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Research article

Agroindustrial biomass for xylanase production by *Penicillium chrysogenum*: Purification, biochemical properties and hydrolysis of hemicelluloses

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ABSTRACT

Background: In this work, the xylanase production by *Penicillium chrysogenum* F-15 strain was investigated using agroindustrial biomass as substrate. The xylanase was purified, characterized and applied in hemicellulose hydrolysis.
Results: The highest xylanase production was obtained when cultivation was carried out with sugar cane bagasse as carbon source, at pH 6.0 and 20°C, under static condition for 8 d. The enzyme was purified by a sequence of ion exchange and size exclusion chromatography, presenting final specific activity of 834.2 U·mg·prot^{-1}. The molecular mass of the purified enzyme estimated by SDS-PAGE was 22.1 kDa. The optimum activity was at pH 6.5 and 45°C. The enzyme was stable at 40°C with half-life of 35 min, and in the pH range from 4.5 to 10.0. The activity was increased in the presence of Mg^{+2} and Mn^{+2} and reducing agents such as DTT and β-mercaptoethanol, but it was reduced by Cu^{+2} and Pb^{+2}. The xylanase presented K_m of 2.3 mM and V_{max} of 731.8 U·mg·prot^{-1} with birchwood xylan as substrate. This xylanase presented differences in its properties when it was compared to the xylanases from other *P. chrysogenum* strains.
Conclusion: The xylanase from *P. chrysogenum* F-15 showed lower enzymatic activity on commercial xylan than on hemicellulose from agroindustry biomass and its biochemistry characteristics, such as stability at 40°C and pH from 4.0 to 10.0, shows the potential of this enzyme for application in food, feed, pulp and paper industries and for bioethanol production.

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1. Introduction

Lignocellulosic materials are mainly composed by cellulose, hemicellulose and lignin, and smaller portion of pectin, waxes and mineral salts [1]. Hemicellulose is a class of structurally variable heteropolysaccharides that, in association with cellulose and lignin, aid in the adhesion and cohesion of plant fibers [2]. Xylan is the most abundant hemicellulose in lignocellulosic biomass from many agroindustrial wastes. These polysaccharides represent up to 50% of tissues from monocots, grasses and cereals [3]. It is composed by a backbone of (1–4) linked β-D-xylanopyranosyl residues, with substituents such as glucuronosyl, arabinosyl and acetyl groups, among others [4,5,6,7]. The complete and efficient xylan biodegradation requires the action of several enzymes, known as xylanolytic system. The main enzyme is endo-1,4-β-xylanase (1,4-β-D-xylan xylanohydrolases, EC 3.2.1.8) which hydrolyze the xylan backbone, reducing the degree of polymerization and releasing smaller oligosaccharides and xylobiose [5,7,8,9]. Xylanases have been used for many biotechnological applications such as treatment of juices, beer and wine; in bakery industries; for production of xylooligosaccharides (XOS) and xylitol; improvement of animal feed digestibility; and bleaching of cellulosic pulp. These enzymes also have been used to degrade polysaccharides from lignocellulosic materials to produce second-generation bioethanol [3,6,7,10,11,12,13]. Fungi are important xylanolytic enzyme producers, because they secrete the

enzymes to the external medium, and the levels are much higher than those verified in yeasts and bacteria [14,15]. Several fungal producers such as *Aspergillus niger*, *Humicola insolens*, *Termonospora fusca*, *Trichoderma reesei*, *Trichoderma longibrachiatum* and *Trichoderma koningii* have been manipulated to produce commercial xylanases [11, 16]. The production of xylanolytic enzymes by *Penicillium* has also been explored in many species [17] such as *Penicillium purpurogenum*, *Penicillium janthinellum*, *Penicillium funiculosum*, *Penicillium herquei*, and *Penicillium capsulatum* [18,19,20,21,22]. Some *Penicillium* species such as *Penicillium sclerotiorum* [23], *Penicillium janczewskii* [24] and *Penicillium glabrum* [25] have been cultivated in brewer's spent grains for xylanolytic enzymes production. *Penicillium chrysogenum* was previously reported as producer of xylanase and other lignocellulosic enzymes with potential application to degradation winery-derived biomass waste [26,27]. Biomass degradation studies are required due to the high demand for alternative treatments for agricultural and industrial wastes. This degradation is important because it allows the reuse of these materials and the rational use of the degradation by-products. During a screening trial, a *P. chrysogenum* strain demonstrated to be a notable xylanase producer. The aim of this work was to investigate the behavior of a *P. chrysogenum strain* in the agroindustrial wastes biodegradation for xylanases production. The study was performed with wastes from different agroindustry, an industrial sector with large waste production. In addition to the production, a xylanase purification protocol was stablished, and its physicochemical properties were determined. This knowledge can allow a targeted enzyme application in which the enzyme characteristics are compatible and adequate to be applied in specific industrial sectors.

