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Research Paper

Efficient production of lignocellulolytic enzymes xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by the mutant strain *Aspergillus awamori* 2B.361 U2/1

Leda Maria Fortes Gottschalk[1], Raquel de Sousa Paredes[2], Ricardo Sposina Sobral Teixeira[2], Ayla Sant'Ana da Silva[2], Elba Pinto da Silva Bon[2]

[1]Bioprocess Laboratory, Embrapa Food Technology, Rio de Janeiro, RJ, Brazil.
[2]Chemistry Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

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Abstract

The production of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by *Aspergillus awamori* 2B.361 U2/1, a hyper producer of glucoamylase and pectinase, was evaluated using selected conditions regarding nitrogen nutrition. Submerged cultivations were carried out at 30 °C and 200 rpm in growth media containing 30 g wheat bran/L as main carbon source and either yeast extract, ammonium sulfate, sodium nitrate or urea, as nitrogen sources; in all cases it was used a fixed molar carbon to molar nitrogen concentration of 10.3. The use of poor nitrogen sources favored the accumulation of xylanase, β-xylosidase and ferulic acid esterase to a peak concentrations of 44,880; 640 and 118 U/L, respectively, for sodium nitrate and of 34,580, 685 and 170 U/L, respectively, for urea. However, the highest β-glucosidase accumulation of 10,470 U/L was observed when the rich organic nitrogen source yeast extract was used. The maxima accumulation of filter paper activity, xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by *A. awamori* 2B.361 U2/1 was compared to that produced by *Trichoderma reesei* Rut-C30. The level of β-glucosidase was over 17-fold higher for the *Aspergillus* strain, whereas the levels of xylanase and β-xylosidase were over 2-fold higher. This strain also produced ferulic acid esterase (170 U/L), which was not detected in the *T. reesei* culture.

Key words: *Aspergillus awamori* 2B.361 U2/1, cellulases, hemicellulases, β-glucosidase, nitrogen nutrition.

Introduction

The demand to establish renewable feedstock for the production of chemicals and fuels is pressing on the development of the necessary technologies to process biomass both efficiently and economically. The potential use of these residues worldwide is indeed significant; considering biomass availability in Brazil the agroindustry of corn, sugarcane, rice, cassava, wheat, citrus, coconut and grass collectively generate 597 million tons of residues per year (Ferreira-Leitão *et al.*, 2010). For biomass processing, efforts have been made towards the production of efficient and low cost enzyme blends to hydrolyse both the cellulose and the hemicellulose part of the biomass. Efficient cellulose hydrolysis requires the cooperative action of endoglucanases (EC 3.2.1.4) and exoglucanases (EC 3.2.1.91), which release cellooligosaccharides and cellobiose, respectively. The enzyme β-glucosidase (EC 3.2.1.21) cleaves the disaccharide cellobiose into two molecules of glucose - the end product of cellulose hydrolysis (Zhang *et al.*, 2009). The enzymes used for the cellulose degradation have been traditionally produced mostly by fungi belonging to the genus *Trichoderma* (Gosh and Gosh, 1992) nevertheless its low β-glucosidase titre (Stockton *et al.*, 1991). As such, β-glucosidase supplementation is necessary to hydrolyze cellobiose, which is also a strong inhibitor of endo- and exo-glucanase (Howell and Stuck, 1975). To overcome the

Send correspondence to: L.M.F. Gottschalk. Embrapa Food Technology, Bioprocess Laboratory, Av. das Américas 29501, CEP 23020-470, Rio de Janeiro, RJ, Brazil. E-mail: leda@ctaa.embrapa.br.

Trichoderma enzyme pool deficiency, studies have been carried out on its supplementation with *Aspergillus* enzymes (Duff *et al.*, 1987; Duenas *et al.*, 1995; Gutierrez-Correa and Tengerdy, 1997; Wen and Chen, 2005; Bon *et al.*, 2009; Gottschalk *et al.*, 2010).

