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Research Paper

Screening of thermotolerant and thermophilic fungi aiming β-xylosidase and arabinanase production

Vivian Machado Benassi[1], Rosymar Coutinho de Lucas[1], João Atílio Jorge[2], Maria de Lourdes Teixeira de Moraes Polizeli[2]

[1]Departamento de Bioquímica e Imunologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.
[2]Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

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Abstract

Plant cell wall is mainly composed by cellulose, hemicellulose and lignin. The heterogeneous structure and composition of the hemicellulose are key impediments to its depolymerization and subsequent use in fermentation processes. Thus, this study aimed to perform a screening of thermophilic and thermotolerant filamentous fungi collected from different regions of the São Paulo state, and analyze the production of β-xylosidase and arabinanase at different temperatures. These enzymes are important to cell wall degradation and synthesis of end products as xylose and arabinose, respectively, which are significant sugars to fermentation and ethanol production. A total of 12 fungal species were analyzed and 9 of them grew at 45 °C, suggesting a thermophilic or thermotolerant character. Additionally *Aspergillus thermomutatus* anamorph of *Neosartorya* and *A. parasiticus* grew at 50 °C. *Aspergillus niger* and *Aspergillus thermomutatus* were the filamentous fungi with the most expressive production of β-xylosidase and arabinanase, respectively. In general for most of the tested microorganisms, β-xylosidase and arabinanase activities from mycelial extract (intracellular form) were higher in cultures grown at high temperatures (35-40 °C), while the correspondent extracellular activities were favorably secreted from cultures at 30 °C. This study contributes to catalogue isolated fungi of the state of São Paulo, and these findings could be promising sources for thermophilic and thermotolerant microorganisms, which are industrially important due to their enzymes.

Key words: screening, β-D-xylosidase, arabinanase, thermotolerant, thermophilic.

Introduction

The plant cell wall polysaccharides biodegradation is an important biotechnological process for obtaining monosaccharides which are useful in different industrial processes, such as fermentation for the generation of bioethanol, and biological activities including antioxidant activity, blood and skin related effects, anti-allergy, anti-infection, anti-inflammatory properties and selective cytotoxic activities (Chapla *et al.*, 2012; Ranaval *et al.*, 2010).

The plant cell wall lignocelulolytic biomass is mainly composed by cellulose (30-45%); hemicellulose (20-30%); and lignin (5-20%). The heterogeneous structure and composition of the hemicellulose are key impediments to its depolymerization and subsequent utilization in fermentation processes (Barr *et al.*, 2012).

Xylan, a major component of hemicellulose, is a highly branched β-1,4-linked D-xylose polymer with substituents that include acetyl, arabinosyl, and the glucuronyl groups. The complete degradation of xylan requires the action of several types of enzymes: endo-β-1,4-xylanase, β-xylosidase, α-arabinofuranosidase, α-glucuronidase, acetylxylan esterase, and ferulic acid esterase (Kim and Yoon, 2010). β-D-Xylosidases (β-D-xyloside xylohydrolase; EC 3.2.1.37) hydrolyze short xylooligosaccharides

Send correspondence to M.L.T.M. Polizeli. Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, 14040-901, Ribeirão Preto, SP, Brazil. E-mail: polizeli@ffclrp.usp.br.

and xylobiose from the non-reducing end to liberate xylose. True β-xylosidases are able to cleave artificial substrates like *p*-nitrophenyl-β-D-xyloside. These enzymes appear to be mainly cell associated in bacteria and yeasts. However, extracellular xylosidase activities have also been reported (Knob *et al.*, 2010).

Arabinan is one the most important primary cell wall components of different families of plants in seeds, fruits, and roots. This polysaccharide is associated to pectic substances side-chains or as free polymers unattached to pectic domains. Its chemical structure usually consists of α-1,5-linked L-arabinofuranosyl units, but variably branched at *O*-2 and/or *O*-3 by single arabinosyl residues. Arabinan is linked to rhamnogalacturonan in the plant cell walls, but it is possible to find some phenolic esters between the rhamnogalacturonan polymers, such as feruloyl or coumaroyl (Damásio *et al.*, 2012; Hara *et al.*, 2013).

There are two types of arabinases, the exo-acting α-L-arabinofuranosidase (EC 3.2.1.53), which is active against *p*-nitrophenyl-α-L-arabinofuranosides and on branched arabinans, and the endo-1,5-α-arabinanase (EC 3.2.1.99), which is active only toward linear arabinans. These enzymes hydrolyze 1,5-α-arabinans, but they are not able to hydrolyze the chromogenic substrate phenyl-α-L-arabinofuranoside or gum arabic (Wong *et al.*, 2008).

Many microorganisms including bacteria, yeasts and fungi are known to produce different types of hemicellulases and the nature of the enzymes varies among different organisms. In the case of arabinan-degrade enzymes, little is known about this group, some researchers have been isolating them from different sources, and others have been trying to clone and express these enzymes. It can be reported, for example, the study of Hong *et al.* (2009), which expressed a thermotolerant recombinant endo-1,5-α-L-arabinanase from *Caldicellulosiruptor saccharolyticus*. Other study from Wong *et al.* (2008), showed a novel exo-α-1,5-L-arabinanase that was cloned and expressed heterologously, the gene was isolated from rumen microbial metagenome. In this case this enzyme did not work at high temperatures, but show exo-activity, which is few reported in the literature. Most of the xylan-degrading enzymes are produced by mesophilic and thermophilic microorganisms, and have been found and studied in bacteria and fungi, and filamentous fungi have been widely used as enzyme producers and generally are considered more potent producers of xylanases than bacteria and yeasts (Polizeli *et al.*, 2005; Pedersen *et al.*, 2007). Among the mesophilic fungi, the commercial production of xylanases is highlighted in the genera *Aspergillus* and *Trichoderma*, due to its potential for thermotolerance and production of thermostable enzymes (Pedersen *et al.*, 2007). These enzymes are of interest because the increase of reaction temperature, generally increases the reaction rate and reduces the risks of microbial contamination (Collins *et al.*, 2005).

