

Full Length Research Paper

## Xylanase from *Fusarium heterosporum*: Properties and influence of thiol compounds on xylanase activity

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The properties of xylanase purified from *Fusarium heterosporum* that was grown in barley-brewing residue under solid-state fermentation and the effects of thiol compounds on the reactivation of the metal ion-inhibited xylanase were investigated. Xylanase was purified to homogeneity by ion exchange chromatography, and its molecular mass was estimated to be 19.5 kDa by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The optimum pH for the xylanase was 5.0, and it was stable in acidic pH (4.5 to 5.5), where it retained more than 87% of its activity after 24 h. The optimum temperature was 50°C, and it had a half-life of 53 min at 45°C. The apparent Km and Vmax values for the xylanase were 5.63 mg/ml and 800 µmol/mg/min, respectively. Ba<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and the thiol compounds β-mercaptoethanol and dithiothreitol (DTT) enhanced xylanase activity, while Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup> strongly inhibited enzyme activity. Furthermore, this xylanase had an alternative mode of regulation in the presence of thiol compounds because the enzyme was able to recover its catalytic activity after inhibition by heavy metal ions.

**Key words:** Hemicellulase, fungus, solid-state fermentation, barley brewing residue, thiol compounds.

### INTRODUCTION

Xylan is the major component of hemicellulose, which is abundant in the cell walls of monocot plants and hardwoods. This heteropolymer is composed of xylose units that are connected by a β -1, 4 linkages in the backbone and can be found in the side chains of glucuronic acid, α-arabinose, acetyl, feruloyl and p-coumaroyl residues (Dutta et al., 2007). Due to the structural complexity of xylan, its complete hydrolysis

requires the synergistic action of several enzymes, including the endo-1, 4-β-D-xylanases (EC 3.2.1.8), which are important for cleavage of the glycosidic β-1, 4 bonds of the backbone of xylan to produce short-chain xylooligosaccharides of various lengths; β-xylosidase, α-arabinofuranosidase, α-methylglucuronidase and acetyl xylan esterase (Collins et al., 2005; Lafond et al., 2011). These xylanases are classified as Glycosyl Hydrolases

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**Abbreviations:** DTT, Dithiothreitol; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

(GH) based on their amino acid sequences (<http://www.cazy.org>) and belong to families 5, 7, 8, 10, 11 and 43 (Cai et al., 2011). Xylanase is an industrially important enzyme with many applications, such as the bioconversion of lignocellulosic material or agro-wastes to sugar, bio-bleaching in the paper industry, the improvement of texture and loaf volume of bread (Dobrev et al., 2007), the clarification of juice and wine, the improvement of the nutritional value of animal feed stock, and the extraction of plant oil, coffee and starch (Ahmed et al., 2012).

Filamentous fungi secrete higher levels of xylanase than bacteria and yeasts (Polizeli et al., 2005), and xylanases are produced by several fungi including *Aspergillus*, *Trichoderma*, *Penicillium*, *Aureobasidium*, *Fusarium*, *Chaetomium*, *Phanerochaete*, *Rhizomucor*, *Humicola* and *Talaromyces* (Kheng and Omar, 2005). Likewise, a large number of fungal xylanases have been studied and purified from organisms such as those of the *Trichoderma* sp. (Xiong et al., 2004; Zhou et al., 2011), *Aspergillus carneus* M34 (Fang et al., 2008) and some species of *Fusarium* (for example, *F. verticillioides* and *F. proliferatum*) (Saha, 2001, 2002). *Fusarium* fungi show a cosmopolitan distribution in soil and are associated with plants, as described by Nees in 1832 (Feldman et al., 2008). Some *Fusarium* species are considered plant pathogens [for example, *Fusarium solani* (Bogale et al., 2009) and *Fusarium graminearum* (Kikot et al., 2009)]; however, a few studies have reported the use of *Fusarium* species in the production of hemicellulases under solid-state fermentation.

Recently, a novel isolate of *Fusarium heterosporum* was obtained from local soil and showed the potential to produce xylanase using barley-brewing residue under solid-state fermentation. To our knowledge, no report in the literature concerning xylanase production by *F. heterosporum* exists. However, lipase production by this fungus has been widely studied. Some fungal xylanases have been reported to enhance their activity in the presence of thiol compounds such as dithiothreitol (DTT) and  $\beta$ -mercaptoethanol; however, these enzymes are strongly inhibited by certain heavy metal ions. Thus, in this study, the influence of thiol compounds on xylanase from *F. heterosporum* that had been inhibited by heavy-metal ions and the recovery of the catalytic activity of metal-ion-inhibited xylanase is reported.

## MATERIALS AND METHODS

### Fungal strain and culture conditions

The fungus *F. heterosporum* was newly isolated from local soil from Cascavel, Paraná state, Brazil and identified at the Instituto de Botânica (Institute of Botany), São Paulo, Brazil. The fungus strain was cultivated in potato-dextrose-agar (PDA) at 28°C for 7 days, and after growth, its spores were harvested in sterile, distilled water. Solid-state fermentation (SSF) was carried out by seeding  $10^5$  spores  $\text{ml}^{-1}$  of *F. heterosporum* on 5 g of various carbon sources (agro-industrial residue) and moistened with distilled water (1:1 w/v).

