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ARTICLE

Production of enzymes by filamentous fungus using sugarcane and sugarcane bagasse as substrate

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ABSTRACT:(Production of enzymes by filamentous fungus using sugarcane and sugarcane bagasse as substrate). The production of enzymes by bioprocesses is a good alternative to add value to agroindustrial waste. Sugarcane bagasse, an abundant and cheap by-product of the sugar industry, was tested as a carbon source for the production of biotechnological interesting enzymes. In this work, fungi were isolated from anatomical parts of sugarcane (root, steam and leaf) and, then, were assessed for enzyme production. The isolated and identified fungi were *Fusarium* sp., *Penicillium* sp., *Trichoderma auroviride* and *Cladosporium cladosporioides*. *Trichoderma auroviride* was used for enzyme production (xylanase, invertase and protease) using sugarcane as substrate. Xylanase production (2037 U) by *Trichoderma auroviride* was higher than invertase and protease production; thus, this enzyme was selected for the further studies. The study of the influence of variables (temperature and stirring intensity) on xylanase production by *Trichoderma auroviride*, using sugarcane bagasse as substrate, showed that the most favorable xylanase production conditions were observed at 25 °C, without stirring intensity and using saline and Tween for enzyme extraction, which led to a 1980 U xylanase activity.
Key words: xylanase production, sugarcane bagasse, filamentous fungi, *Trichoderma auroviride*, enzymes.

RESUMO: (Produção de enzimas por fungos filamentosos utilizando cana-de-açúcar e bagaço de cana-de-açúcar como substrato). A produção de enzimas por bioprocessos é uma boa alternativa para agregar valor a resíduos agroindustriais. O bagaço da cana-de-açúcar, um abundante e barato subproduto da indústria de açúcar, foi testado como fonte de carbono para a produção de enzimas de interesse biotecnológico. Neste trabalho foi realizado o isolamento e identificação de fungos a partir de peças anatômicas (caule, raiz e folha) da cana-de-açúcar e em seguida foi realizada a investigação da produção de enzimas por esses microrganismos. *Fusarium* sp., *Penicillium* sp., *Trichoderma auroviride* e *Cladosporium cladosporioides* foram os fungos isolados e identificados. *Trichoderma auroviride* foi utilizado para a produção de enzimas (xilanase, invertase e protease) utilizando cana-de-açúcar como substrato. A produção de xilanase (2037 U) por *Trichoderma auroviride* foi maior que a produção de protease e de invertase, portanto, essa enzima foi selecionada para estudos posteriores. O estudo da influência das variáveis temperatura e intensidade de agitação na produção da xilanase por *Trichoderma auroviride* usando bagaço da cana-de-açúcar como substrato demonstrou que a condição mais favorável para a produção de xilanase foi observada a 25 °C, sem agitação e utilizando solução salina e Tween para extração da enzima, o que levou a uma produção de xilanase de 1980 U.
Palavras-chaves: produção de xilanases, bagaço de cana-de-açúcar, fungos filamentosos, *Trichoderma auroviride*, enzimas.

INTRODUCTION

The search for cheaper procedures for the hydrolysis of bagasse to fermentable sugar that can still avoid formation of toxic compounds for the subsequente fermentation has been increasing. The use of microbial enzymes such as cellulases and xylanases meets these requirements; however, this technology is not ready to be applied (Pávon-Orozco *et al*. 2012).

In some works, a variety of microorganisms including bacteria (Mattéotti *et al*. 2012), yeast (Menon *et al*. 2010), actinomycetes (Kapoor *et al*. 2008) and filamentous fungi (Silva *et al*. 1999) have been reported to produce xylanolytic enzymes. Because of their natural ability to produce plant polysaccharide-hydrolyzing enzymes, filamentous fungi such as *Trichoderma* (Hung *et al. 20*08), *Fusarium* (Carapito *et al*. 2009), *Penicillium* (Mesharam *et al*. 2008) and *Aspergillus* (Téo *et al*. 2000) have been the organisms of choice for investigating xylan hydrolysis applications. Different biotechnological applications for microbial xylanases have been found in the last two decades. They are widely used in the cellulose biobleaching process in the pulp and paper industry to reduce the usage of chlorine (Beg *et al*. 2001), as well as in others industries, such as brewing

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(Qiu *et al*. 2010), bakery (Dalfré *et al*. 2007), fruit and vegetable processing (Dhiman *et al*. 2011), juice and wine (Hung *et al*. 2008), and as feed additives in broiler and animal diets (Ncube *et al*. 2012).