Hemicellulose, which is, after cellulose the second most abundant polysaccharide available in nature (Bastawde, 1992), is processed by the depolymerizing xylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37) and debranching enzymes such as acetyl esterases (EC 3.1.1.6), α-arabinofuranosidases (EC 3.2.1.55), ferulic acid esterases (EC 3.2.1.73) *p*-coumaroyl esterases (EC 3.2.1.73) and α-glucuronidase (EC 3.2.1.139). The importance of developing balanced xylanase enzyme pools, containing endo-acting, exo-acting and debranching activities, is crucial to hydrolyse the heterogeneous hemicelluloses structure, allowing the use of the rich C5 sugars syrups stream as feedstock in industrial yeast or bacterial fermentations, for the production of a range of fuels and chemicals such as ethanol, xylitol, 2,3-butanediol, acetone, isopropanol, butanol and hydrogen. Other products include carbon dioxide and organic acids, such as butyric acid, acetic acid, formic acid, succinic acid and lactic acid (Rosenberg, 1980), which presents a higher commercial value in comparison to the ethanol fuel. *Aspergillus* spp have been widely used as sources of industrial enzymes such as β-xylosidases (Kurakake *et al.*, 2005), β-D-manosidases; β-D-mananases (Kurakake and Tomaki, 2001), α-galactosidases (Neustroev *et al.*, 1991), acetyl esterases (Koseki *et al.*, 1997), ferulic acid esterases (Koseki *et al.*, 2006), β-glucosidases (Anindyawati *et al.*, 1998) and proteinases (Ahmed *et al.*, 2011). The industrial strain *Aspergillus awamori* 2B.361 U2/1 stands out for its ability to secrete high levels of glucoamylase (Bon and Webb, 1989, 1993; Pavezzi *et al.*, 2008), xylanases and polygalacturonases (Lemos *et al.*, 2000; Botella *et al.*, 2007; Umsza-Guez *et al.*, 2011).

This work aimed to further the knowledge on the ability of the industrial strain *Aspergillus awamori* 2B.361 U2/1 to efficiently secrete an enzyme pool containing xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase, which act on biomass. The fungus physiological response was studied concerning the accumulation of these enzymes, under comparative and selected cultivation conditions regarding the nitrogen nutrition, using amino nitrogen, NH_4+, NO_3- or urea. For comparison, the enzymes activity profile of *Aspergillus awamori* 2B.361 U2/1 was compared to that produced by *Trichoderma reesei* Rut-C30.

Materials and Methods

Microorganisms, maintenance and propagation

Trichoderma reesei Rut-C30 (ATCC 56765) and *Aspergillus awamori* 2B.361 U2/1, a sequential mutant of *A. awamori* 3112 (Bon and Webb, 1933; Bon *et al.*, 2007; Gottschalk *et al.*, 2010) were cultured in Petri dishes containing PDA (Potato Dextrose Agar) for seven days at 30 °C. Spore suspensions were obtained by addition of NaCl 0.9% (w/v) in sporulating Petri plates and subsequently lightly scraping the cultures. The suspensions were centrifuged for 15 min in a Beckman-Coulter Allegra 6R centrifuge and the spores were preserved in glycerol 20% (v/v) at -18 °C.

Enzymes production

The effect of the nitrogen sources on the production of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by *A. awamori* 2B.361 U2/1 was evaluated in a buffered growth media containing 30 g wheat bran/L (WB) as carbon source, and either yeast extract (YE), $(NH_4)_2SO_4$, $NaNO_3$ or urea as nitrogen sources, presenting in all cases a fixed millimolar carbon / millimolar nitrogen ratio (C/N) of 10, plus salts (g/L): 3.0 KH_2PO_4, 6.0 K_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.05 $CaCl_2.2H_2O$ (Table 1). For comparison *Trichoderma reesei* Rut-C30 was cultivated in a liquid medium containing (g/L): 30 lactose, 6.0 yeast extract, 0.3 urea, 0.6% (v/v) corn steep liquor, plus salts (g/L): 1.4 $(NH_4)_2SO_4$, 2.0 KH_2PO_4, 0.3 $CaCl_2$ and 0.3 $MgSO_4.7H_2O$, and trace elements (mg/L): 5.0 $FeSO_4.7H_2O$, 20 $CoCl_2$, 1.6 $MnSO_4$ and 1.4 $ZnSO_4$ (Mandels and Weber, 1969). Enzymes production by both fungi was carried out in 1000 mL Erlenmeyer flasks con-

Table 1 - Percentage of carbon and nitrogen concentration in mmol/L of carbon and nitrogen in different nitrogen sources and C/N ratio of each medium.

