Sugarcane bagasse as a source of carbon for enzyme production by filamentous fungi

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ABSTRACT - (Sugarcane bagasse as a source of carbon for enzyme production by filamentous fungi). The aim of the present work was to assess the enzymatic activity of six strains of filamentous fungi grown in liquid media containing 1% sugarcane bagasse as the sole carbon source. All fungal strains were able to use this agro-industrial residue, producing various types of enzymes, such as cellulases, xylanases, amylases, pectinases, and laccases. However, *Aspergillus japonicus* Saito was the most efficient producer, showing the highest enzymatic activity for laccase (395.73 U L^{-1}), endo-β-1,4-xylanase (3.55 U mL^{-1}) and β-xylosidase (9.74 U mL^{-1}) at seven, fourteen and twenty-one days in culture, respectively. Furthermore, the endo-β-1,4-xylanases and β-xylosidases of *A. japonicus* showed maximum activity at 50°C, and pH 5.5 and pH 3.5-4.5, respectively. Thus, these results indicate that *A. japonicus* has a great biotechnological potential for the production of these enzymes using sugarcane bagasse as the sole source of carbon.
Keywords: anamorphic fungi, *Aspergillus japonicus,* endo-β-1,4-xylanase, enzymes, laccase*,* β-xylosidase

RESUMO - (O bagaço de cana-de-açúcar como fonte de carbono para a produção de enzimas por fungos filamentosos). O presente trabalho teve como objetivo avaliar a atividade enzimática de seis cepas de fungos filamentosos cultivados em meio liquido contendo 1% de bagaço de cana-de-açúcar como a única fonte de carbono. Todas as cepas fúngicas foram capazes de usar este resíduo agroindustrial, produzindo vários tipos de enzimas, como celulases, xilanases, amilases, pectinases e lacases. No entanto, *Aspergillus japonicus* Saito foi o produtor mais eficiente, mostrando as maiores atividades enzimáticas para lacase (395,73 U L^{-1}), endo-β-1,4-xilanase (3,55 U mL^{-1}) e β-xilosidase (9,74 U mL^{-1}) com sete, catorze, e vinte e um dias de cultura, respectivamente. Além disso, as endo-β-1,4-xilanases e as β-xilosidases de *A. japonicus* apresentaram atividade máxima a 50°C, e pH 5,5 e pH 3,5-4,5, respectivamente. Assim, estes resultados indicam que *A. japonicus* tem um ótimo potencial biotecnológico para a produção destas enzimas utilizando o bagaço de cana-de-açúcar como a única fonte de carbono.
Palavras-chave: fungos anamórficos, Aspergillus japonicus, endo-β-1,4-xilanase, enzimas, lacase, β-xilosidase

Introduction

Sugarcane is the main agricultural crop cultivated in Brazil. The sugarcane stem is milled to obtain the cane juice, which is either used for ethanol or sugar production (Canilha *et al*. 2012). Each ton of sugarcane processed generates approximately 270-280 kg of bagasse (Rodrigues *et al*. 2003). This bagasse is normally burned in the industries to produce the energy required in the process (Canilha *et al*. 2012). However, this agro-industrial by-product may have more valuable uses than the direct energy generation through combustion (Gamez *et al*. 2006).

Sugarcane bagasse contains approximately 50% cellulose, 25% hemicellulose and 25% lignin. Due to its high availability, it could serve as a substrate for microbial production of value-added products, such as protein-rich animal feed, enzymes, amino acids, organic acids and compounds of pharmaceutical importance (Parameswaran 2009), and also source of carbon for the growth of filamentous fungi (Martins *et al*. 2011). Filamentous fungi are particularly useful producers of enzymes from the industrial point of view, due to the high production level and extra cellular secretion of enzymes, as well as relative ease

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of cultivation (Tallapragada & Venkatesh 2011). These fungi produce high levels of polysaccharide-degrading enzymes and are frequently used in the production of industrial enzymes (Tsukagoshi *et al.* 2001), such as amylases, cellulases, xylanases, pectinases, and lignin-modifying enzymes from lignocellulosic biomass. These enzymes have several biotechnological applications, for instance, they have been used in the production of textiles, detergents, paper, food for animals and humans for decades (Bocchini *et al.* 2003, Graminha *et al.* 2008). They can also be applied in industrial processes to eliminate the use of high temperatures, organic solvents, and extreme pH, while at the same time offering increased reaction specificity, product purity and reduced environmental impact (Cherry & Fidantsef 2003).