The cultures were incubated at 28°C for six days, and after incubation and growth, 50 ml of cold, sterile, distilled water was added and agitated in an orbital shaker (150 rpm) at 20°C for 60 min. The solid materials and fungal biomass were subsequently vacuum-filtered on filter paper, the filtrate was centrifuged at 5,000 x g for 10 min, and the clear supernatant was used to determine the enzymatic activity.

### Enzymatic assay and protein quantification

The xylanase activity was assayed by analyzing the reducing sugars that were released after incubation in a properly diluted enzyme solution containing 1% (w/v) birchwood xylan in 50 mM acetate buffer (pH 5.0) at 50°C for 10 min. The amounts of reducing sugars were determined using the dinitrosalicylic acid (DNS) method by Miller (1959). One unit of xylanase was defined as the amount of enzyme that was capable of releasing 1  $\mu\text{mol}$  of D-xylose and served as the standard under the assay conditions. The amount of protein was estimated by the Bradford method (1976) using bovine serum albumin as a standard, and an absorbance of 280 nm was used for monitoring the protein in the column eluates.

### Purification of xylanase from *F. heterosporum*

The crude extract of *F. heterosporum* was cultured using barley-brewing residue under solid-state fermentation (fungal strain and culture conditions) after incubation for six days. Subsequently, the culture was filtered and centrifuged at 5000 g for 10 min at 4°C. The supernatant was then dialyzed using 25 mM sodium acetate buffer (pH 5.5), loaded onto a diethylaminoethyl cellulose (DEAE-Cellulose) chromatographic column (2.0 x 20 cm) and eluted using a linear gradient of NaCl (0 to 0.5 M) in the same buffer. 5 ml fractions were collected at a flow rate of 1.0 ml/min, and those with xylanase activity were pooled, dialyzed overnight using 25 mM sodium acetate buffer (pH 5.5) at 4°C, applied onto a carboxymethyl-cellulose (CM-cellulose) chromatographic column (2.0 x 20 cm), and eluted using a linear gradient of NaCl (0 to 0.5 M) in the same buffer. 3 ml fractions were collected at a flow rate of 0.5 ml/min, and those with the highest activity were pooled, lyophilized and used for biochemical characterization of the purified xylanase.

### Effect of pH and temperature on enzyme activity and stability

The optimum pH for xylanase was determined to be 50°C using the McIlvaine buffer (1921) at pH values ranging from 2.2 to 8.0. The optimum temperature was determined by assaying for xylanase activity at temperatures ranging from 40 to 65°C. The thermal stability of xylanase was determined by pre-incubating the enzyme samples at 45, 50 and 55°C; aliquots were withdrawn at various time points, and the residual activity was measured under standard conditions.

### Electrophoresis

Samples of purified enzyme were subjected to 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970). The gels were silver-stained according to Blum et al. (1970), and the utilized molecular mass markers were as follows: phosphorylase B (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.1 kDa) and  $\alpha$ -lactalbumin (14.1 kDa).

### Kinetic parameters

The kinetic parameters of the purified enzyme were determined

**Table 1.** Effect of carbon source on xylanase production by *F. heterosporum*.

Carbon source	Xylanase activity <sup>a</sup> (U/ml)
Sugar cane bagasse	0.12 ± 0.01
Barley-brewing residue	6.94 ± 0.21
Peanut husks	0.66 ± 0.02
Passion fruit husks	0.66 ± 0.05
Wheat bran	3.58 ± 0.27
Corn straw	0.82 ± 0.03

<sup>a</sup>Mean and standard deviation of three replications.

**Table 2.** Summary of the purification of xylanase from *F. heterosporum*.

Purification step	Total protein (mg)	Total activity (units)	Specific activity (U/mg prot.)	Yield (% recovery)	Purification (fold)
Crude extract	45.11	2,526.55	56.09	100	1
DEAE-Cellulose	18.91	1,781.20	94.21	72.8	1.7
CM Cellulose	1.09	139.29	127.78	21.1	2.3

Total U: U ml<sup>-1</sup> × volume of extract; total protein: mg ml<sup>-1</sup> × volume of extract.

using birchwood xylan as the substrate for the xylanase assay, and the concentrations of xylan ranged from 1 to 20 mg/ml. The  $K_m$  and  $V_{max}$  values were determined using the Lineweaver-Burk plot (1934).

#### Effects of metal ions and other compounds on xylanase activity

Salts [BaCl<sub>2</sub>, CaCl<sub>2</sub>, CoCl<sub>2</sub>, HgCl<sub>2</sub>, KCl, MgCl<sub>2</sub>, NaCl, PbCl<sub>2</sub>, ZnCl<sub>2</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] and other compounds [β-mercaptoethanol, dithiothreitol (DTT), cystine, L-cysteine, ethylenediaminetetraacetic acid (EDTA), iodoacetamide and sodium dodecyl sulfate (SDS)] were also tested. The metal ions and compounds (1 or 5 mM) were pre-incubated with the enzyme for 15 min. After incubation, an aliquot was withdrawn and chilled on ice, and the hydrolytic activity was determined by the standard assay with xylan as the substrate.