Medium	Nitrogen and carbon sources	Concentration (g/L)	% C	% N	[C] (mmol/L)	[N] (mmol/L)	C/N ratio
$NaNO_3$	sodium nitrate	3.5	0.0	16.48	0.0	41.18	10.3
	wheat bran	30	57.31	4.6	1431.44	98.52	
YE	yeast extract	15	45.0	8.9	561.98	95.31	10.3
	wheat bran	30	57.31	4.6	1431.44	98.52	
$(NH_4)_2SO_4$	ammonium sulphate	2.7	0.0	21.18	0.0	40.83	10.3
	wheat bran	30	57.31	4.6	1431.44	98.52	
Urea	urea	1.29	20.0	46.65	21.48	42.96	10.3
	wheat bran	30	57.31	4.6	1431.44	98.52	

taining 300 mL of growth medium. After sterilization, the culture media were inoculated with a 1% (v/v) of spore suspension such that it was obtained a final concentration of 10^6-10^7 spores/mL growth medium. Triplicate cultures were incubated for seven days in a rotary shaker (New Brunswick model INNOVA 4340) at 30 °C, 200 rpm. The wheat bran used in this work presented a composition of 70% of carbohydrates and 20% of proteins. To calculate the carbon and nitrogen millimolar concentration in each media, the studies of Jones and Gersdorff (1925) and Nandini and Salimath (2001) were considered. According to Jones and Gersdorff (1925), wheat bran presents 1.6% of free amino nitrogen and its proteins contain 53% of carbon and 15% of nitrogen. Thus, considering only the protein fraction, the wheat bran used in this study presented 10.6% of carbon and 4.6% of nitrogen. According to Nandini and Salimath (2001) the carbohydrate fraction of wheat bran is composed of 37% of arabinose, 27% of xylose and 26% of glucose. Based on these data, the amount of carbon on each sugar was calculated, resulting in a total carbon of 46.71%, considering only the carbohydrate fraction.

Enzymes activity assays

The culture supernatants were used for the determination of FPase, CMCase, xylanase, β-glucosidase, β-xylosidase and ferulic acid esterase activities. All measurements were performed in duplicates. Filter paper activity (FPA), carboxymethyl cellulase activity (CMCase) and β-glucosidase (BGU) were determined according to standard IUPAC procedures and expressed as international unit (U) (Ghose, 1987). One unit of FPase or CMCase activity corresponded to the formation of 1μmol of reducing sugar (glucose equivalent) per min using as substrates a 6.0 x 1.0 cm filter paper Whatman No.1 strip or 4% carboxymethylcellulose, respectively. Xylanase activity was determined by mixing 50 μL of enzyme solution with 100 μL of soluble fraction of oat spelt xylan (1%, w/v) in 100 mM sodium acetate buffer, pH 5.0 at 50 °C for 30 min (Teixeira *et al.*, 2010). One unit of xylanase activity was defined by the formation of 1μmol of reducing sugar (xylose equivalent) per minute. Reducing sugars were estimated by 3,5-dinitrosalicylic acid (DNS) method prepared without phenol and metabisulfite (Teixeira *et al.*, 2012). Glucose or xylose were used as standard. One unit of β-glucosidase activity corresponded the formation of 1 μmol of glucose per min using cellobiose as substrate. Glucose concentrations were measured using a Biochemistry Analyzer YSI 2700. Ferulic acid esterase (FAE) activity was assayed by measuring the release of ferulic acid in a reaction mixture containing 10 μL of enzyme solution, 20 μL of 1% of ethyl ferulate in dimethylsulfoxide (DMSO), 100 μL of 1 M acetate buffer (pH 5.0), plus 870 μL of water, at 50 °C for 20 min. Reaction was terminated by boiling the reaction mixture for 5 min and the ferulic acid quantified by HLPC. One unit of FAE corresponded to the formation of 1μmol of ferulic acid per minute. β-xylosidase activity was determined in a reaction mixture containing 50 μL of an appropriately diluted enzyme solution, 100 μL of 10 mM p-nitrophenyl-β-D-xylopyranoside, 200 μL of 0.5 M sodium acetate buffer pH 5.0 plus 650 μL Milli-Q water, at 50 °C for 10 min. Reaction was terminated by adding 500 μL of 1 M Na_2CO_3. The concentration of p-nitrophenol, which is the reaction product, was measured at 400 nm. One unit of β-xylosidase was defined as the amount of enzyme that released 1 μmol of *p*-nitrophenol at 50 °C in 1 min.