Xylan is one of the major components of hemicelluloses in plant cell walls – the second most abundant polysaccharide after cellulose – and it accounts for 20-30% of their total dry mass. In nature, complete hydrolysis of xylan requires the synergistic action of different xylanolytic enzymes, including endoxylanase, β-xylosidase, and accessory enzymes, such as alpha-arabinofuranosidase, acetyl esterase, and alpha-glucuronidase. Among them, endo-β-1,4-xylanase (EC 3.2.1.8) catalyzes the hydrolysis of long-chain xylan into short xylooligosaccharides; it has been studied due to the extensive interest arising from its wide applications in pulp bleaching, bioethanol production and oligosaccharides production (Jun *et al*. 2009, Qiu *et al. 20*10, Kumar *et al*. 2012).

Each organism or strain has its own special conditions for maximum enzyme production. Therefore, optimization of medium composition has to be carried out in order to maintain a balance among various medium components, hence minimizing the amount of non-utilized components at the end of fermentation. An important factor is the selection of the most suitable medium composition to decrease the cost of the bioprocess (Müllen & Silva 2005). This can be achieved by using cheaply available agroindustrial waste. Part of this waste is composed by sugarcane bagasse (SCB), which, in Brazil, comes from the production of sugar and alcohol from sugarcane. SCB is an abundant and low-cost lignocelullosic material, which has about 30–50% of cellulose and 20–24% of lignin (Hernández-Salas *et al*. 2009, Vieira *et al*. 2007).

The main proposals of this study were isolating and identifying fungus species able to produce extracellular enzymes, determining the best conditions for xylanase production using sugarcane bagasse as substrate, and comparing xylanase extraction types.

MATERIAL AND METHODS

Materials

Birchwood xylan used as substrate for xylanase assay was obtained from Sigma Aldrich (São Paulo, São Paulo, Brazil). All chemicals used were of analytical grade.

Fungi identification

The filamentous fungi used in this work were isolated from sugarcane (*Saccharum* sp.), which was provided by the Company of Agricultural Research of Pernambuco (Empresa Pernambucana de Pesquisa Agropecuária-IPA). The sugarcane was removed with all the anatomical parts (leaf, stem and root) and were treated separately with 15% (v/v) sodium hypochlorite solution. Soon after, the samples were crushed. The liquid obtained from the maceration was diluted with 5 mL of sterilized distillated water and inoculated using Drigalsky spatial (1 mL) in Petri dish with Sabouraud agar medium with 0.05% (w/v) yeast extract and 0.005% (w/v) of chloramphenicol and ampicillin, for 3 days at 30 ºC.

The macroscopic study was carried out from purified colonies of filamentous fungi isolated from sugarcane, on Sabouraud agar medium. They were later cultivated on Czapek agar (Bernfeld 1955) composed of 0.3% (w/v) sucrose, 0.03% (w/v) $NaNO_3$, 0.005% (w/v) KCl, 0.005% (w/v) $MgSO_4$, 0.001% (w/v) $FeSO_4.7H_2O$, 0.01% (w/v) K_2HPO_4, 0.2% (w/v) and PDA-potato dextrose agar (Lacaz *et al*. 2002) with 14% (w/v) potato, 0.2% (w/v) dextrose, 0.2% (w/v) agar at 30 ºC for 10 days. The following morphological characteristics were evaluated: colony growth (length and width); presence or absence of aerial mycelium; colony color; presence of wrinkles and furrows; pigment production; and other characteristics, according to Booth (1971), Ellis (1971), Rapper and Fennell (1977), Domsch and Gams (1993) and Pitt (1998).

The microscopic study were carried out from Fungi colonies fragments cultivated at 30 ºC for 7 days on Czapek agar and PDA media in glass microchamber. Previously sterilized coverslips (18 x 18 mm) were placed on the slide with the fragments (Domsch & Gams 1993). Mycelium germination and growth were observed using Amann blue dye under an optical microscope.