#### Influence of thiol compounds on the recovery of heavy metal ion-inhibited xylanase activity

Xylanase was pre-incubated for 10 min with heavy metal ions (Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>) at 0.5 mM to promote enzymatic inhibition. Subsequently, the recovery of xylanase activity was assayed by incubating the enzyme with thiol compounds (DTT and β-mercaptoethanol; 0.5 mM) for 20 min and then measuring the enzyme activity under standard conditions. The protective effect of the thiol compounds on metal-inhibited xylanase was assayed by pre-incubating the enzyme with DTT and β-mercaptoethanol (0.5 mM) 15 min before the addition of heavy metal ions (Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>). Then, the enzymatic activity was assayed under standard conditions as described in 'enzymatic assay and protein quantification'. The activity recovery of xylanase was observed against a control; that is, the absence of the heavy metal ions).

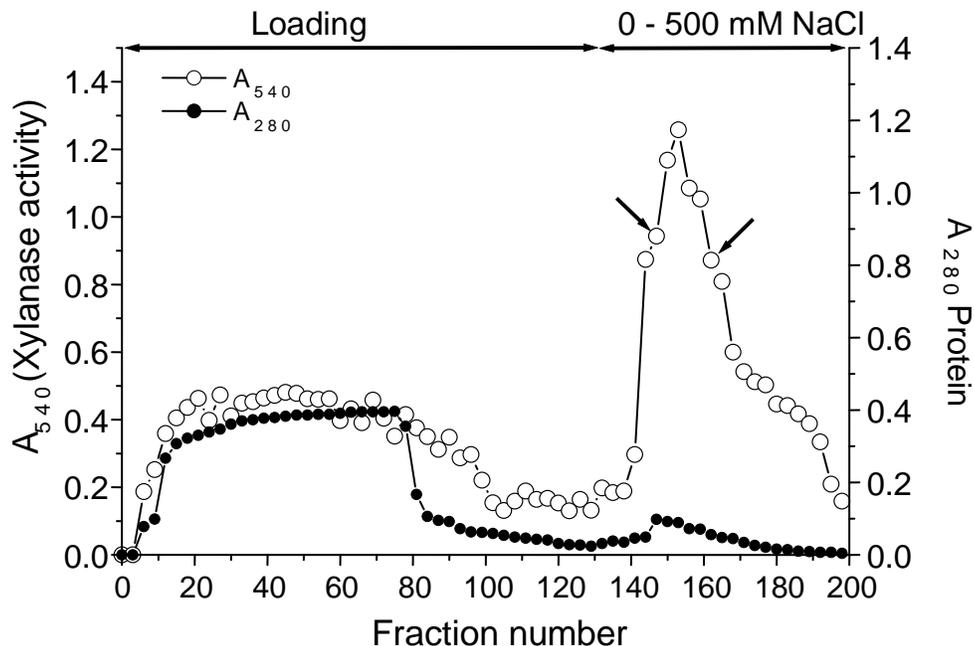
## RESULTS AND DISCUSSION

### Effect of an alternative carbon source on the production of xylanase

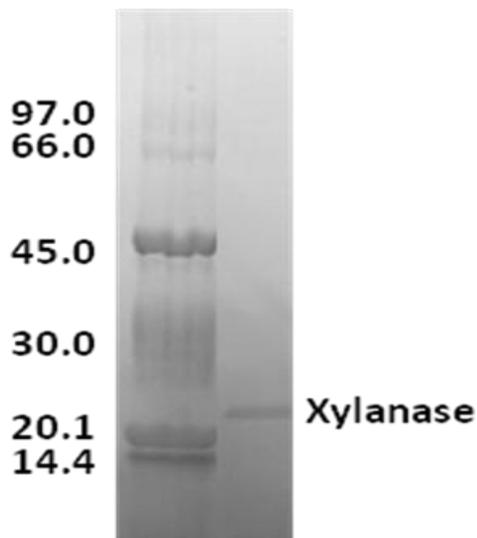
The ability of the new strain of soil *F. heterosporum* to produce xylanase under SSF was studied using a variety of carbon sources such as sugar cane bagasse, barley-brewing residue, peanut husks, passion fruit husks, wheat bran and corn straw. *F. heterosporum* was capable of growing and producing xylanase (6.94 U/ml) in a culture containing barley-brewing residue, which is an inexpensive substrate that is disposed of in large scale from the brewing industry in Brazil. This result indicates that a substrate such as barley-brewing residue, which is rich in cellulosic and non cellulosic polysaccharides, can be efficiently used to induce xylanase production by *F. heterosporum* (Table 1). Similarly, Silva et al. (2005) have obtained high yields of xylanase using different wastes such as corncob, green grass, dried grass, corn straw and wheat bran as raw materials for SSF using the thermophilic fungus *Thermoascus aurantiacus*.

### Purification of xylanase

The xylanase from *F. heterosporum* was purified to apparent homogeneity by ion exchange chromatography, and a summary of the purification procedure is presented in Table 2. Xylanase was purified 2.3-fold with a recovery of 21.1% by two chromatographic, ion-exchange purification steps: a DEAE-cellulose chromatographic column



**Figure 1.** Chromatographic profiles of the xylanase that was produced by *F. heterosporum* using a CM-Cellulose column. The collected fractions are indicated by arrows ( $\rightarrow$ ).