2. Materials and methods

2.1. Microorganisms and growth

P. chrysogenum F-15 strain was isolated from soil of caatinga biome located in Northeast Brazil (Floresta Nacional Contendas do Sincorá, Bahia). It is available in the Culture Collection of Environmental Studies Center – CEA/UNESP, Brazil. Conidia production was carried out on solid Vogel medium [28] containing 3.0% (*w/v*) wheat bran and 1.5% (*w/v*) agar at 25°C for 7 d and inoculum corresponded to a 5.10^7 conidia·mL^{-1} suspension. Xylanolytic strains from previous studies such as *Aspergillus giganteus* [29], *Aspergillus versicolor* [30], *Trichoderma inhamatum* [31], *P. janczewskii* [24] and *P. sclerotiorum* [23] were used for hydrolysis hemicelluloses comparison.

2.2. Submerged cultivation

Cultures of *P. chrysogenum* were prepared in Vogel medium [28] containing 1% (*w/v*) of each substrate and the pH was adjusted for each experiment, as below. Erlenmeyer flasks (125 mL) containing 25.0 mL of medium were inoculated with 1.0 mL of the conidia suspension and incubated at different conditions, as indicated subsequently. All experiments were performed in triplicate and the results were mean values. The other strains were cultivated under conditions previously established for high xylanase production [23,24, 29,30,31].

2.3. Enzyme preparations and assays

Cultures were harvested by filtration and the culture filtrate was used to assay extracellular enzyme activity and protein. The mycelium was washed with water, frozen and ground with sand in 0.05 M sodium phosphate buffer pH 6.0. Then, the samples were centrifuged (3.900 xg, Mach 1.6, Sorvall, Kendro, Hanau, Germany) at 4°C and the supernatants were used as intracellular protein source.

2.4. Xylanase activity and protein assays

Xylanase activity was determined with 1% (*w/v*) of substrates prepared in 0.05 M sodium phosphate buffer pH 6.0 at 50°C, according to Bailey et al. [32]. Substrates were commercial beechwood, birchwood and oat spelts xylans from Sigma–Aldrich Chemical Co (St. Louis, MO, USA) and in-house extracted hemicellulose from sugar cane bagasse, brewer's spent grain and corncobs [33]. After enzymatic reaction, reducing sugars were quantified with dinitro salicylic (DNS) acid [34] and the absorbance was read at 540 nm (spectrophotometer Ultrospec 3000, Amersham Pharmacia Biotech, Little Chalfont, UK). One unit of enzyme activity (U) was defined as the amount of enzyme which releases 1 μmol of reducing sugars per min. Specific activities were expressed as enzyme units per milligram of protein (U·mg·prot^{-1}). Protein was determined by the Lowry method [35] using bovine serum albumin (Sigma–Aldrich) as standard. During purification chromatography, protein in the fractions was followed by reading absorbance at 280 nm.

2.5. Xylanase production on different substrates and influence of particle size

Vogel medium [28] was supplemented with 1.0% (*w/v*) glucose, xylose, Avicel®, carboxymethyl cellulose (CMC), xylan from oat spelts, oat bran, wheat bran, sugar cane bagasse, brewer's spent grain, orange peel and corncobs. The influence of particle size on enzyme production was verified with sugar cane bagasse and brewer's spent grain particulate in the following ranges: higher than 10 mesh, between 10 and 18 mesh, and between 18 and 45 mesh.

2.6. Effect of culture conditions, pH and temperature on xylanase production

Cultivation was carried out under static (15 d) and shaking (9 d, 120 rpm, incubator shaker MA 830/A, Marconi, Piracicaba, SP, Brazil) conditions. The effect of initial pH on enzyme production was analyzed in the range from 2.0 to 10.0; and the influence of temperature was verified from 15 to 30°C.

2.7. Xylanase purification

The culture filtrate was dialyzed overnight against 0.05 M sodium acetate buffer pH 4.5 at 4°C. The dialyzed sample was applied to cationic exchange chromatography in a CM Sephadex C-50 column (17.0 × 2.2 cm) equilibrated in the same buffer. Proteins were eluted with a 0.0 to 0.7 M linear NaCl gradient. Fractions exhibiting xylanase activity were pooled, freeze-dried (Thermo Savant, Micro Modulyo, Waltham, MA, USA), suspended in 3 mL of ammonium acetate buffer 0.05 M pH 4.5 and submitted to size exclusion chromatography on a Sephadex G-100 column (64.0 × 2.6 cm) equilibrated in the same buffer. Fractions exhibiting xylanase activity were pooled and the resulting sample was submitted to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). All purification steps were carried out at 4°C, with 10% (*v/v*) glycerol (Merck, Darmstadt, Germany) in solution and 1 mM dithiothreitol (DTT) (Sigma-Aldrich) were added to the samples and solutions each 24 h.