Substrate preparation for microorganism growth

In order to increase sugarcane bagasse digestibility, alkaline hydrolysis was performed at 121 ºC for 4h after mixing 100g over-dried chopped bagasse with 1L 0.25 N NaOH solution according to Hernández-Salas *et al.* (2009). After heating, the solid waste was separated by filtration. The hydrolyzate bagasse was washed with deionized water to remove NaOH. The hydrolyzate bagasse remained in heater for 24h at 80 ºC, to remove the excess of water. The hidolyzate bagasse was used as substrate for enzymes productions studies.

Screening for enzyme production by selected Fungi

In the first step, *Penicillium* spp., *Trichoderma auroviride* and *Cladosporium cladosporioides* were selected for enzymes production using sugarcane steam as substrate. The fungi were inoculated in a spore suspension (10^6 spores/mL) and cultured in submerged fermentation (SmF) using Erlenmeyer flasks (250 mL) with 10g sugarcane steam and 25 mL distilled water, which were sterilized at 121 ºC for 20 min. The cultures were incubated in an orbital shaker (180 rpm) at 30˚C for 5 days. Samples were collected every 24h and 2 mL were used for enzyme extraction in an orbital shaker for 10 min in the same culture conditions, with 27 mL of 0.1% (v/v) Tween 80 in 0.15 M saline solution. Supernatant (enzyme extract) was obtained after centrifugation (5500 x

g) for 15 min and assessed to determine the total protein and xylanase, protease and invertase activity.

Enzyme activities

Xylanase activity was assessed using 1% Birchwood xylan (Sigma, USA) in 0.05 M citrate buffer (pH 5.3), according to Bailey *et al*. (1992). The release of reducing sugars was determined by Miller (1959) using 3,5-dinitrosalicylic acid (DNS). A unit of enzymatic activity (U) was defined as the amount of enzyme producing 1 μmol of xylose per minute.

Protease activity on azocasein as a substrate was determined according to Leighton *et al*. (1973) using 1% (w/v) azocasein in a 0.1 M Tris-HCl buffer (pH 7.2). One unit (U) of protease activity was defined as the amount of enzyme required to produce a 0.1 increase in the optical density after 1h at 440 nm.

Invertase activity (β-D-fructofuranosidase fructohydrolase, EC 3.2.1.26) was determined as descried by Robinson *et al*. (1988). Crude extract (1.5 mL) was added to 2.5 mL sucrose solution (0.25 M acetate buffer, pH 5.0) and incubated for 30 min at 37 °C. The reducing sugars produced by sucrose hydrolysis were measured by the DNS method (Bailey *et al*. 1992). One unit of enzyme (U) was defined as the quantity of enzyme that hydrolyses 1 μmol of sucrose to glucose and fructose per minute.

Effect of stirring and temperature on xylanase production using sugarcane bagasse hydrolyzate as substrate

In the second step, the influence of stirring intensity and temperature on xylanese production by *Trichoderma aureoviride* was evaluated at different time intervals. The fermentations were carried out in Erlenmeyer flasks (250 mL) with the production medium with the following composition: 2.5g sugarcane bagasse hydrolyzate, enriched with 25 mL Vogel minimum salts medium. After inoculating spores up to a concentration of 10^6 cells/mL in Erlenmeyer flasks with the production medium, fermentations were performed in an orbital shaker at different stirring intensity (96 and 150 rpm) and temperature (25 and 35 ˚C). Samples were collected after 120h of fermentation and used for the enzyme extraction step, once previous experiments demonstrated that, at this time, xylanase production was high.

Enzyme extraction was obtained after fermentations; 3 mL of the homogenized fermented were suspended and used in the enzyme extraction step, in three different methods: 27 mL of sterilized distilled water, 27 mL of 0.1% (v/v) Tween 80 in 0.15 M saline solution,

Table 1. Macromorphological and micromorphological characteristics of isolated fungi.