**Figure 2.** Polyacrylamide gel electrophoresis 10% SDS-PAGE. The xylanase that was produced by *F. heterosporum* is shown in lane B; and molecular mass markers are shown in lane A: phosphorylase B (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (21.1 kDa) and  $\alpha$ -lactalbumin (14.1 kDa).

followed by a CM-cellulose chromatographic column. The enzyme eluted as a single peak with 75 mM NaCl (Figure 1), and this peak showed a single band with an apparent

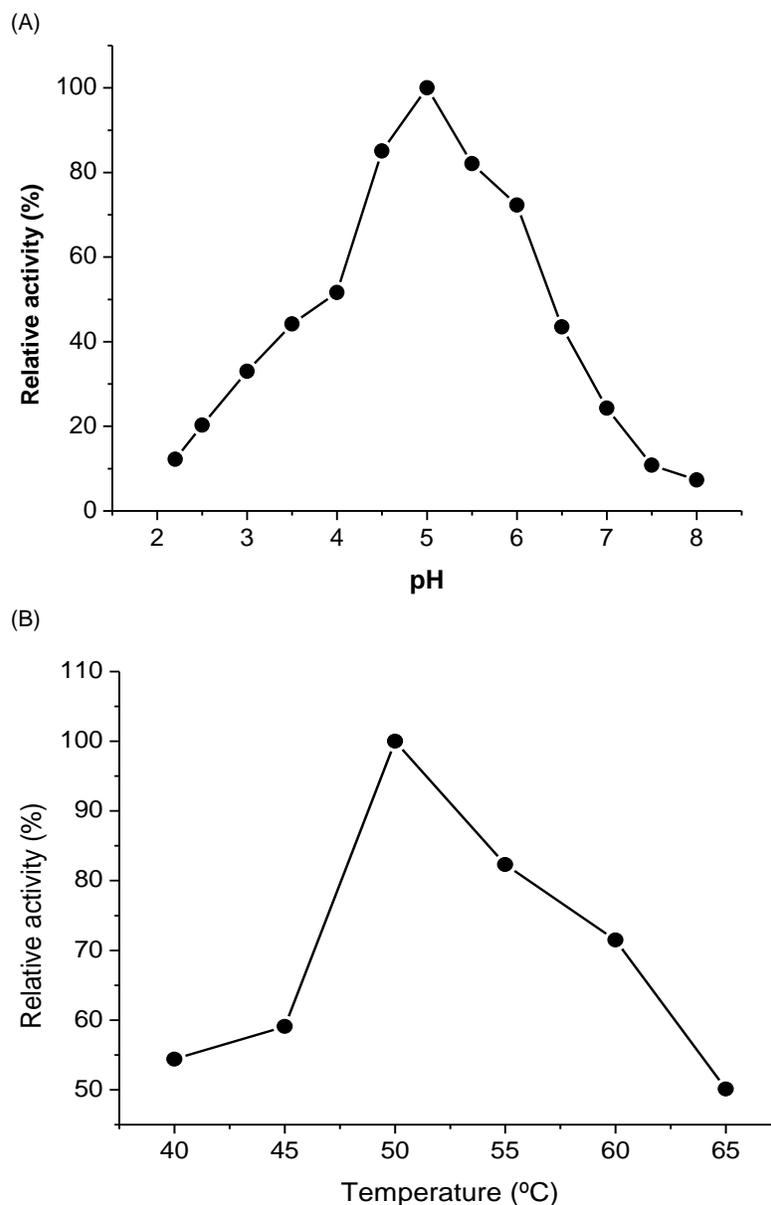
molecular mass of 19.5 kDa by 10% SDS-PAGE (Figure 2). The molecular mass of this xylanase was lower than that of xylanases produced from *Thermomyces lanuginosus* SS-8 (23.79 kDa) (Shrivastava et al., 2011) and *Penicillium occitanis* Pol6 (30 kDa) (Driss et al., 2012).

#### Effect of pH on the activity and stability of xylanase

The optimum pH of the purified xylanase from *F. heterosporum* was 5.0 (Figure 3A). The enzyme was active within the acidic pH range of 4.5 to 5.5 and retained more than 87% of its activity after 24 h of incubation (Figure 4A). This optimum pH value was similar to that described for xylanase produced by *F. solani* SYRN7 (Arabi et al., 2011) and the optimum pH for xylanases of other *Fusarium* species has been reported to be within the range of 4.5 to 8.0; for example, *Fusarium oxysporum* f. sp. *lycopersici*, pH 4.5 (Ruiz et al., 1997) and *F. solani*, pH 8.0 (Bakri et al., 2013).