2.8. Enzyme characterization

2.8.1. Electrophoresis

SDS-PAGE was performed [36] with 8 to 18% (*w/v*) polyacrylamide gels. Low molecular weight proteins kit (GE Healthcare UK Limited Little Chalfont, Buckinghamshire, UK) containing phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) e α-lactalbumin (14.4 kDa) was

Table 1
Influence of some carbohydrates and agro-industrial biomass on xylanase production by *Penicillium chrysogenum* F-15.

Substrate	Intracellular protein (mg)	Xylanase activity (U·mL^{-1})	Specific xylanase activity (U·mg·prot^{-1})
Glucose	1.42 ± 0.17	0.15 ± 0.01	0.44 ± 0.01
Xylose	2.57 ± 0.07	3.59 ± 0.60	6.59 ± 0.22
Avicel	0.09 ± 0.01	ND	ND
CM-cellulose	0.34 ± 0.06	ND	ND
Oat spelts xylan	0.25 ± 0.02	0.47 ± 0.13	0.73 ± 0.14
Brewer's spent grain	1.87 ± 0.18	2.28 ± 0.06	3.83 ± 0.04
Corncobs ground	1.26 ± 0.01	2.74 ± 0.53	6.11 ± 0.43
Oat bran	1.47 ± 0.02	4.56 ± 0.19	4.96 ± 0.48
Orange peel	1.62 ± 0.06	0.24 ± 0.02	0.12 ± 0.02
Sugar cane bagasse	1.12 ± 0.06	3.33 ± 0.14	8.75 ± 0.10
Wheat bran	2.37 ± 0.14	1.63 ± 0.24	2.21 ± 0.18

Data are mean and standard deviation of three cultures. Cultivation was carried out under static condition in Vogel medium pH 6.5 supplemented with 1% (w/v) of each substrate for 7 d at 28°C. ND: activity not detectable under the assay conditions. CM-cellulose: carboxy methyl cellulose.

Table 2
Influence of particle size of sugar cane bagasse on xylanase production by *Penicillium chrysogenum* F-15.

Carbon source	Particle size (mesh)	Intracellular protein (mg)	Xylanase activity (U·mL^{-1})	Specific xylanase activity (U·mg·prot^{-1})
Sugar cane bagasse	>10	0.53 ± 0.02	1.79 ± 0.06	6.89 ± 0.27
	10–18	0.85 ± 0.12	4.23 ± 0.27	10.00 ± 0.19
	18–45	0.27 ± 0.01	2.85 ± 0.26	8.37 ± 0.21

Data are mean and standard deviation of three cultures. Static cultivation in Vogel's medium, pH 6.5 with 1% carbon source (w/v) for 7 d at 28°C.

used as standard. Proteins were stained with Coomassie brilliant blue R-250 (J.T. Baker) 0.1% (w/v) in ethanol: acetic acid: water (3:1:6, v/v/v).

2.8.2. pH and temperature optima

Xylanase activity was measured at 50°C in different pH values using the following 0.05 M buffer systems: glycine-HCl (pH 2.0–3.5), sodium acetate (pH 4.0–5.5), imidazole (pH 6.0–7.0), Tris–HCl (pH 7.0–9.0), glycine-NaOH (pH 9.0–10.0), at 50°C. Temperature optimum was determined by carrying out enzymatic reactions from 20 to 75°C, with 5°C intervals, at pH 6.5.

2.8.3. Thermal and pH stability

Thermal stability was evaluated in absence of substrate by incubating enzyme samples from 40 to 60°C. Samples were collected during 60 min and residual activity was determined. Half-lives were determined after plotting residual activity against time. The xylanase was 1:2 diluted (v/v) in different buffers to compose the pH range

from 2.0 to 10.0 (same buffers cited in 2.9.1 item) in absence of substrate, to determine the enzyme stability at different pH after incubation for 24 h at 4°C.

2.8.4. Effect of ions and substances

Xylanase activity was assayed in the presence of NH$_4$Cl, HgCl$_2$, CaCl$_2$, BaCl$_2$, CuCl$_2$, MgSO$_4$, MnSO$_4$, ZnSO$_4$ and Pb(CH$_3$COOH) (J.T. Baker), sodium dodecyl sulfate (SDS) (Sigma-Aldrich), tetra sodium ethylenediaminetetraacetate (EDTA) (Merck), phenylmethanesulfonylfluoride (PMSF) (USB, Cleveland, Ohio, USA), β-mercaptoethanol (Sigma-Aldrich) and 1,4-dithiothreitol (DTT) at 2 and 10 mM final concentrations. The activity was assayed at 45°C in 0.05 M imidazole buffer (J.T. Baker) pH 6.5 and expressed in relation to the control (without any substance).