In the last few years, research has also been focused on the potential use of these fungal enzymes for the degradation of lignocellulosic materials, aiming at releasing fermentable sugars that can be converted to second-generation ethanol by the action of fermentative microorganisms (Buaban *et al.* 2010, Talebnia *et al.* 2010). The breakdown of lignocellulose in the plant cell wall requires the recruitment of glycoside hydrolases with different mechanisms of action in a concerted action with ligninases (Gupta *et al.* 2016). Moreover, pre-treatment using microbial enzymes for ethanol production is a promising technology due to its several advantages, like being an eco-friendly and economically viable strategy for enhancing enzymatic saccharification rates (Sindhu *et al.* 2015).

Lastly, there has been increasing interest in obtaining new, stable and more specific enzymes using low-cost carbon sources, such as the sugarcane bagasse, and in searching for new and suitable microbial strains for large-scale cultivation that might be able to enzymes with the appropriate characteristics for biotechnological processes (Parameswaran 2009). Therefore, the isolation of new fungal strains is the first step for obtaining more efficient and economically accessible enzymes for biotechnological and industrial purposes. Thus, the aim of this study was to assess the enzymatic activity of some strains of filamentous fungi, using sugarcane bagasse as the sole source of carbon.

Materials and methods

Strains and culture conditions - Strains of *Aspergillus japonicus* Saito, *Fusarium solani* (Mart.) Sacc.,

Fusarium sp. Link *ex* Fr., *Fusarium oxysporum* Schltdt., *Pestalotiopsis* sp. Steyaert, and *Trichoderma pseudokoningii* Rifai were obtained from the culture collection of the Laboratory of Microbiology of Universidade Metodista de São Paulo, São Bernardo do Campo, Brazil. Isolates were maintained in the laboratory on solid potato dextrose agar (PDA) slants at 25 °C. Three discs (5 mm diameter) obtained from the cultures were transferred into 250 mL Erlenmeyer flasks containing 50 mL of the following medium (g L^{-1}): (6) NaNO$_3$, (1.5) KH$_2$PO$_4$, (0.5) KCl, (0.5) MgSO$_4$.7H$_2$O, (0.01) FeSO$_4$, (0.01) ZnSO$_4$, and 1% sugarcane bagasse. The sugarcane bagasse was dried in an oven at 35 °C until constant weight and then crushed into particles of an average diameter of 4 mm (Alexandrino *et al.* 2007), before adding to the liquid medium (Alexandrino *et al.* 2007). Flasks were kept at 25 °C for 21 days under static conditions (Menezes *et al.* 2009). Culture growth was interrupted by filtration every 7 days and the crude culture filtrates used for enzymatic assays. All experiments were performed in triplicate.

Protein determination - Protein concentrations in the culture filtrate were estimated by the Bradford method using bovine serum albumin as standard (Bradford 1976). Triplicates of samples were analyzed in a spectrophotometer at 595 nm.

Enzymatic assays - For all assays, one unit of enzymatic activity (U) was defined as the amount of enzyme that releases 1 μmoL of the corresponding product (glucose, xylose, galacturonic acid, ρ-nitrophenol, and oxidized syringaldazine), per minute under the assayed conditions.

Amylase, Endo-β-1,4-xylanase, Endo-β-1,4-glucanase, Pectinase, and Filter paper activity (FPA) Activities - The amylase and endo-β-1,4-xylanase activities were determined by incubating 50 μL of the crude culture filtrate, 50 μL of starch, and birchwood xylan 1% (w/v), respectively, in 50 mM sodium citrate buffer (pH 5.5) at 40 °C for 30 minutes. The endoglucanase and pectinase activities were determined by incubating 50 μL of the crude culture filtrate, 50 μL of carboxymethyl cellulose, and pectin 1% (w/v), respectively, in 50 mM sodium citrate buffer (pH 5.5) at 50 °C for 30 minutes. Filter paper activity (FPA) was determined by incubating 50 mg of Whatman No.1 filter paper strips (1.0 × 6.0 cm) in 500 μL of the crude culture filtrate, and 500 μL of sodium citrate buffer 50 mM (pH 5.5) at 50 °C for 60 minutes (Ghose 1987). All reactions were stopped by

adding 1 mL of 3,5-dinitrosalicylic acid (DNS) and then boiled for 10 min, according to Miller (1959). The reducing sugar released from the substrate was measured in a spectrophotometer at 540 nm.