#### Effect of temperature on the activity and stability of xylanase

The xylanase exhibited optimum activity at a temperature of 50°C (Figure 3B), and the enzyme was stable within a temperature range of 50 to 55°C for 15 min. However, at 45°C, the half-life ( $t_{1/2}$ ) of the xylanase was 53 min (Figure 4B). Likewise, xylanases from *Aspergillus niger* (Lopes et



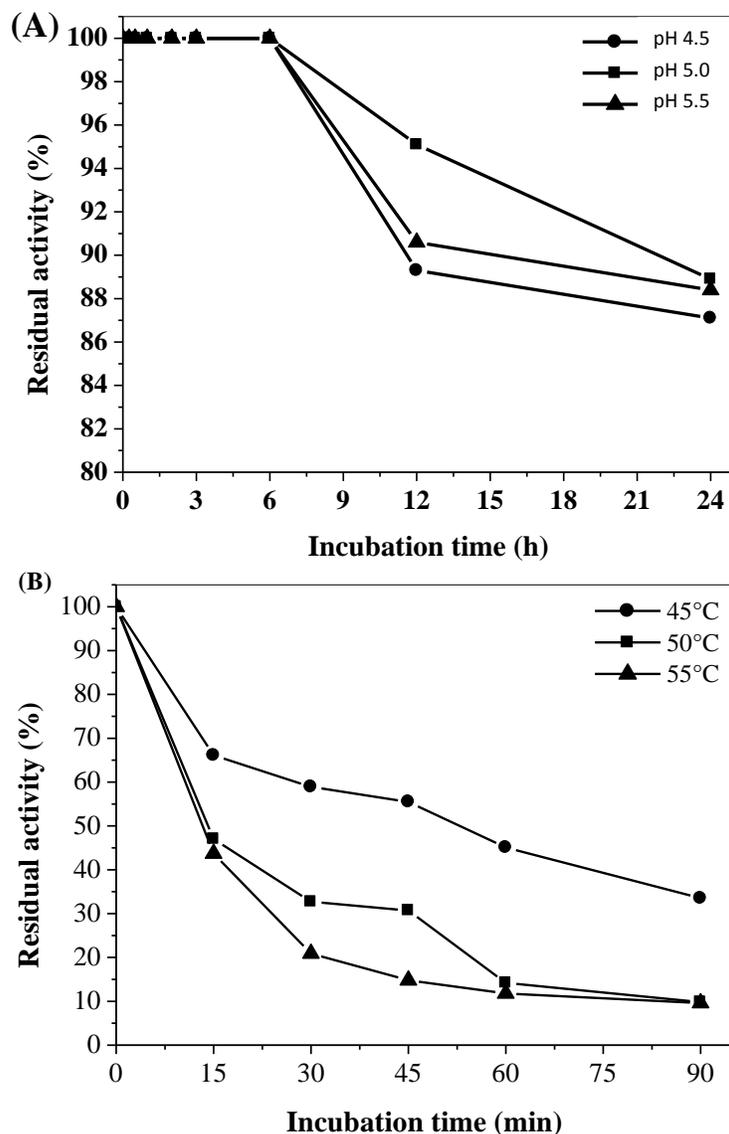
**Figure 3.** Effect of pH (A) and temperature (B) on the activity of xylanase from *F. heterosporum*.

al., 2013) and *A. carneus* M34 (Fang et al., 2008) showed optimum activity at 50°C. Interestingly, similar result regarding optimum temperature was obtained with xylanase from *F. verticillioides* NRRL 26518 (Saha, 2001). In the contrast, xylanase from *F. solani* showed optimum temperature at 25°C (Bakri et al., 2013).

### Kinetic parameters

The apparent  $K_m$  and  $V_{max}$  values for this xylanase were found to be 5.63 mg/ml and 800  $\mu\text{mol}/\text{mg}/\text{min}$ , respectively; when birchwood xylan was used as the

substrate. The  $K_m$  value obtained for xylanase from *F. heterosporum* shows that the enzyme has a higher affinity for xylan than xylanases produced from *Streptomyces cyaneus* SN32 ( $K_m = 11.1$  mg/ml) (Ninawe et al., 2008) and *Humicola grisea* var. *thermoidea* ( $K_m = 10.87$  mg/ml) (Lucena-Neto and Ferreira, 2004). The products released after hydrolysis of birchwood xylan were analyzed by thin layer chromatography and were found to be xylooligosaccharides (X2 to X5) but no free xylose, indicating that this enzyme is typically an endo-xylanase (results not shown). Similar results have been obtained for xylanase from *F. oxysporum* f. sp. *ciceris* (Jorge et al., 2005).



**Figure 4.** pH (A) and thermal stability (B) of xylanase from *F. heterosporum*.

#### Effect of metal ions and other compounds on xylanase activity

Metallic ions and some compounds influenced the xylanase activity of *F. heterosporum* (Table 3).  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{Mg}^{2+}$  (1 mM) increased the enzyme activity by 20, 28 and 38%, respectively. However,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Hg}^{2+}$  were the most effective inhibitors (27, 39 and 74% inhibition, respectively). This inhibition by heavy-metal ions may occur due to complex formation with reactive groups of the enzyme, for example, metals of group IIb exhibit high affinity for SH,  $\text{CONH}_2$ ,  $\text{NH}_2$ ,  $\text{COOH}$ ,  $\text{PO}_4$ , and this effect is similar to that of mercaptides (Khasin et al., 1993). Furthermore, the heavy metal ions may bind non-specifically to regions other than the cysteine thiol group of the enzyme to induce inhibition (Krajewska,

2008). SDS also inhibited (70%) enzyme activity. Similarly, a total loss of xylanase activity from *Penicillium glabrum* was observed in the presence of SDS, indicating that hydrophobic interactions may be important in maintaining the structure of xylanase (Knob et al., 2013). Among the amino acids tested, L-cysteine and cystine did not increase enzyme activity.