2.8.5. Substrate specificity and kinetic parameters

The xylanase specificity was verified by assaying the activity against birchwood and oat spelt xylan, carboxymethylcellulose (CMC) (Sigma-Aldrich) and Avicel® (Fluka Analytical). The assays were carried out with 1% (w/v) substrates in 0.05 M imidazole buffer pH 6.5 at 45°C. The reducing sugars were quantified with the DNS acid reagent [34]. The enzyme was incubated with birchwood xylan at concentrations varying from 4.0 to 30.0 mg·mL^{-1} The Michaelis–Menten constant (K$_m$) and maximal velocity (V$_{max}$) were estimated from Lineweaver–Burk reciprocal plots [37] by linear regression (Microsoft Office Excel) using mean values from triplicates.

3. Results

3.1. Effect of substrates on xylanase production

When *P. chrysogenum* cultivation was performed in different carbohydrates (Table 1), the highest xylanase production was observed with oat bran (4.56 U·mL^{-1}) and intermediate levels of xylanase activity were verified with xylose (2.57 U·mL^{-1}) and sugar cane bagasse (1.12 U·mL^{-1}). Besides cultivation with sugar cane bagasse resulted in the highest specific activity (8.75 U·mg·prot^{-1}), an important factor for purification; and the highest *P. chrysogenum* growth, measured by the intracellular protein concentration, was verified with xylose (2.57 mg of protein). The influence of particle size on xylanase production was evaluated using sugar cane bagasse and brewer's spent grain, which are extremely abundant residues in Brazil. It was observed (Table 2) that only the particle size of sugar cane bagasse influenced xylanase production. Cultivations on brewer's spent grain (data not shown) with different particle size rendered very similar activity with mean values 2.7 U·mL^{-1}. However, with sugarcane bagasse, the highest enzyme production was observed by using 10–18 mesh particles (4.23 U·mL^{-1} and 9.99 U·mg·prot^{-1}), while intermediated values were verified with substrate particles
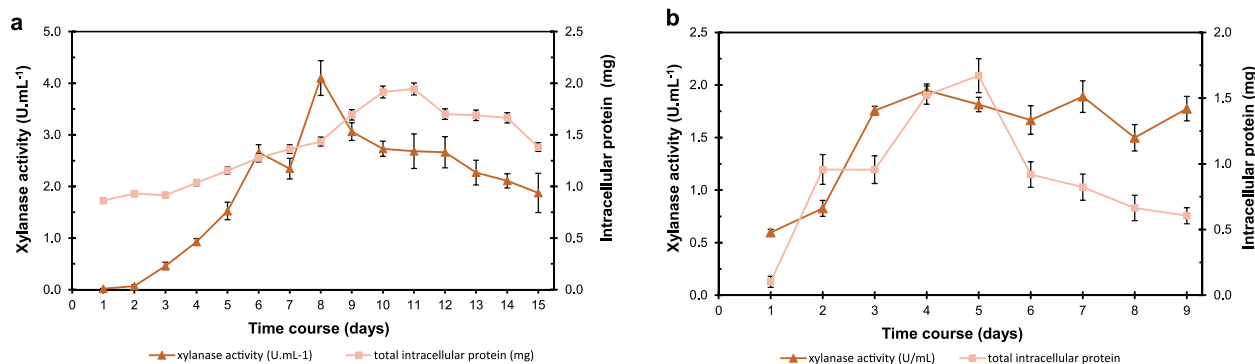