β-glucosidase and β-xylosidase activities - The activity of β-glucosidases and β-xylosidases was determined by incubating 125 µL of ρ-nitrophenyl-β-D-glucopyranoside and 2 mM ρ-nitrophenyl-β-D-xylopyranoside, respectively, 125 µL of the crude culture filtrate, and 250 µL of 50 mM sodium citrate buffer (pH 5.5) at 40 °C for 30 minutes. Reactions were stopped by adding 500 µl of 0.5 M Na_2CO_3 (Mayrink 2010). The p-nitrophenolate ion released from the substrate was measured in a spectrophotometer at 405 nm.

Laccase Activity - The laccase activity was determined by incubating 0.2 mL of syringaldazine in 0.5 mM ethanol, 0.1 mL of the crude culture filtrate, and 1.5 mL of 100 mM phosphate buffer (pH 6.5) at 30 °C for 30 minutes (Leonowicz & Grzywnowicz 1981). Oxidation of syringaldazine was measured in a spectrophotometer at 525 nm. One unit of laccase activity was defined as 1 µmol of syringaldazine oxidized per min.

Enzymatic characterization - Samples of a 7-day crude culture filtrate from *A. japonicus* were used to assess the effect of pH and temperature on the endo-β-1,4-xylanase and β-xylosidase activities. For determining the optimum temperature of these enzymes, samples were incubated for 30 min in a range of temperatures from 20 °C to 60 °C, and a pH of 5.5 held with sodium citrate buffer. The optimum pH of these enzymes was determined by incubating the samples at 50 °C and in

a pH range of 2 to 7 with citrate-phosphate buffer, for 30 minutes. The enzymatic activities were performed as previously described, and the values were expressed as relative percentages.

Data Analysis - Data were analyzed by a one-way ANOVA (Sodek® Scopus Tecnologia S.A, version 3.02), comparing the enzymatic activities between days of culture of the same strain and between different strains, and then the average of three replicates was examined with Tukey´s test (p ≤ 0.05).

Results

Enzymatic Activities - Table 1 shows the cellulase activity of the six filamentous fungi grown in liquid medium with sugarcane bagasse as the sole carbon source. Most fungi showed a maximum FPA between 14 and 21 days. The highest FPA activities were produced by *A. japonicus* (0.14 U mL⁻¹) and *F. oxysporum* (0.13 U mL⁻¹) at days 14 and 21, respectively, whereas the lowest FPA was exhibited by *Fusarium* sp. and *F. solani*. The strains of *F. oxysporum*, *T. pseudokoningii*, and *Pestalotiopsis* sp. showed a gradual increase in the enzyme activity over time, while *A. japonicus*, *F. solani* and *Fusarium* sp. showed the least variation in their enzymatic activities. In addition, most fungi reached their maximum endo-β-1,4-glucanase activity at 21 days of culture, but the highest activity of this enzyme (0.10 U mL⁻¹) was exhibited by *Aspergillus japonicus* at 7 days of culture. *Pestalotiopsis* sp. and all *Fusarium* strains showed no endo-β-1,4-glucanase activity during some/all days of culture. On the other hand, the only strains that showed β-glucosidase activity were *F. oxysporum*

Table 1. β-glucosidase, endoglucanase, and FPA activities of fungal isolates grown in submerged cultures of sugarcane bagasse as the sole carbon source for seven, fourteen, and twenty-one days.

Fungal Species	β-glucosidase (U mL⁻¹)			Endoglucanase (U mL⁻¹)			FPA (U mL⁻¹)		
	7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
Aspergillus japonicus	0.20 Aa*	0.21 Aa	0.28 Aa	0.10 Aa	0.06 Ab	0.06 Ab	0.12 Aa	0.14 Aa	0.13 ABa
Fusarium sp.	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Cb	0.00 Cb	0.02 CDa	0.04 Db	0.04 Db	0.05 DEa
Fusarium solani	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Ca	0.00 Ca	0.00 Da	0.03 Da	0.04 Da	0.04 Ea
Fusarium oxysporum	0.00 Bb	0.06 Bab	0.19 Aa	0.00 Cc	0.02 Bb	0.04 ABCa	0.07 BCb	0.11 ABa	0.13 ABa
Pestalotiopsis sp.	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Cc	0.02 Bb	0.04 ABCa	0.05 CDa	0.07 Cb	0.09 BCc
Trichoderma pseudokoningii	0.00 Ba	0.00 Ba	0.00 Ba	0.04Bab	0.03 Bb	0.05 ABa	0.05 Db	0.07 Ca	0.08 CDa