Fungi (Genus)	Macroscopic characteristics	Microscopic characteristics
Fusarium	Produces cotton- and velvet-like colonies, which are smooth and scattered. In the front, the color of the colony may be white, cream, salmon, yellow, red, violet, pink, or purple. The back may be colorless, red, dark purple, or brown. (De Hoog *et al.* 2000a, Larone *al.* 1995, Sutton *et al.* 1998)	Hyphae, conidiophores, phialides, macroconidia, and microconides are septate and hyaline. In addition to these basic elements, chlamydospores are also produced by some species. Phialides are cylindrical – with a small collarette –, lonely or produced as components of a complex branched system. The color of the colony, length and shape of macroconidia, the number, shape and arrangement of the fusiform and the presence or absence of chlamydospores are key features for *Fusarium* species differentiation. (De Hoog *et al.* 2000a, Larone *al.* 1995).
Cladosporium	The colonies texture is velvety. Similar to other Deuteromycetes fungi, the colony has an olive green color and its back ranges from dark green to black. (Collier *et al.* 1998, Dixon *et al.* 1991, Sutton *et al.* 1998).	The genus produces brown hyphae, septate, erect and pigmented conidiophores, and conidia. Conidiophores of some species are geniculate, and present conidia, light-colored to brown. The conidial wall is smooth or, occasionally, spiculated. (Collier *et al.* 1998, Sutton *et al.* 1998).
Trichoderma	The colonies are velvety and become compact over time. The front can display white. While the spore form patches bluish green or yellowish green. These patches can sometimes form concentric rings. The back is pale or yellowish. (De Hoog *et al.* 2000a, Larone *al.* 1995, St-Germain-Germain *al.* 1996, Sutton *et al.* 1998).	Hyphae, conidiophores, phialides, and conidia are septate and hyaline can also produce chlamydospores. Conidiophores are branched and may occasionally indicate a pyramidal arrangement. Phialides are attached to the conidiophores at right angles. Phialides may be solitary or arranged in clusters. Spores are round or ellipsoidal. They are smooth or rough, grouped in sticky heads at the tips of phialides. Conidia are mostly green. (De Hoog *et al.* 2000a, Larone *al.* 1995, St-Germain-Germain *al.* 1996, Sutton *et al.* 1998).
Penicillium	*Penicillium* colonies may have smooth, filamentous, velvet- or cotton-like textures. The colonies are initially white and become blue green, green-gray, gray-olive or yellow with time. The back of the card is usually pale to yellowish (De Hoog *et al.* 2000a, Larone *al.* 1995, St-Germain-Germain *al.* 1996, Sutton *et al.* 1998).	They have septate and hyaline hyphae and conidiophores. The conidia and phialides may be simple or branched; conidiophores are formed by the secondary phialides. The organization of the phialides at the tips of conidiophores is very common. (De Hoog *et al.* 2000a, Larone *al.* 1995, St-Germain-Germain *al.* 1996, Sutton *et al.* 1998).

and 27 mL of 0.5 M acetate buffer (pH 5.5). After that, each homogenized fermented was stirred at 110 rpm in shaker for 10 min. The supernatant (enzyme extract) was obtained after centrifugation (5500 x g) for 15 min and used as the enzymatic crude extract.

The protein concentration was determined according to Bradford (1976), using bovine serum albumin as standard.

In addition, all of the analyses and experiments were performed in triplicate, and the results were expressed as mean values. The experimental data errors from the mean values were expressed as standard deviation, using the Microsoft Excel 2000 software (MapInfo Corporation, Troy, NY, USA) and illustrated as error bars.

RESULTS AND DISCUSSION

Fungi isolation and identification

The microorganisms found isolated from all anatomical parts (leaf, stem and root) sugarcane were divided into: bacteria and filamentous fungi in the leaf; bacteria, fungi and yeasts in the root; and only yeast in the stem. Five fungi, isolated from root and leaf, were selected for the purification and identification steps. The isolated fungi were identified for genus and species, if possible, based on the macromorphological and micromorphological characteristics presented in specific culture medium (Table 1).

The purified and identified fungi in Czapek and Sabouraud agar are shown in figure 1. Among the isolated microorganisms, the fungi *Fusarium* oxysporum (Fig. 1A), *Cladosporium cladosporioides* (Fig. 1B), *Penicillium* sp. (Fig. 1C), and *Trichoderma auroviride* (Fig. 1D), were identified. Only the three latter were used for the study of enzyme production, once they presented the lowest degree of pathogenicity. *Cladosporium cladosporioides* culture in Sabouraud agar shows olive-green color colonies and its reverse ranges, from dark green to black texture, different from the others. *Penicillium* sp. Sabouraud agar culture showed macroscopic characteristics different from the same culture in Czapek agar. The colonies are white to yellow in Sabouraud agar culture medium, and blue-green, green-gray and gray-olive in Czapek agar. The reverse of the colonies in both culture media is usually pale to yellowish.