Fig. 1. Time course of xylanase production by *Penicillium chrysogenum* F-15 in static (a) and shaking culture at 120 rpm (b). Cultivation in Vogel's medium with 1% (w/v) sugar cane bagasse, pH 6.5 at 28°C.
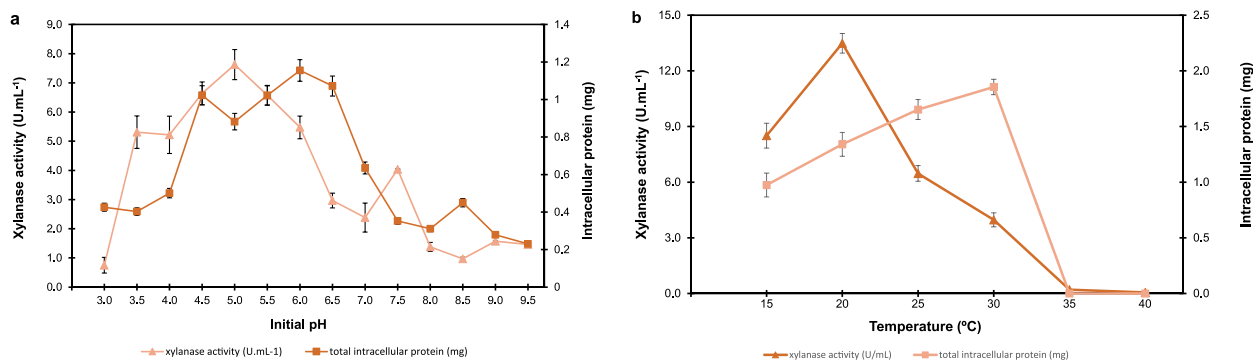
Fig. 2. Influence of initial pH (a) and temperature (b) on xylanase production by *Penicillium chrysogenum* F-15. Static cultivation in Vogel medium with 1% (w/v) sugar cane bagasse for 8 days at 28°C (a) and pH 5.0 (b).
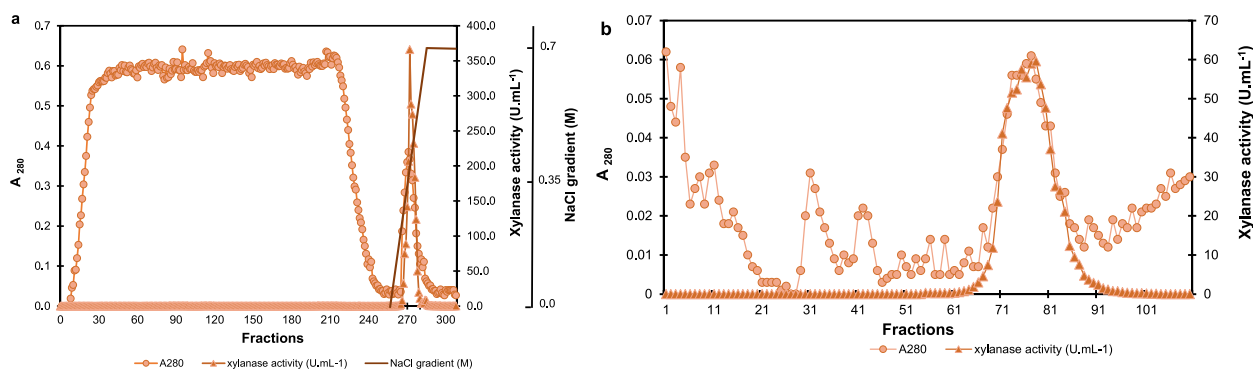

Fig. 3. Elution profiles of the xylanase from *Penicillium chrysogenum* F-15 chromatographed on CM Sephadex C-50 (a) and Sephadex G-100 (b) columns. The columns were equilibrated and eluted with 0.05 M sodium acetate buffer pH 4.5.

between 18–45 mesh (2.85 U·mL^{-1}) and lower levels (1.79 U·mL^{-1}) with substrate particles <10 mesh. When cultured under static conditions in liquid cultures with sugar cane bagasse (10–18 mesh) for 15 days, the highest xylanase production was obtained in 8 d-old cultures (4.10 U·mL^{-1} and 11.86 U.mg prot^{-1}) and the highest growth was reached after 11 days cultivation (1.94 mg of proteins) (Fig. 1a). In shaking condition, the highest xylanase production was on the 4th d (1.95 U·mL^{-1}) and the specific activity on the 3rd d (5.62 U·mg·prot^{-1}); maximum fungal growth was reached by 5 d-old cultures (1.67 mg of proteins) (Fig. 1b). Considering the xylanase production in static conditions was 2-fold higher than that on shaking cultures, the subsequent experiments were carried out in this condition. When *P. chrysogenum* was cultivated in pH from 3.0 to 9.5 (Fig. 2a), both xylanase activity and growth were detected in all pH range. The highest xylanase activity was observed at pH 5.0 (7.62 U·mL^{-1}) and higher fungal growth was verified in medium with slightly acid initial pH. The effect of temperature on xylanase production by *P. chrysogenum* is presented in Fig. 2b. The highest xylanase activity was obtained at 20°C (13.48 U·mL^{-1}), while the maximum growth was observed at 30°C (1.85 mg of proteins). At 40°C the strain was not able to grow, confirming its mesophilic characteristic [38].