Interestingly, compounds containing thiol groups such as  $\beta$ -mercaptoethanol and DTT enhanced the activity of xylanase from *F. heterosporum* by 55% and 54%, respectively, and a similar effect was observed for xylanase from *Termitomyces* spp. in the presence of reducing agents (Faulet et al., 2006). Likewise, crude xylanase from *T. longibrachiatum* and *A. niger* were activated by L-cysteine, DTT and  $\beta$ -mercaptoethanol (Medeiros et al., 2003). In contrast, DTT (5 mM) inhibited

**Table 3.** Effects of various compounds on the activity of xylanase from *F. heterosporum*.

Compound	Residual activity (%)	
	1 mM	5 mM
Control	100	100
BaCl <sub>2</sub>	128 ± 3.1	132 ± 2.8
CaCl <sub>2</sub>	120 ± 1.8	146 ± 1.4
CoCl <sub>2</sub>	111 ± 0.2	112 ± 0.4
HgCl <sub>2</sub>	26 ± 0.4	6 ± 0.3
KCl	112 ± 2.6	115 ± 2.3
MgCl <sub>2</sub>	138 ± 3.3	148 ± 3.7
NaCl	110 ± 2.6	117 ± 2.4
PbCl <sub>2</sub>	61 ± 0.4	52 ± 0.5
ZnCl <sub>2</sub>	73 ± 2.0	62 ± 2.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	118 ± 0.4	118 ± 0.4
β-Mercaptoethanol	133 ± 2.7	155 ± 2.6
DTT	141 ± 2.7	154 ± 2.3
Cystine	99 ± 2.7	82 ± 2.4
L-cysteine	97 ± 2.4	81 ± 2.5
Iodoacetamide	84 ± 1.9	82 ± 1.7
SDS	30 ± 1.5	12 ± 1.3
EDTA	91 ± 1.0	89 ± 1.5

Results are expressed as a percentage of the control, which is 100%. Xylanase used: 2 U ml<sup>-1</sup>.

<sup>a</sup>Residual activity is expressed as a percentage of the control.

xylanase from *F. proliferatum* NRRL, 26517 by 23% (Saha, 2002).

### Influence of thiol compounds on xylanase activity

In this study, we observed that the activity of xylanase from *F. heterosporum* was inhibited by metal ions (Hg<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>), but this activity was subsequently restored to control levels after exposure to DTT and β-mercaptoethanol (Figure 5A). Furthermore, our studies show the protective effect of thiol compounds on this enzyme when these compounds are previously pre-incubated with heavy metals (Figure 5B). These results suggest that compounds such as β-mercaptoethanol and DTT can interact with the enzyme at a higher affinity and prevent the formation of heavy metal - enzyme complexes. This behavior can be explained by a model proposing the non-essential enzyme activation of asparaginase from *Erwinia carotovora* (Warangkar and Khobragade, 2010). When β-mercaptoethanol and DTT are added, they bind to another site other than the site of the enzyme substrate, a conformational change in the enzyme results in decreased binding affinity for the heavy metal, and the enzyme can recover its catalytic activity. Therefore, the finding of a xylanase with an alternative form of regulation due to a higher affinity for thiol