These results are in accordance with some results reported in the literature. *Fusarium, Penicillium, Trichoderma* and *Cladosporium* genus, as well as *Aspergillus* (Damen *et al*. 2012), *Geotrichum*, *Mucor,* and *Oidium* (Dalfré *et al*. 2007), and endophytical fungi like *Curvularia, Monilia, Trichophyton*, and *Mycelia* (Müllen & Silva 2005) were the most frequently encountered ones in the sugarcane and derivates samples.

Yeasts and bacteria genus were also observed in sugarcane agroecosystem. Eighty-two bacteria strains were isolated, such as: Bacillus, Pseudomonas, Serratia, Enterobacter, among others (Lima *et al*. 1999). De Azeredo *et al*. (1998) studied yeast communities associated with sugarcane leaves, stems and rhizosphere and observed that the prevalent genus isolated from sugarcane were *Cryptococcus, Rhodotorula, Debaryomyces, Candida, Sacharomyces*, and *Trichosporon*.

Production of fungi extracellular enzymes using sugarcane steam as substrate

The first step was carried out using sugarcane steam as substrate for xylanase, invertase, and protease production by selected fungi *Penicillium* sp., *Trichoderma auroviride* and *Cladosporium cladosporioides*. Figure 2

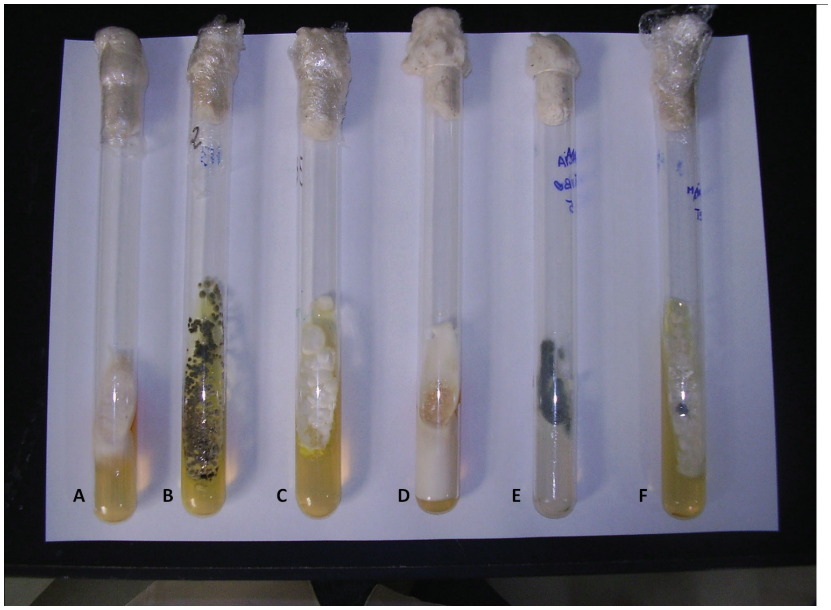

Figure 1. Fungi isolated from sugarcane in Sabouraud and Czapek agar. A. *Fusarium* spp. B. *Cladosporium cladosporioides.* C. *Penicillium* spp. (n. 1). D. *Trichoderma auroviride.* E. *Penicillium* spp. (n. 2) in Czapek agar. F- *Penicillium* spp. (n. 2).