Results

Effect of YE, $(NH_4)_2SO_4$, $NaNO_3$ or urea on the production of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by *A. awamori*

The maximal xylanase, ferulic acid esterase and β-xylosidase produced by *A. awamori*, as well as the cultivation time to reach the corresponding peak activities using YE, $(NH_4)_2SO_4$, $NaNO_3$ or urea as nitrogen sources, are presented in Figure 1a, 1b and 1c. Ammonium, nitrate or urea resulted in high levels of xylanase (U/L) (28,300 ± 3,950, 44,880 ± 1,620 and 34,580 ± 1,880), β-xylosidase (U/L) (390 ± 120, 640 ± 70 and 685 ± 110) and ferulic acid esterase (U/L) (183 ± 19, 118 ± 3 and 170 ± 32), respectively. Media containing inorganic nitrogen or urea favored these enzymes accumulation in comparison to that containing the more expensive YE (12,900 ± 330 U/L for xylanase; 210 ± 20 U/L for β-xylosidase and 63 ± 2 U/L for ferulic acid esterase). Nitrate favored xylanase (44,880 ± 1,620 U/L), urea favored β-xylosidase (685 ± 110 U/L) and ammonium favored ferulic acid esterase (183 ± 19 U/L) accumulation, whose levels were over three-fold higher than that observed for the use of YE.

The maximal β-glucosidase produced by *A. awamori*, using YE, $(NH_4)_2SO_4$, $NaNO_3$ or urea as nitrogen sources, as well as the cultivation time to reach the corresponding peak activities, are presented in Figure 1b. The response for β-glucosidase production was quite the opposite, as the medium containing yeast extract greatly favored the accumulation of this enzyme. As such, higher levels of β-glucosidase activity were obtained with the YE medium (10,470 ± 490 U/L). The aforementioned average levels were 2 to 3 fold higher than that observed for the use of $NaNO_3$ (4,460 ± 110 U/L), $(NH_4)_2SO_4$ (3,610 ± 870 U/L) or urea (4,770 ± 940 U/L) as nitrogen source.

Correlation of pH profiles of cultivations on media containing different nitrogen sources and xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase production by *A. awamori*

The pH profiles of the cultivations which were conducted in this study are presented in Figure 2. The metabolism of wheat bran, used as carbon source, jointly with

