peralta et al. 1997; Iwashita et al. 1998; Kimura et al. 1999).

In relation to the hydrolysis of the natural substrates, xylobiose was the favorite substrate for β-xylosidase. However, the enzyme was able to hydrolyze xylan of different sources, as birchwood xylan, larchwood xylan, oat spelt xylan and eucalyptus xylan, respectively (Table 4), which is in contrast to the properties of true β-xylosidases.

Table 3 Hydrolysis of synthetic substrates by *A. ochraceus* β-xylosidase

Synthetic substrates	%
p-nitrophenyl-β-D-xylopyranoside	100.00
p-nitrophenyl-β-D-glucopyranoside	53.95
p-nitrophenyl-β-D-arabinopyranoside	10.53
p-nitrophenyl-β-D-galactopyranoside	7.89
p-nitrophenyl-β-D-cellobiopyranoside	nd
4-*O*-methyl-D-glucurono-D-xylan	1.32
4-nitrophenyl-acetate	nd

nd not detected

The assays were performed at 70 °C and pH 4.5

Table 4 Hydrolysis of natural substrates by *A. ochraceus* β-xylosidase

Natural substrates	%
Xylobiose	100.00
Birchwood xylan	13.11
Larchwood xylan	6.89
Oat spelt xylan	5.57
Eucalyptus xylan	2.30

The assays were performed at 70 °C and pH 4.5

Xylans are heteropolysaccharides constituted by a main backbone of xylose residues with distinct degree of substituents according to the origin of plant. These substituents can include acetyl groups, 4-*O*-methyl-α-D-glucuronopyranosyl units and α-L-arabinofuranosyl groups (Polizeli 2009). The knowledge of the xylans composition from different sources may explain the differences in specificity observed for the β-xylosidase.

Birchwood xylan presented the highest content of xylose (89.1 %), and it was xylan which had higher affinity by the enzyme. Oat spelt xylan, besides the high xylose content (65.2 %), also showed a high content of arabinose (20.1 %). This is according to the literature, since arabinoxylans have been identified in wheat, rye, barley, oat, rice and sorghum (Polizeli et al. 2005). Similar composition was reported by Li et al. (2000). They reported that larchwood xylan presents in its constitution high content of mannose (26 %) and glucose (26.5 %) besides xylose (47.5 %). The *A. ochraceus* β-xylosidase hydrolyzed oat spelt xylan and larchwood xylan in similar degree. However, eucalyptus xylan showed the smallest hydrolysis rate, and its composition proved to be the most complex among the xylans and besides the high content of xylose (63 %), it also presented a high content of acetic acid (16 %), showing to be an acetylated xylan (Table 5).

Table 5 Chemical compositions of the xylans

	Carbohydrate residues (molar %)		
	Birchwood xylan	Oat spelt xylan	Eucalyptus xylan
Arabinose	nd	20.10	0.75
Glucose	2.35	5.95	3.25
Galactose	8.56	6.94	7.95
Xylose	89.09	65.21	62.70
Mannose	nd	nd	9.35
Galacturonic acid	nd	nd	0.25
Acetic acid	nd	1.80	15.75

nd not detected

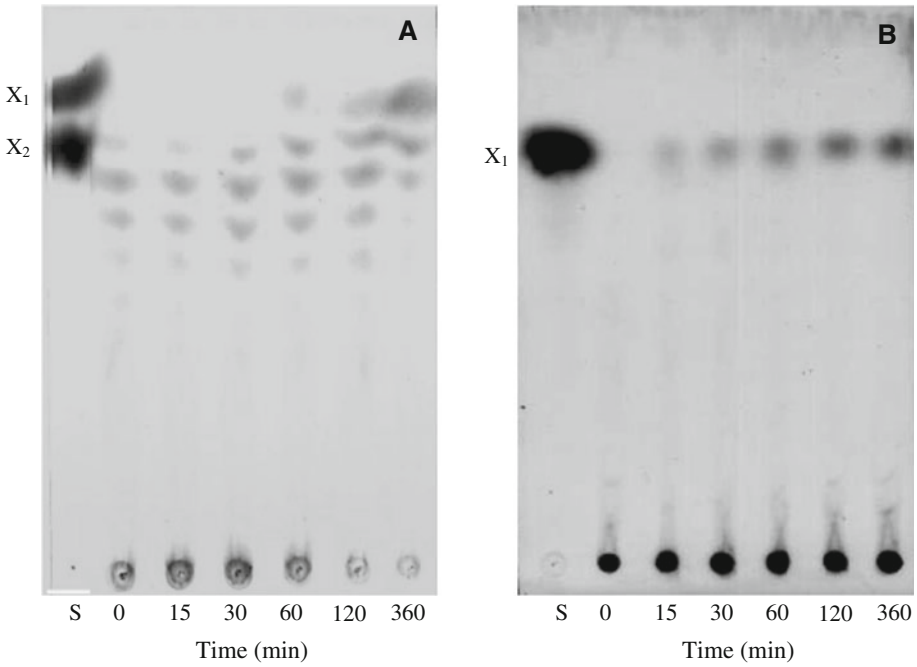

Fig. 4 Thin layer chromatography of the hydrolysis products of xylo-oligosaccharides (**a**) and xylan (**b**) by the purified β-D-xylosidase. Hydrolysis times were 0, 15, 30, 60, 120 and 360 min. Standards (S) were a mixture of 1 mg ml^{-1} of xylose (X_1) and xylobiose (X_2). The assays were performed at 70 °C and pH 4.5

Kinetic parameters

The purified enzyme exhibited a typical Michaelis–Menten kinetics, with K_m and V_{max} values for *p*-NP-xyl of 0.66 mM and 39 U (mg protein)$^{-1}$, respectively. This K_m value suggests higher affinity for *p*-NP-xyl for the β-xylosidase from *A. ochraceus* than that reported to *T. thermophilus* (Guerfali et al. 2008), *Scytalidium thermophilum* (Zanoelo et al. 2004) and *Aspergillus phoenicis* (Rizzatti et al. 2001).

Analysis of the hydrolysis products

Thin layer chromatography of the products resulting from the action of *A. ochraceus* purified β-D-xylosidase on xylo-oligosaccharides mixtures showed that the enzyme hydrolyzed these substrates until xylopentaose. It is possible be observed a reduction and/or disappearance in the concentration of large xylo-oligosaccharides, such as xylopentaose and xylotetraose, after 6 h; and an increase of smaller xylo-oligosaccharides, as xylobiose and xylose, as a consequence of these hydrolysis (Fig. 4a).

The *A. phoenicis* β-xylosidase hydrolyzed only up to xylotriose (Rizzatti et al. 2001), while the *S. thermophilum* β-xylosidase hydrolyzed up to xylotetraose (Zanoelo et al. 2004) and *Fusarium proliferatum* β-xylosidase hydrolyzed up to xylohexaose (Saha 2003b). According to Yan et al. (2008), β-xylosidases that hydrolyze up to xylopentose seem to be more applicable for xylan saccharifications.

Then, true β-D-xylosidases release xylose from xylobiose and xylo-oligosaccharides, a typical feature of an exo-type xylanolytic enzyme (Herrmann et al. 1997). However, the *A. ochraceus* β-xylosidase is able to release xylose from xylan (Fig. 4b), confirming the exo-character of the enzyme and showing a novel β-xylosidase that is able to hydrolyze xylan besides xylobiose and short xylo-oligosaccharides.

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