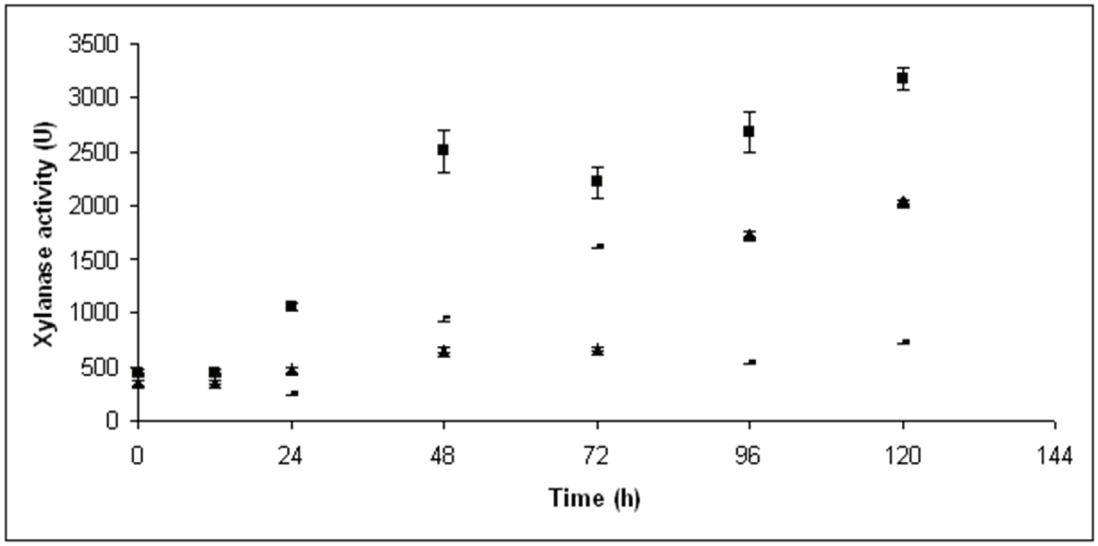

Figure 2. Xylanase production by *T. aureoviride* (▲), *C. cladosporides* (-) and *Penicillium* (■) using sugarcane steam as substrate.

shows xylanase production by *Penicillium* sp., *Trichoderma auroviride* and *Cladosporium cladosporioides* during 120h of cultivation. The highest fungus producer of xylanase was *Penicillium* sp. (3181 U) after 120h of cultivation, followed by *Trichoderma auroviride* (2039.5 U) and *Cladosporium cladosporioides* (1637.5 U) after 120h and 72h of cultivation, respectively. This activity was much higher than that reported by Oliveira *et al*. (2006) for xylanase production by *Penicillium janthinellum* (23 U) using sugarcane bagasse as substrate after 132h of cultivation.

To verify the co-production of other enzymes, invertase and protease concentration were investigated by the selected fungi at the maximum xylanase production time (Table 2). Invertase production obtained by *Trichoderma auroviride* (62.3 U) and *Cladosporium cladosporioides* (58.4 U) was lower than that the one observed with *Penicillium* sp. (762.6 U).

Alegre *et al*. (2009) observed the highest levels of extracellular invertase activity (301 U) under submerged fermentation when *Aspergillus caespitosus* was cultured using agroindustrial wastes, such as wheat bran, as carbon source. The best result was obtained in Khanna medium supplemented with nitrogen ($(NH_4)_2SO_4$ and peptone), phosphate (KH_2PO_4 and Na_2PO_4) and glucose after 72h with wheat bran. Other agroindustrial wastes used as carbon sources in Khanna medium, such as oat meal (6.2 U), rice straw (4.2 U), sugar cane bagasse (2.2 U), among others, also stimulated invertase production and secretion.

Table 2. Invertase and protease detection by the selected fungi at the maximum xylanase production time.

Fungi/Enzymes	Invertase (U)	Protease (U)
Penicillium sp.	762.6	0.04
Trichoderma auroviride	62.3	0.33
Cladosporium cladosporioides	58.4	0.27

In this work, the medium used for enzymes production by the selected fungi was only sugarcane steam plus distillated water, and the activity obtained was much higher than that reported by Alegre *et al*. (2009) for invertase production by *Aspergillus caespitosus* (301 U), using wheat bran medium supplemented with nitrogen ($(NH_4)_2SO_4$ and peptone), phosphate (KH_2PO_4 and Na_2PO_4) and glucose.

A co-production of high proteolytic activity may be a serious problem to maintain the stability of xylanase. However, low levels of protease activity were obtained during the cultivation of three selected fungi (Table 2). These results may be due to the chemical composition of the culture medium (sugarcane steam), which contains low levels of protein. According to Oliveira *et al*. (2006), protease production was very low in all agro-industrial residues because of the low nitrogen source. Similar behavior was also observed by Ferreira *et al*. (1999) using sugarcane bagasse for the production of xylanase by *Aspergillus tamarri.*

Influence of temperature, stirring intensity and enzyme extraction method on xylanase production using sugarcane bagasse as substrate

The goal of this part of the work was to explore the influence of temperature, stirring intensity, and types of enzyme extraction on xylanase production by *Trichoderma aureoviride* (URM 5351), using sugarcane bagasse as substrate. Even though *Penicillium* sp. has presented the highest yields of xylanase and invertase, it was not used for further studies due to the impossibility of identifying it as a species, thus belonging to the fungi pathogenic group.