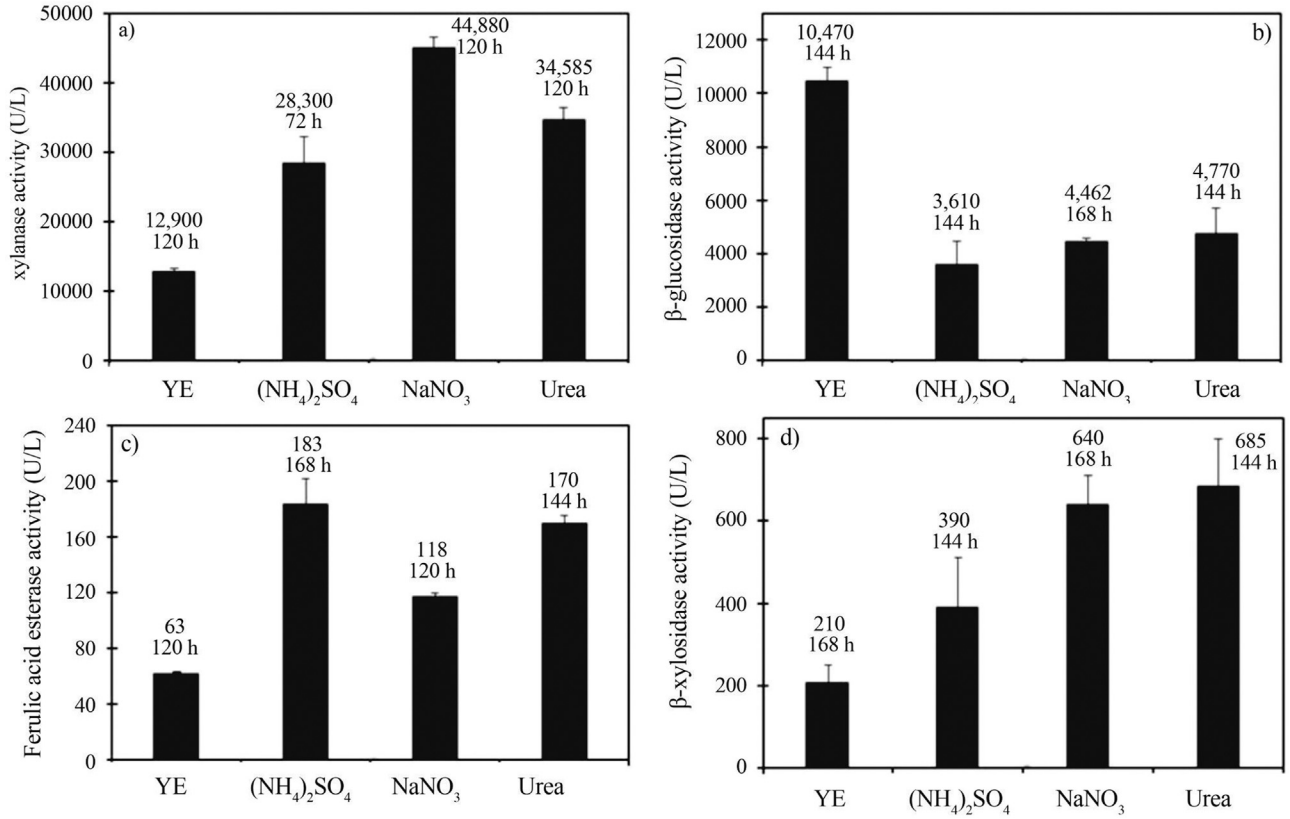

Figure 1 - Maximal accumulation of xylanase (a), β-glucosidase (b), ferulic acid esterase (c) and β-xylosidase (d) in the culture supernantants of *Aspergillus awamori* 2B.361 U2/1 comparing media with yeast extract (YE), sodium nitrate ($NaNO_3$), ammonium sulphate ($(NH_4)_2SO_4$) or urea as nitrogen sources.

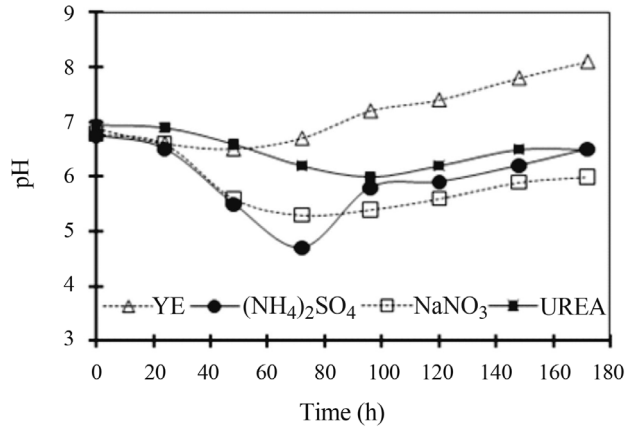

Figure 2 - pH profiles throughout the *A. awamori* fermentations using wheat bran as carbon source and yeast extract (YE), sodium nitrate ($NaNO_3$), ammonium sulphate ($(NH_4)_2SO_4$) or urea (UREA) as nitrogen sources.