*Means followed by the same letters, lowercase in rows and capital letters in columns, showed no differences of statistical significance by Tukey's test (p < 0.05).

and *A. japonicus,* and their maximum activities were achieved at 21 days of culture. These two fungi also showed a higher activity of β-glucosidase than of endoglucanases and FPA.

The ability to produce amylase, pectinase, and laccase of the fungi in the culture medium is shown in table 2. *Aspergillus japonicus* was the only strain that produced amylase, with a maximum activity at 14 days of culture (0.35 U mL^{-1}) (data not shown). Notwithstanding, all strains showed the maximum pectinase activity at 7 days of culture, and thereafter this activity decreased. The highest pectinase activity was observed in *A. japonicus* (0.53 U mL^{-1}), while the other species tested showed low activity. Nonetheless, an optimum laccase activity was registered for almost all strains at 14 days of culture. The highest activity of this enzyme was observed for *A. japonicus* (395.73 U L^{-1}) at 7 days, but it decreased at 14 and 21 days of culture.

The endo-β-1,4-xylanase and β-xylosidase activities of the strains in the liquid medium is shown in table 3. The highest endo-β-1,4-xylanase (3.55 U mL^{-1}) and β-xylosidase (9.74 U mL^{-1}) activities were observed for *A. japonicus* at 14 and 21 days of culture, respectively. The rest of the strains showed low enzymatic activities of both enzymes. The strain of *A. japonicus* presented a higher production of β-xylosidases than of endo-β-1,4-xylanases at all days of culture, while the other species presented higher activities of endo-β-1,4-xylanase than of β-xylosidase. We also registered lower cellulase activities than of these two enzymatic activities.

Enzymatic characterization - In *A. japonicus*, the endo-β-1,4-xylanases and β-xylosidase were the enzymes

with the highest activity, and both tended to increase over time (table 3). Based on these results, we only evaluated the specific activity of endo-β-1,4-xylanase and β-xylosidase (table 4). The specific activity of endo-β-1,4-xylanase and β-xylosidase was consistent with the pattern of enzymatic activity exhibited, as shown in table 3.

Effects of temperature and pH - Both endo-β-1,4-xylanases and β-xylosidases showed their maximum activity at 50 °C, and at pH 5.5 and 3.5 to 4.5, respectively (figure 1a and b).

Discussion

The FPA activities of *Aspergillus japonicus* and *F. oxysporum* are shown in table 1. Our results are in agreement with previous studies, which showed an activity of 0.16 FPA mL^{-1} for *Trichoderma reesei* QM9414 grown in sugarcane bagasse (Basso *et al.* 2010), and of 0.159 FPA mL^{-1} for *A. terreus* in banana stems (Siqueira *et al.* 2010). However, Mayrink (2010) found a higher FPA for *A. japonicus* (3.26 FPA mL^{-1}), when grown in wheat bran. Previous studies also indicated that *A. japonicus* has the potential for cellulase production (Herculano *et al.* 2011, Kumar *et al.* 2008).

The endoglucanase activity has been previously reported for several fungi grown in different agro-industrial residues. For example, Stroparo *et al.* (2012) reported endoglucanase activity in *Penicillium miczninscii* grown in pineapple peel (0.18 U mL^{-1}) and orange peel (0.15 U mL^{-1}). These findings are consistent with the endoglucanase activity of *A. japonicus* found in this work (table 1). In contrast, Gomes *et al.* (2007) found a higher endoglucanase activity (0.49

Table 2. Pectinase and laccase activities of fungal isolates grown in submerged cultures of sugarcane bagasse as the sole carbon source for seven, fourteen, and twenty-one days.