A 25 mL working volume in 250 mL Erlenmeyer flasks under 150 rpm stirring condition was found to be suitable for more efficient xylanase production by *Trichoderma aureoviride* (600 U). Stirring rates below

Table 3. Relationship between the stirring intensity variable and two different types of enzyme extraction selected at 30 ˚C.

Agitation intensity (rpm)	Tween 80 + saline solution	Sodium acetate buffer pH 5.5	Destillated water
96	420 U	460 U	240 U
150	540 U	600 U	260 U

150 rpm, regardless of the extraction system used (Table 3), resulted in lower xylanase yields, probably due the difficulty in maintaining sufficient dissolved oxygen (DO) level for cell growth. The stirring and aeration processes are used to meet the demand of oxygen during the fermentation processes. Moreover, mechanical stirring is reported to be crucial in fermentative processes due to its effectiveness in mixing contents of media and prevention of cell clumping (Kapoor *et al*. 2008).

Moreover, it is known that temperature is also one of the most critical parameters that must be controlled in the fermentation process (Chi & Zhao 2003). Not only does temperature regulate the synthesis of the enzyme but possibly the secretion of the enzyme, by changing the properties of the cell wall (Anandan *et al*. 2007). In this work, temperature showed a positive effect on xylanase production, and the maximum yield (1980 U) was obtained at 35 ºC (Table 4). It is likely that xylanase secretion was improved at higher temperatures to maintain adequate metabolic fluxes under these stress conditions.

The literature on the production of xylanase from filamentous fungi is extremely broad. To provide a few examples, the maximum xylanase productions from *Penicillium oxalicum* (31.1 U) (Li *et al*. 2007), *Penicilium janthinellum* (28.98 U) (Menon *et al*. 2010) and *Trichoderma reesei* (630 U) (Xiong *et al*. 2005) were found at 31.1 ºC, 28 ºC and 30 ºC, respectively.

The extraction method after enzyme production was another parameter evaluated. Three different methods were employed: sterilized distilled water; 0.1% (v/v) Tween 80 in 0.15 M saline solution; and 0.5 M sodium acetate buffer (pH 5.5). The best enzyme recovery (1900 U) was obtained using the sodium acetate buffer method, followed by Tween 80 in saline solution (1840 U) (Table 4). The distillated water extraction method was not as efficient as the other two. This observation is pursuant to the results of Rezende *et al*. (2002), who obtained a maximum xylanase extraction (15 U) using both extraction methods (Tween 80, 0.1% (v/v), in physiological saline and 50 mM sodium acetate buffer, pH 5.0). Silva *et al*. (1999) also efficiently extracted the xylanase produced (1234 U) by *Aspergillus fumigatus* from the fermentation broth using 0.025 M sodium acetate buffer, pH 5.0.

Among the results of the influence of variables on the cultivation step and the enzyme extraction method used, the best result of xylanase production by *Trichoderma aureoviride* (1900 U) in sugarcane bagasse as substrate was observed at 35 °C, at150 rpm stirring intensity after 120h of cultivation using sodium acetate buffer solution as enzyme extraction method.

Table 4. Relationship between the temperature variable and two different types of enzyme extraction selected at 150 rpm.

Temperature (˚C)	Tween 80 + saline solution	Sodium acetate buffer pH 5.5	Destillated water
25	540 U	600 U	340 U
35	1840 U	1900 U	380 U

CONCLUSION

The results obtained in the present paper reveal the potential of sugarcane steam and sugarcane bagasse as an alternative and cheap substrate for the production of enzymes, especially xylanase by fungus isolated from sugarcane. The influence of two variables - temperature and stirring intensity - showed a considerable impact on xylanase production by the new isolate *Trichoderma aureoviride*, using sugarcane bagasse hydrolyzed as substrate. The best result of xylanase production (1900 U) was obtained at 35 °C with 150 rpm stirring intensity, after 120h of cultivation using sodium acetate buffer solution as enzyme extraction method by *Trichoderma aureoviride*.

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