either YE, $NaNO_3$, $(NH_4)_2SO_4$ and urea resulted on an initial pH decrease which was followed by pH increase whose range and time scale responded to each particular the nitrogen source. Minima and maxima pH values for the YE containing medium were in the range of 6.5 to 8.1, whereas for nitrate and ammonium were of 5.3 to 6.8 and 4.7 to 6.8, respectively. Urea showed the smallest pH range variation from 6.0 to 7.0. This is a quite interesting feature at industrial scale as the fermentation pH control could not be necessary. Peak enzyme activities for the YE, $(NH_4)_2SO_4$, $NaNO_3$, and urea media were observed on the following pH ranges: 5.6 to 6.7 (xylanase), 6.0 to 7.8 (β-xylosidase), 5.6 to 8.1 (ferulic acid esterase) and 5.9 to 8.1 (β-glucosidase). Higher xylanase, β-xylosidase and ferulic acid esterase activity levels were observed in the pH range 5.5 to 6.5 which might indicate a higher stability of the enzyme protein under this pH range. As for β-glucosidase, the higher activity level was observed at pH values around 8.0, suggesting both enzyme release due to cell lyses and the enzyme protein high stability at this alkaline pH value.

Time course for the accumulation of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase in growth medium containing YE or urea as nitrogen source

According to data presented on Figure 3a the medium containing 30 g WB/L and 15 g YE/L significantly favored β-glucosidase accumulation (10,470 ± 490 U/L), which was build up after the 3rd fermentation day and concomitant to pH rise (Figure 2) suggesting enzyme release via cell lyses. β-xylosidase accumulation showed a similar pattern

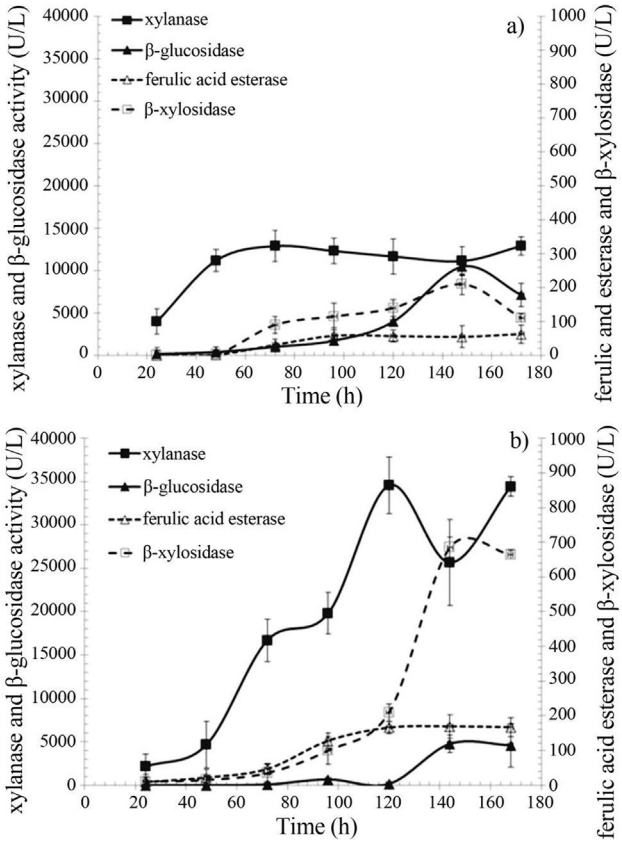

Figure 3 - Xylanase, β-glucosidase, ferulic acid esterase and β-xylosidase production profile of in the culture supernantants of *Aspergillus awamori* 2B.361 U2/1 using wheat bran as carbon source and (a) YE or (b) Urea as nitrogen source.

and peaked during the pH rise stage, reaching 210 ± 20 U/L. Xylanase and ferulic acid esterase accumulation, which showed to be growth associated, picked within 72 h of cultivation with maximal enzymes concentrations of 12,900 ± 330 and 63 ± 2 U/L, respectively. All enzymes, except β-xylosidase, were quite stable under the cultivation conditions, which were carried out at 30 °C and presented a pH span from 6.2 to 8.1.