Fungal Species	Pectinase (U mL^{-1})			Laccase (U L^{-1})		
	7 days	14 days	21 days	7 days	14 days	21 days
Aspergillus japonicus	0.53 Aa*	0.24 Ab	0.28 Ab	395.73 Aa	165.81 ABb	100.00 Cb
Fusarium sp.	0.10 BCa	0.08 Ba	0.09 Ba	82.91 CDc	131.28 ABb	209.40 Aa
Fusarium solani	0.19 Ba	0.09 Bb	0.11 Bb	194.87 BCa	217.09 Aa	205.13 ABa
Fusarium oxysporum	0.19 Ba	0.09 Bb	0.12 Bb	126.15 CDb	258.12 ABa	229.91 Ba
Pestalotiopsis sp.	0.00 Ca	0.00 Ca	0.00 Ca	28.72 Db	126.50 Ba	111.11 Ca
Trichoderma pseudokoningii	0.08 Ba	0.02 Cb	0.03 Cb	182.05 BCb	227.35 ABab	257.26 ABa

*Means followed by the same letters, lowercase in rows and capital letters in columns, showed no differences of statistical significance by Tukey's test ($p < 0.05$).

Table 3. Endo-β-1,4-xylanase and β-xylosidase of fungal isolates grown in submerged culture of sugarcane bagasse as the sole carbon source for seven, fourteen, and twenty-one days.

Fungal Species	Endo-β-1,4-xylanase (U mL^{-1})			β-xylosidase (U mL^{-1})		
	7 days	14 days	21 days	7 days	14 days	21 days
Aspergillus japonicus	2.47 Ab*	3.55 Aa	2.94 Ab	4.48 Ab	8.31 Aa	9.74 Aa
Fusarium sp.	0.13 Cb	0.13 Db	0.30 Ba	0.00 Ba	0.00 Ba	0.00 Ba
Fusarium solani	0.20 BCc	0.45 BCb	0.50 Ba	0.53 Ba	0.32 Bab	0.32 Bb
Fusarium oxysporum	0.45 Bb	0.56 Bab	0.63 Ba	0.04 Bb	0.24 Ba	0.25 Ba
Pestalotiopsis sp.	0.07 Cc	0.26 CDb	0.45 Ba	0.06 Bb	0.15 Ba	0.00 Bc
Trichoderma pseudokoningii	0.07 Cc	0.26 CDb	0.47 Ba	0.00 Ba	0.00 Ba	0.00 Ba

*Means followed by the same letters, lowercase in rows and capital letters in columns, showed no differences of statistical significance by Tukey's test ($p < 0.05$).

Table 4. Specific activity (U mg^{-1}) of endo-β-1,4-xylanase and β-xylosidase obtained from crude culture filtrates of *Aspergillus japonicus* grown in sugarcane bagasse for seven, fourteen, and twenty-one days.

Enzymes	Specific Activity (U mg^{-1})		
	7 days	14 days	21 days
Endo-β-1,4-xylanase	173.81 ± 8.72	172.06 ± 24.30	163.40 ± 12.84
β-xylosidase	315.39 ± 48.26	402.30 ± 77.80	540.82 ± 95.28
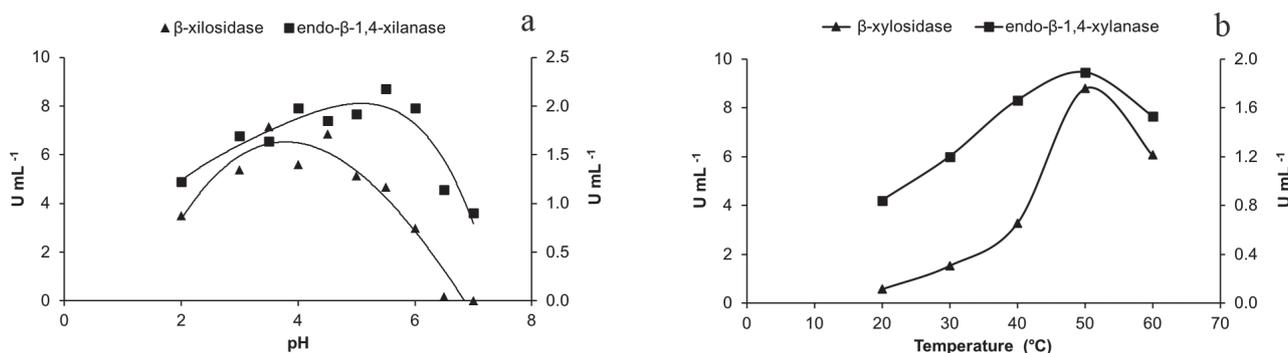
Figure 1. Effect of temperature (a) and pH (b) on the endo-β-1,4-xylanase and β-xylosidase activities of *Aspergillus japonicus* grown in sugarcane bagasse as the sole carbon source for seven days. The values are means of three experiments (n = 3).