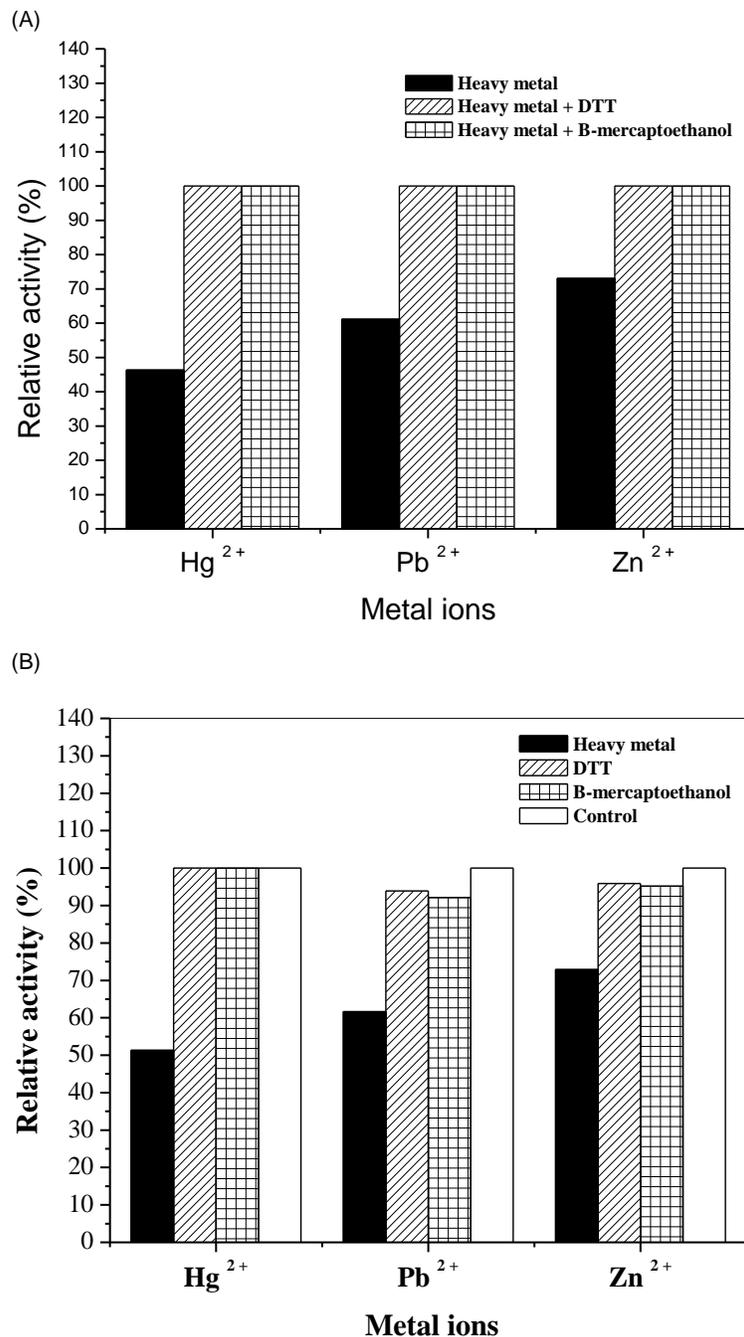
compounds will be particularly useful because of the potential to improve and promote the recovery of the catalytic efficiency of the enzyme after it has been inhibited by metallic ions.

### Conclusion

The new isolate *F. heterosporum* proved to be a promising strain in producing xylanase using a low-cost, alternative substrate such as barley-brewing residue. Interestingly, the enzyme showed the versatility of interacting with thiol compounds and promoting the recovery of the catalytic efficiency of xylanase that had been inhibited by heavy metal ions, which thereby accentuates the biotechnological potential of this enzyme.

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**Figure 5.** Recovery of heavy metal-inhibited xylanase activity by thiol compounds (A). Protective effect of the thiol compounds on heavy metal-inhibited xylanase (B). The control ( $\square$ ) is the xylanase activity in the absence of a heavy metal ion. <sup>a</sup>Relative activity is expressed as a percentage of the control.

## REFERENCES

- Ahmed S, Imdad SS, Jamil A (2012). Comparative study for the kinetics of extracellular xylanases from *Trichoderma harzianum* and *Chaetomium thermophilum*. *Electron. J. Biotechnol.* 15:3.
- Arabi MIE, Bakri Y, Jawhar M (2011). Extracellular Xylanase Production by *Fusarium* species in Solid State Fermentation. *Pol. J. Microbiol.* 60(3):209-212.
- Bakri Y, Jawhar M, Arabi MI (2013). Xylanase production by *Fusarium solani* in solid state fermentation. *Res. Biotechnol.* 4(1):31-37.
- Blum H, Beier H, Gross B (1987). Improved silver staining of plant proteins, RNA and DNA in polyacrylamide gels. *Electrophoresis* 81:93-99.
- Bogale M, Steenkamp ET, Wingfield MJ, MJWingfield BD (2009).