The use of urea as nitrogen source (Figure 3b) favored significantly the build up of xylanase and ferulic acid esterase in the early cultivation stages, reaching 34,580 ± 1,880 and 170 ± 32 U/L, respectively. The production of β-xylosidase was also greatly improved by the use of urea, a poor nitrogen source, such that it reached 685 ± 110 U/L, a value three-fold higher than that observed using the yeast extract. However, β-glucosidase accumulation of 4,770 ± 940 U/L decreased two-fold.

Comparison between *A. awamori* 2B.361 U2/1 and *T. reesei* Rut-C30 enzymes production profile

The profile for cellulases and xylanases accumulation by *T. reesei* Rut-C30 and *A. awamori* 2B.361 U2/1 were compared to further the understanding on their enzyme pools characteristics and activities balance towards biomass hydrolysis (Table 2). The culture media used for *T. reesei* Rut-C30 enzyme production was chosen in accordance with the most commonly described conditions for cellulases production by this microorganism. Thus, by comparing the *A. awamori* 2B.361 U2/1 and *T. reesei* Rut-C30 enzymes production profile it is possible to assess the adequacy of *A. awamori* crude extract to supplement *T. reesei* Rut-C30 enzymes in order to obtain an effective blend for biomass hydrolysis. It was found that *A. awamori* 2B.361 U2/1, grown in YE produced outstanding β-glucosidase levels (10,470 ± 490 U/L) as compared to *T. reesei* Rut-C30 (600 ± 50 U/L). However, the levels of CMCase (2,500 ± 140 U/L) and FPase (190 ± 10 U/L), in this medium were approximately 5- to 10- fold lower than those observed for *T. reesei* Rut-C30 (25,000 ± 1,970 U/L of CMCase activity and 1,200 ± 140 U/L of FPase activity).

Table 2 - Maximal accumulations of FPase, CMCase, β-glucosidase, xylanase, β-xylosidase, ferulic acid esterase in the culture supernantants of *A. awamori* 2B.361 U2/1 and *T. reesei* RUT-C30 (means values ± standard deviations).

Enzyme activities	*Aspergillus awamori* 2B.361 U2/1	*Trichoderma reesei* Rut-C30
	Supernatant concentration (U/L)	
Filter paper activity	190 ± 10[a]	1,200 ± 140
Carboxymethyl cellulase	2,500 ± 140[a]	25,000 ± 1,970
β-glucosidase	10,470 ± 490[a]	600 ± 50
Xylanase	34,580 ± 1,880[b]	15,000 ± 700
β-xylosidase	685 ± 110[b]	290 ± 25
Ferulic acid esterase	170 ± 32[b]	0

[a]Activities levels obtained using medium containing YE as nitrogen sources.
[b]Activities levels obtained using medium containing urea as nitrogen source.

Xylanase and β-xylosidase accumulation was also high in the urea medium, reaching 34,590 ± 3,250 and 685 ± 110 U/L, respectively, that compares well to those produced by the *T. reesei* Rut-C30, of 15,000 ± 700 and 290 ± 25 U/L, respectively. Moreover, *A. awamori* produced high levels of ferulic acid esterase (170 ± 32 U/L), an enzymatic activity that was not detected in the *T. reesei* supernatant. The use of a concentrate enzymatic blend which was obtained with the supernatants of *A. awamori* 2B.361 U2/1 and *T. reesei* Rut-C30 and presenting the following activity profile (2,000 U/L of FPase, 24,000 U/L of CMCase, 23,000 U/L of β-glucosidase, 52,000 U/L of xylanase) has been already used to efficiently hydrolyze sugarcane bagasse (Gottschalk *et al.*, 2010).