U mL^{-1}) in *Trichoderma viride* grown in sugarcane bagasse, while Siqueira *et al*. (2010) in *Aspergillus oryzae* (0.223 U mL^{-1}) and *A. terreus* (0.519 U mL^{-1}) grown in cotton and banana stems, respectively. Singh *et al*. (2009) and Castro *et al*. (2010) found that *Aspergillus* species are the best producers of exo and endoglucanases, and that the concentrations of β-glucosidases produced by species of this genus are particularly high, which may represent an advantage in the process of biomass saccharification.

Only *A. japonicus* and *F. oxysporum* exhibited β-glucosidase activity (table 1), which was lower than reported in previous studies. Some authors have reported higher β-glucosidase activities for *A. niger* (3 U mL^{-1}) and for *A. japonicus* (1.79 U mL^{-1}) both grown in wheat bran (Bansal *et al*. 2011, Mayrink 2010), and for *F. proliferatum* (3.31 U mL^{-1}) grown in corn stover (Gao *et al*. 2012). Among the fungi analyzed, *A. japonicus* and *F. oxysporum* seem to be the most efficient cellulose degraders. Bansal *et al*. (2011) observed that *Aspergillus* usually present a high β-glucosidase activity but low endoglucanase levels, whereas *Trichoderma* has high endoglucanase and exoglucanase activities but low β-glucosidase levels,

and hence, this latter fungus has a limited efficiency in cellulose hydrolysis. The same pattern was registered in this study, indicating a greater saccharification of cellulose by *A. japonicus*. The characteristics of the β-glucosidase produced by *A. japonicus* have been previously assessed for a potential use in biomass saccharification (Korotkova *et al.* 2009).

Pasin *et al.* (2014) reported high levels of amylase activity in *A. japonicus* when cultivated in different agro-industrial residues. The presence of this activity in the cultures might be due to the fact that amylases are constitutive enzymes, which are generally located in the fungal cell walls (Benassi *et al.* 2012).

Our results of pectinase activity from *A. japonicus* are in agreement with Teixeira (2000), who reported a pectinase activity of 0.50 U mL^{-1} for *A. japonicus* 586, in the presence of 0.5% pectin and 0.5% glucose (table 2). However, higher fungal pectinase activities have been reported in the literature; for instance, *Penicillium verruculosum* reached 10.32 U mL^{-1} in culture media supplemented with orange peel (Stroparo *et al.* 2012), and *Aspergillus niger* LB-02-SF exhibited 14 U mL^{-1}, when pectin was added to the medium (Reginatto *et al.* 2017). Despite this, several pectinases have been hitherto reported for *A. japonicus*, such as endopolygalacturonases and pectinesterases (Hasunuma *et al.* 2003, Semenova *et al.* 2003, Teixeira *et al.* 2011), pectin lyases (Semenova *et al.* 2003, Ishii & Yokotsuka 1975), and exo-polygalacturonases (Teixeira *et al.* 2011), indicating that this species can be highly promising for the production of these enzymes.

Interestingly, the studied strains showed a higher laccase activity than reported by Okamoto *et al.* (2000) and Mansur *et al.* (2003), who found a laccase activity of 130 U L^{-1} and 310 U L^{-1}, respectively for *P. ostreatus* (table 2). According to Kiiskinen *et al.* (2004), the majority of the laccases characterized so far have been found in white-rot fungi, which are efficient lignin degraders. Unlike results from previous studies, we observed that the fungi analyzed in the present study might have the potential to produce this enzyme, especially in the case of *A. japonicus*, which showed the highest laccase activity, and has been previously reported as an efficient degrader of phenolic compounds using wheat straw as source of carbon (Milstein *et al.* 1983).