3.2. Xylanase purification

The crude filtrate containing the extracellular xylanase from *P. chrysogenum* F-15 (44.5 U·mg·prot^{-1}), was submitted to ion exchange chromatography with CM-Sephadex C-50. The elution of bounded proteins was performed with an ascending NaCl gradient giving rise to one peak with xylanase activity (Fig. 3a). The sample from the pooled fractions of this step presented 438.3 U·mg·prot^{-1}. This sample was

then applied to a Sephadex G-100 column eluting in only one peak with xylanase activity (Fig. 3b) that was pooled and showed electrophoretic homogeneity (Fig. 4). The process presented 31.1% yield and the xylanase 18.7-fold purified presenting 834.2 U·mg·prot^{-1} (Table 3).

3.3. Properties of purified xylanase

The molecular mass (MM) estimated by PAGE-SDS was 22.1 kDa. The pH optimum for activity was 6.0–6.5. Intermediate activity levels
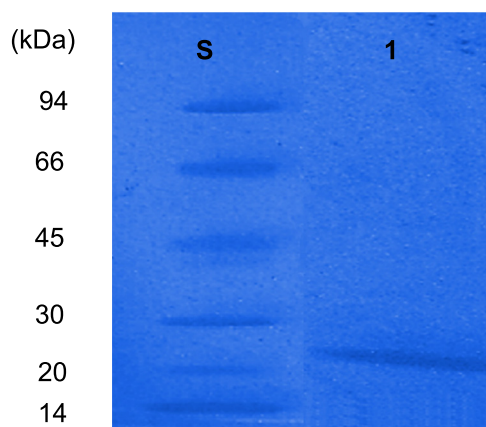

Fig. 4. SDS-PAGE (8–18%) of the purified *Penicillium chrysogenum* xylanase. Lane S: low molecular weight standard proteins: phosphorylase b (94 kDa), albumin (66 kDa), ovoalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa) and lactalbumin (14 kDa); Lane 1: purified xylanase.

Table 3
Purification of the xylanase from *Penicillium chrysogenum* F-15.

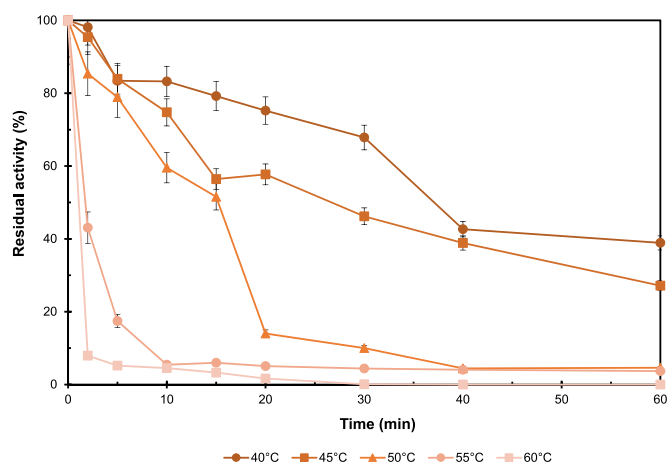| Step	Total activity (U)	Total protein (mg)	Specific activity (U·mg·prot⁻¹)	Recovery (%)	Fold change
Crude filtrate	6024.7	135.3	44.5	100.0	1.0
CM- Sephadex C-50	2972.8	6.8	438.3	49.3	9.8
Sephadex G-100	1872.0	2.2	834.2	31.1	18.7

Fig. 5. Thermal stability of the purified xylanase from *Penicillium chrysogenum*. The enzyme was incubated at 40°C, 45°C, 50°C, 55°C and 60°C without substrate. Residual xylanase activity was assayed with 0.05 M imidazole buffer pH 6.5 at 45°C.

were observed in pH 5.0, 5.5 and 7.0 and lower levels were verified in pH below 5.0 and above 7.0. The temperature optimum was 45°C (23.6 U·mL⁻¹). At 50 and 55°C the activity also was high, with values of 20.1 and 17.1 U·mL⁻¹, respectively. At all other temperatures the activity decreased to levels below 15.0 U·mL⁻¹. When thermal stability was assayed, the enzyme presented half-life of 35 min at 40°C (Fig. 5) and the half-lives were 31, 10, 3 and 2 min at 45, 50, 55 and 60°C, respectively. The enzyme retained from 30 to 50% of activity in the pH range from 4.5 to 10.0, with the highest stability in pH 6.5, in which more than 55% of the activity was preserved. When incubated with different chemical compounds, Mg^{+2}, Mn^{+2}, DTT and β-mercaptoethanol activated xylanase activity, while Cu^{+2}, Pb^{+2} and Hg^{+2} were strong inhibitors (Table 4). EDTA and SDS slightly affected the xylanase activity in both concentrations.