Discussion

In this work, the production of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase by *Aspergillus awamori* 2B.361 U2/1 was evaluated using selected conditions regarding nitrogen nutrition. Nitrogen nutrition is an important factor when industrial fermentation process are taken into account as it has a high impact on costs and it may also selectively affect both, cell growth and product formation in response to the basic biochemical steps related to their use by the cell. As far as amino acids are concerned, they are assimilated and directly incorporated into proteins, and are not first degraded into ammonia. This process favors biomass accumulation, and the available carbon source is highly allocated into it. Abundant nitrogen is incorporated into cell constituents with a consequent increase in the rate of respiration and the carbon source consumption. With respect to ammonium, nitrate and urea, their use is limited by the rate at which these components are incorporated into their organic counterparts. Furthermore, nitrate and urea goes, beforehand, through specific metabolic pathways which convert its nitrogen content into the readily metabolized ammonia (Bon and Webb, 1993). In this work, media containing inorganic nitrogen or urea favored enzymes accumulation in comparison to that containing the more expensive yeast extract. The advantages of urea for enzymes production are well orchestrated with the need to decrease the cost of fermentations for industrial enzymes production. Indeed, while the cost of YE can reach US$ 1,900 per ton, the cost of urea of US$ 360-380 per ton, is five-fold lower (Nascimento *et al.*, 2010).

Concerning the carbon source, preliminary experiments carried out in our laboratory showed that wheat bran was an adequate carbon source for *A. awamori* cultivation. Wheat bran is composed predominantly of non-starch carbohydrates, starch and crude protein. The non-starch carbohydrates are primarily arabinoxylans, cellulose and β-(1-3)(1-4)-glucan, which may induce xylanase production (Haltrich *et al.*, 1996; Sun *et al.*, 2008). Indeed, high levels of xylanase were obtained in this work when wheat bran was used as carbon source. It was also reported that *A. awamori* produces high levels of ferulic acid esterase when cultivated on wheat bran medium (Kaunachi *et al.*, 2008).

In uncontrolled pH fermentations, variations in the early stages are mostly related to the microorganism physiological response towards substrates uptake, whereas in the later stages cell lyses may play a bigger role. Lyses are followed by proteolysis and aminoacids deamination with their conversion into the corresponding keto acid by the removal of the amine functional group as ammonia. This augment on ammonia concentration in the growth media leads to pH increase. Thus, the enzyme activity peaks presented in here might reflect a balance between the excretion of the enzyme and its external inactivation by a denaturating pH environment. Regarding xylanase activity, it was reported that the crude xylanase from *A. carneus* M34 was stable in the range of 3-10 (> 70% relative activity) for 24 h at room temperature. It is also reported that *A. japonicus* xylanase retained more than 80% of its original activity over a wide pH range, from 2.0 to 9.0 (Wakiyama *et al.*, 2010). These results substantiate the finding that most crude xylanases of fungal origin are stable over a broad pH range. Concerning the β-xylosidase and ferulic acid esterase, *A. japonicus* β-xylosidase had retained more than 90% of its original activity between pH 2.0 and 7.0 at room temperature for 3 hours (Wakiyama *et al.*, 2008) and the *A. awamori* ferulic acid esterase showed to be stable within the pH range 4.5 to 6.5 (Fazary *et al.*, 2009). The pH studies for fungal β-glucosidase showed the enzyme to be stable up to 60 min at 50 °C over a pH range of 4.0-8.0 (Peralta *et al.*, 1997) and a maximum activity in an acidic pH range 4.0-6.0 (Joo *et al.*, 2010; Teixeira *et al.*, 2010). The *A. awamori* β-glucosidase produced in this work showed to be quite stable in a pH value as high as 8.1, in accordance to the literature.

Additionally, enzyme levels of *A. awamori* were compared to that of *T. reesei* Rut-C30 aiming to find out complementary activities and activity levels to design a complete blend for biomass enzymatic hydrolysis. *A. awamori* produces β-glucosidase in high yields which is of importance for the supplementation of the *T. reesei* cellulases pool. However, the highest β-glucosidase accumulation was observed when yeast extract was used, which is a rich organic nitrogen source that favors fungal biomass accumulation but very costly for industrial processes.

In conclusion, *A. awamori* 2B.361 U2/1 showed to be an outstanding fungal strain for the production of xylan hydrolyzing enzymatic pool. It was shown that *A. awamori* performed well when urea was used as a nitrogen source, which comparatively presents cost advantage and keeps the cultivation pH within a desirable fluctuation range. Under these conditions, this work presented a feasible process for the production of xylanase, ferulic acid esterase and β-xylosidase, which are of outmost importance for the supplementation of cellulases preparations aiming for the biomass saccharification.

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