It is important to note that in *A. japonicus* we found higher activities of endo-β-1,4-xylanase and β-xylosidase than of other enzymes, except in the case of laccase (table 3). Siqueira *et al.* (2010) found similar

activities of endo-β-1, 4-xylanases produced by various strains of *Aspergillus* grown in banana stems, cotton residues, and sugarcane bagasse. Other studies have also found a high endo-β-1,4-xylanase activity from fungi grown in sugarcane bagasse; for example, Lemos *et al.* (2000) in *A. awamori* (30 U mL^{-1}), Biswas *et al.* (1988) in *A. ochraceus* (8.5 U mL^{-1}), and Kadowaki *et al.* (1997) in *A. tamarii* (16.7 U mL^{-1}). We also noted that the activity of β-xylosidase produced by *A. japonicus* in sugarcane bagasse was higher than reported in previous studies. Biswas *et al.* (1988) and Smith & Wood (1991) have reported the production of β-xylosidases by *A. ochraceus* (5.70 U mL^{-1}) and *A. awamori* (2.86 U mL^{-1}) when grown in submerged cultures of milled oat straw.

Aspergillus japonicus showed a higher activity of β-xylosidase than of endo-β-1,4-xylanase when grown in sugarcane bagasse (table 3). This can be due to the fact that β-xylosidases are responsible for removing xylooligosaccharides, which are inhibitors of xylanases, and thus allowed a more efficient hydrolysis of xylan. According to Polizeli *et al.* (2005) the ideal microorganism for biotechnological purposes would be the one that produces an adequate amount of each of the enzymes of the xylanolytic complex. In this sense, *A. japonicus* appears to be an efficient producer of β-xylosidase and endo-β-1,4-xylanase, and this might be a strategy of this fungus for breaking down hemicelluloses from sugarcane bagasse in order to use them for rapid growth. In addition, we found a lower activity of cellulases than of endo-β-1,4-xylanases and β-xylosidases (table 1 and 3). This may be related to different factors that prevent the hydrolysis of cellulose, such as, cellulose crystallinity, accessible surface area, protection by lignin, and sheathing by hemicellulose (Kumar *et al.* 2009).

Regarding the specific activity of endo-β-1,4-xylanase and β-xylosidase, we found that *A. japonicus* showed a high specific activity for both enzymes when grown in sugarcane bagasse (table 4) and that these activities were higher than reported in previous studies. For example, Guimaraes *et al.* (2013) reported that the specific activity of xylanase for *Aspergillus niger* and *A. flavus* in different sources of carbon did not exceed 20 U mg^{-1}, while Rizzatti *et al.* (2001) reported a β-xylosidase activity for *A. phoenicis* of 219.9 U mg^{-1} grown in sugarcane bagasse and of 112.3 U mg^{-1} in maize pith as sole carbon sources.

Remarkably, we found that *A. japonicus*, when grown in sugarcane bagasse, has a lower temperature for endo-β-1,4-xylanase and β-xylosidase activities (figure 1a and b)

than reported by Wakiyama *et al.* (2008) and Semenova *et al.* (2009), although the pH range for these enzymes was similar. These authors did not use any agroindustrial residue as source of carbon and found optimal endo-β-1,4-xylanase and β-xylosidase activities for the same strain at 60 °C - 70 °C and pH 4.0-5.0. Polizeli *et al.* 2005 also reported maximum activity of both endoxylanase and β-xylosidase between 40 °C and 80 °C, and pH 4.0 and 6.5. Some species of *Aspergillus* have shown an optimum temperature for endo-β-1,4-xylanase activity of 50 °C (Polizeli *et al.* 2005), as in the case of *A. fumigatus* at 55 °C and pH 5.5 (Silva *et al.* 1999). In the case of β-xylosidase, the optimum temperature and pH range for *A. phoenicis* were at 75 °C and pH 4.0-4.5 (Rizzatti *et al.* 2001), and for *A.* cf. *tubingensis* LAMAI 31 at 55 °C and pH 5.0 (Santos *et al.* 2016).

Conclusion

Our results showed that *Aspergillus japonicus*, when cultivated in sugarcane bagasse as the sole source of carbon, presented the most promising results for enzymatic activities. This fungus showed the highest activities of laccase, endo-β-1,4-xylanase, and β-xylosidase at 7, 14 and 21 days of culture, respectively. This strain was also able to produce the maximum activity of endo-β-1,4-xylanases and β-xylosidases at low temperature when compared to other studies. Therefore, *A. japonicus* appears to be an effective enzymatic producer when grown in sugarcane bagasse, suggesting its potential application in biotechnological processes that require the production of these enzymes.

Acknowledgments

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