The xylanase presented activity of 12.1 and 9.5 U·mL⁻¹ on birchwood and oat spelt xylan, respectively, and no activity against CMC and Avicel. The Michaelis–Menten constant (K_m) and maximal velocity (V_{max}) estimated with birchwood xylan were 2.3 mM and 731.8 U·mg·prot⁻¹, respectively. For a comparison, xylanase activity present in crude filtrate from several filamentous fungi, obtained under optimized conditions for xylanase production, were evaluated

on hemicelluloses from different origins (Table 5). Among all xylanase-enriched filtrates, the highest activity was that from *A. versicolor* using brewer's spent grain hemicellulose with 154.4 U·mL⁻¹. The enzymatic activity of *T. inhamatum* in brewer's spent grain hemicellulose was 41.8 U·mL⁻¹, higher than that presented on previous study, which verified 11.4 U·mL⁻¹ using birchwood xylan as substrate [31]. Xylanase activity from *P. janczewskii* performed on birchwood xylan was 15.4 U·mL⁻¹ [24], higher than the activity presented in this work, but using sugar cane bagasse hemicellulose the activity was 22.2 U·mL⁻¹. *P. sclerotiorum* activity was 10.6 U·mL⁻¹ with birchwood xylan, higher than that previously observed [23]. *P. chrysogenum* F-15 strain showed lower enzymatic activity on commercial xylans such as those from beechwood (11.7 U·mL⁻¹), birchwood (12.1 U·mL⁻¹) and oat spelts (9.5 U·mL⁻¹) than on in-house extracted hemicellulose from agroindustry biomass such as sugarcane bagasse (18.7 U·mL⁻¹), brewer's spent grain 15.5 U·mL⁻¹) and corncobs (14.1 U·mL⁻¹).

4. Discussion

In cultures with xylose, xylanase production was ten-fold higher than that with oat spelts xylan and two-fold higher than that with glucose, suggesting that its production is inducible and can undergo to catabolite repression by glucose. The xylanase activity with sugar cane bagasse, brewer's spent grain and corncobs was intermediate, lower than with oat bran but much higher than that observed with oat spelts xylan. This is advantageous for xylanase production because the use of agro-industrial wastes can contribute to reduce the production cost. This work was the first to describe a purification protocol for the xylanase from *P. chrysogenum* produced on cultivation with sugar cane bagasse. Some other studies with filamentous fungi cultivation have been conducted with agro-industrial biomass for xylanase production, e.g., *P. janthinellum* was cultivated on corncobs and corn straw, presenting high xylanase production in these agrowastes [39]; *A. versicolor* showed higher xylanase production in cultures with wheat bran [30]; *A. giganteus* in sugar cane bagasse and corncobs [29] and *P. janczewskii* with wheat bran, oat bran and brewer's spent grain [24]. Also, this work was the first to evaluate the influence of particle size on the xylanase production by fungus. This material characteristic is important for enzyme production in a fermentation process, since it influences the accessibility of the microorganism to the nutrients, the arrangement and adhesion of fungal hyphae to the substrate and the release of metabolites to the extracellular medium. A particle separation allows better homogenization of the material, which is extremely heterogeneous due to the natural origin [14,40]. The molecular weight (MW) estimated for the *P. chrysogenum* xylanase was similar to other microbial xylanases from family GH 11, as described on the Carbohydrate-Active Enzymes (CAZy) database [41]. In characterization studies of xylanases from other *Penicillium* species, similar values for optimal activity are usually verified such as those from *P. purpurogenum* (pH 7.0, 50°C) [22], *P. sclerotiorum* (pH 4.5, 50°C) [23] and *Penicillium ocitanis* (50°C) [42]. Other studies relating the production, purification and characterization of xylanases from *P.*

Table 4
Effect of different substances on the activity of the purified xylanase from *Penicillium chrysogenum* F-15.

Substance	Relative activity (%)		Substance	Relative activity (%)	
	2 mM	10 mM		2 mM	10 mM
Control	100	100	$CuCl_2$	18.9 ± 4.1	7.6 ± 1.0
NaCl	88.0 ± 1.0	87.7 ± 0.5	$HgCl_2$	ND	ND
$CaCl_2$	69.4 ± 1.5	106.5 ± 3.6	$Pb(CH_3COO)_2$	ND	ND
$MgSO_4$	118.9 ± 2.6	175.3 ± 4.1	PMSF	103.6 ± 2.6	98.9 ± 3.1
$ZnSO_4$	100.4 ± 3.1	93.8 ± 10.2	DTT	101.1 ± 6.2	103.7 ± 2.5
NH_4Cl	94.9 ± 2.6	89.5 ± 6.2	EDTA	97.1 ± 6.7	86.5 ± 3.1
$BaCl_2$	96.7 ± 2.0	117.8 ± 5.1	SDS	92.4 ± 5.6	81.1 ± 0.1
$MnSO_4$	88.7 ± 2.1	138.5 ± 3.6	β-mercaptoethanol	101.4 ± 6.7	110.9 ± 3.6

Table 5
Xylanase production by different fungal strains on hemicelluloses from several agroindustrial biomass.