- Diverse *Fusarium solani* isolates colonise agricultural environments in Ethiopia. *Eur. J. Plant. Pathol.* 124:369-378.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
- Cai H, Shi P, Bai Y, Huang H, Yuan T, Yang P, Luo H, Meng K, Yao (2011). A novel thermoacidophilic family 10 xylanase from *Penicillium pinophilum* C1. *Process. Biochem.* 46:2341-2346.
- Collins T, Gerday C, Feller G (2005). Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol. Rev.* 29:3-23.
- Dobrev GT, Pishtiyski IG, Stanchev VS, Mircheva R (2007). Optimization of nutrient medium containing agricultural wastes for xylanase production by *Aspergillus niger* B03 using optimal composite experimental design. *Bioresour. Technol.* 98:2671-2678.
- Driss D, Bhiri F, Siela M, Ghorbel R, Chaabouni SE (2012). Purification and properties of a thermostable xylanase GH 11 from *Penicillium occitanis* Pol6. *Appl. Biochem. Biotechnol.* 168(4):851-863.
- Dutta T, Sengupta R, Sahoo RS, Sinha R, Bhattacharjee A, Ghosh S (2007). A novel cellulase free alkaliphilic xylanase from alkali tolerant *Penicillium citrinum*: production, purification and characterization. *Lett. Appl. Microbiol.* 44:206-211.
- Fang H, Chang S, Lan C, Fang TJ, (2008). Purification and characterization of a xylanase from *Aspergillus carneus* M34 and its potential use in photoprotectant preparation. *Process. Biochem.* 43: 49-55.
- Faulet BM, Niamke S, Gonnety JT, Kouamé LP (2006). Purification and biochemical properties of a new thermostable xylanase from symbiotic fungus, *Termitomyces* sp. *Afr. J. Biotechnol.* 5: 273-282.
- Feldman TS, O'Brien H, E, Arnold AE (2008). Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists. *Microb. Ecol.* 56:742-750.
- Jorge I, Rosa O, Navas-Cortes JA, Jimenez-Díaz RM, Tena M (2005). Extracellular xylanases from two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*: enzyme production in culture and purification and characterization of a major isoform as an alkaline endo-b-(1,4)-xylanase of low molecular weight. *A. Van. Leeuw. J. Microb.* 88:49-59.
- Khasin A, Alchanati I, Shoham Y (1993). Purification and characterization of a thermostable xylanase from *Bacillus stearothermophilus* T-6. *Appl. Environ. Microbiol.* 59:1725-1730.
- Kheng PP, Omar IC (2005). Xylanase production by a local fungal isolate, *Aspergillus niger* USM AI 1 via solid state fermentation using palm kernel cake (PKC) as substrate. *Songklanakarin J. Sci. Technol.* 27 (2):325-336.
- Kikot GE, Hours RA, Alconada TM (2009). Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. *J. Basic Microb.* 49 (3):231-241.
- Knob A, Beitel SM, Fortkamp D, Terrasan CRF, Almeida AF (2013). Production, Purification, and Characterization of a Major *Penicillium glabrum* Xylanase Using Brewer's Spent Grain as Substrate. *Biomed. Res. Int.* 728735.
- Krajewska B (2008). Mono- (Ag, Hg) and di- (Cu, Hg) valent metal ions effects on the activity of jack bean urease. Probing the modes of metal binding to the enzyme. *J. Enzyme Inhib. Med. Chem.* 23(4):535-542.
- Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
- Lafond M, Tauzin A, Desseaux V, Bonnin E, Ajandouz E, Giardina T (2011). GH10 xylanase D from *Penicillium funiculosum*: biochemical studies and xylooligosaccharide production. *Microb. Cell Fact.* 10:20.
- Lineweaver H, Burk D (1934). The determination of the enzyme dissociation constants. *Am. Chem. Soc.* 56:658-666.
- Lopes AM, Valeri D, Pessoa-Júnior A (2013). Comparison of kinetic characteristics of xylanases from *Aspergillus niger* and *Trichoderma* sp. with pH and temperature baking process parameters. *Afr. J. Biotechnol.* 12(19):2640-2645.
- Lucena-Neto SA, Ferreira EXF (2004). Purification and characterization of a new xylanase from *Humicola grisea* var. *thermoidea*. *Braz. J. Microbiol.* 35:86-90.
- McIlvaine T C, (1921). A buffer solution for colorimetric comparison. *J. Biol. Chem.* 49:183- 186.
- Medeiros RG, Hanada R, Ferreira EXF (2003). Production of xylan-degrading enzymes from Amazon forest fungal species. *Int. Biodeter. Biodegr.* 52:97-100.
- Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31:426-428.
- Ninawe S, Kapoor M, Kuhad RC (2008). Purification and characterization of extracellular xylanase from *Streptomyces cyaneus* SN32. *Bioresour. Technol.* 99:1252-1258.
- Polizeli MLTM, Rizzatti ACS, Monti R, Terenzi HF, Jorge JA, Amorim DS (2005). Xylanases from fungi: properties and industrial applications. *Appl. Microbiol. Biotechnol.* 67:577-591.
- Ruiz MC, Di Pietro A, Roncero MI G (1997). Purification and characterization of an acidic endo- $\beta$ -1,4-xylanase from the tomato vascular pathogen *Fusarium oxysporum* f. sp. *lycopersici*. *FEMS Microbiol. Lett.* 148 (1):75-82.
- Saha BC (2001). Xylanase from a newly isolated *Fusarium verticillioides* capable of utilizing corn fiber xylan. *Appl. Microbiol. Biotechnol.* 56:762-766.
- Saha BC (2002). Production, purification and properties of xylanase from a newly isolated *Fusarium proliferatum*. *Process. Biochem.* 37:1279-1284.
- Saha BC (2003). Hemicellulose bioconversion. *J. Ind. Microbiol. Biotechnol.* 30:279-291.
- Shrivastava S, Shukla P, Mukhopadhyay K (2011). Purification and preliminary characterization of a xylanase from *Thermomyces lanuginosus* strain SS-8. *Biotechnology* 1:255-259.
- Silva R, Lago ES, Merheb CW, Macchione MM, Park YK, Gomes E (2005). Production of xylanase and CMCase on solid state fermentation in different residues by *Thermoascus aurantiacus* Miehe. *Braz. J. Microbiol.* 36(3):235-241.
- Warangkar SC, Khobragade DN (2010). Purification, characterization, and effect of thiol compounds on activity of the *Erwinia carotovora* L-asparaginase. *Enzyme Res.* 1-10.
- Xiong H, Weymann N, von Leisola M, Turunen O (2004). Influence of pH on the production of xylanases by *Trichoderma reesei* Rut C-30. *Process. Biochem.* 39:731-736.
- Zhou P, Zhu H, Yan Q, Katrolia P, Jiang Z (2011). Purification and properties of a Psychrotrophic *Trichoderma* sp. xylanase and its gene sequence. *Appl. Biochem. Biotechnol.* 164:944-956.