Microorganism	Substrate					
	BX	BWX	OSX	SCBH	BSGH	CCH
Aspergillus giganteus	0.7 ± 0.0	0.8 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.9 ± 0.1	1.25 ± 0.1
Aspergillus versicolor	88.5 ± 2.6	38.5 ± 0.4	141.4 ± 4.1	97.2 ± 1.5	154.4 ± 3.7	119.6 ± 3.4
Trichoderma inhamatum	4.8 ± 0.1	22.6 ± 0.2	35.5 ± 2.8	24.2 ± 0.9	41.7 ± 2.3	23.3 ± 0.3
Penicillium janczewskii	3.0 ± 0.2	5.7 ± 0.2	10.4 ± 0.1	22.2 ± 0.7	14.3 ± 0.1	13.8 ± 0.2
Penicillium sclerotiorum	6.8 ± 0.2	10.6 ± 0.2	13.4 ± 1.7	8.8 ± 0.1	10.5 ± 0.2	10.7 ± 0.3
Penicillium chrysogenum F-15	11.7 ± 1.1	12.1 ± 1.4	9.5 ± 0.9	18.7 ± 1.2	15.5 ± 1.3	14.1 ± 0.8

BEX: beechwood xylan; BWX: birchwood xylan; OSX: oat spelt xylan; SCBH: sugarcane bagasse hemicelulose; BSGH: brewer's spent grains hemicelulose; CCH: corn cob hemicelulose.

Table 6
Properties of xylanases from different *Penicillium chrysogenum* strains.

Strain	Form	Culture conditions	MW (kDa)	Optimum		Ref.
				pH	Temp (°C)	
P. chrysogenum Q 176	I	Oat spelts xylan, pH 6.0 2 d, 25°C	35	6.0	40	[21]
P. chrysogenum FS010	Recombinant XYL	—	—	5.5	25	[43]
P. chrysogenum F-15	I	Sugar cane bagasse pH 5.0, 8 d, 20°C	22.1	6.5	45	This work

chrysogenum strains, including a recombinant xylanase expressed in *Escherichia coli*, were compared at Table 6. The xylanases usually present MW between 12.6 and 35.0 kDa and most of them are acid enzymes with pH optimum in the range from 4.5 to 6.5. The xylanase produced by *P. chrysogenum* F-15, presents optimum in pH higher than those from others *P. chrysogenum* xylanases. Different strains of the same specie produce enzymes with distinct properties which can be due medium composition, culture period, pH, temperature of cultivation, proteolysis, post-translational modifications (such as glycosylation), or these enzymes can be the product of different genes [14,22]. An interesting procedure applied in this work was the use of DTT and glycerol during all purification steps that preserved the enzymatic activity, since glycerol protects the proteins by decreasing the water activity around the enzyme, maintaining its stability, while DTT, a reducing agent with sulfhydryl groups (-SH), undergoes oxidation rather than amino acids, preventing enzymatic oxidation. These characteristics indicate the presence of cysteines in the active site. The presence of -SH groups in the amino acids of the active site can be evidenced by the inhibition of the enzyme by heavy metals. Another important characteristic verified was that Mg^{+2} increased by 2-fold the xylanase activity, indicating that this ion could be required for catalysis.

In the experiments performed with agroindustrial biomass hemicellulose (Table 5), *A. versicolor* was the one who presented the best performance regarding xylanase activity using all substrates. Notably, hemicellulose from brewer's spent grain was the substrate that underwent to an easier and more extensive enzymatic hydrolysis, since three of the microorganism's xylanases producing studied, *A. giganteus*, *A. versicolor* and *T. inhamatum*, had their greater enzymatic activity by using this substrate. Strains of *P. janczewskii* and *P. chrysogenum* F-15 had its highest enzymatic activity in hemicellulose extracted from sugarcane bagasse and *P. sclerotiorum* in oat bran hemicellulose. This fact may be attributed to the composition of each hemicellulose, which could be more or less branched, allowing lesser or greater accessibility to the xylanases.

5. Conclusion

Penicillium chrysogenum is a good producer of extracellular xylanase using different agroindustrial residues as substrate, especially sugar cane bagasse and brewer's spent grain. The reuse of agrowastes is advantageous and a promising technology around the world. The produced xylanase presents optimum pH between 5.0, stability at alkaline pH, tolerance to some ions and high activity between 40 and 50°C, which makes it suitable for application in the beverage industry, bakery, xylooligosaccharides production, feed industry and for degradation of plant residues aiming bioethanol production.

Conflicts of interest

The authors state not having any conflict of interest in the publication of this article.

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