Most of the microorganisms currently used for ethanol production from lignocellulosic and starchy sugars are mesophiles with optimum of growth and fermentation temperatures varying between 28 °C and 40 °C (Voronovsky *et al.*, 2009). Currently, there is a large industrial interest in the isolation and study of thermophile/thermotolerant microorganisms capable of producing thermostable enzymes which are resistant to high temperatures (Ramírez *et al.*, 2012). This is because the enzymes from thermophilic organisms show resistance to extreme physical and chemical conditions, once these enzymes can work efficiently at high temperatures and low pH, characteristics required for the pretreatment of lignocellulose biomasses (Girfoglio *et al.*, 2012).

Some of the fungi analyzed in this study had already been studied in our laboratory for the production of xylanase, as *A. phoenicis* (Rizzatti *et al.*, 2001); *A. caespitosus* (Sandrim *et al.*, 2005); *A. niveus* (Peixoto-Nogueira *et al.*, 2009); *A. niger* (Betini *et al.*, 2009), *A. terricola* (Michelin *et al.*, 2012b) and *A. ochraceus* (Betini *et al.*, 2009), and these were included in the "screening", to be good producers of xylanases. In addition arabinanases and xylosidases are important enzymes that work hydrolyzing backbone and side chain of hemicellulose, they act helping in the process of lignocellulosic material deconstruction. Accordingly, this work aimed to screen important thermophilic/thermotolerant fungi to study the production of β-xylosidase and arabinanase.

Materials and Methods

Microorganisms and maintenance

The fungi used in this study were collected from several regions of the São Paulo State (Brazil), as established by the SisBiota - FAPESP program. The microorganisms were identified using morphological characteristics by Departamento de Micologia da Universidade Federal de Pernambuco - PE, Brazil, as *Aspergillus caespitosus* USP-RP68, *A. thermomutatus* anamorph de *Neosartorya pseudofischeri* USP-RP69, *A. ochraceus* USP-RP70, *A. phoenicis* USP-RP71, *A. terreus* USP-RP72, *A. parasiticus* USP-RP73, *A. niger* USP-RP67, *Beauveria brongniartii* USP-RP74, *Mucor racemosus* USP-RP75, *Paecilomyces variotii* USP-RP76, *Penicillium purpurogenum* USP-RP77 and *A. niger* var. *awamori* USP-RP78. The fungi have been maintained in silica gel or ultrafreezer -80 °C at the Filamentous Fungi Collection - CFF of Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - Universidade de São Paulo. All fungi were cataloged by Specify software program. On the other hand, the microorganisms were also maintained routinely on solid 4% oatmeal baby food (Quaker) medium or in Vogel com-

plete solid medium (Vogel, 1964) with 2% glucose as the carbon source.

Screening of thermotolerant and thermophilic microorganisms on solid medium

The screening was carried out in Petri dishes containing complete solid medium modified from Selig *et al.* (2008): (total volume of 100 mL of solid medium: 5 mL of salts (0.6 g $NaNO_3$, Sigma S5506; 0.052 g KCl, Sigma 746436; 0.052 g $MgSO_4$, Sigma M7506; 0.152 g KH_2PO_4, Sigma P9791); 0.1 mL of salt minimal solutions (0.0022 g $ZnSO_4$, Sigma 96495; 0.0011 g H_3BO_3, Sigma B6768; 0.0005 g $MnCl_2$, Sigma 244589; 0.0005 g $FeSO_4$, Sigma 12353; 0.00016 g $CoCl_2$, Sigma 60818; 0.00016 g $CuSO_4$, Sigma 61230; 0.00011 g Na_2MoO_4, Sigma 243655; 0.005 g Na_2EDTA, Sigma E5134); 0.1 mL of vitamin solution Sigma V1 (0.02 mM nicotinic acid; 0.01 mM pyridoxine-HCl; 0.02 mM thiamine-HCl; 0.02 mM *p*-aminobenzoic acid; 0.02 mM panthotenate; 0.05 μM folic acid; 0.05 μM riboflavin); 1% glucose, Sigma G8270; 0.5% peptone, Sigma P0556; 0.5% yeast extract, Sigma Y1625; 2.5% agar, Sigma A5306 and distilled water), pH 6.5. Punctual inoculums was carried out at the center of the solid medium (using sticks autoclaved), and the plates with the culture medium were maintained at different temperatures, 30 °C-50 °C, with intervals of 5 °C among them, in a bacteriological incubator. The halo fungi were measured after 24 and 48 h of grown.

Obtainment of β-xylosidase and α-arabinanase from isolate fungi

β-xylosidase and α-arabinanase were obtained when the fungi were growth in Czapek liquid culture medium (Wiseman, 1975), pH 6.0, supplemented with 1% wheat fiber. Then, they were incubated under static conditions (without agitation) for five days, the temperature ranged from 30 °C to 45 °C, with intervals of 5 °C. Afterwards, the cultures were harvested by filtration on Whatman n°1 paper and the filtrates were used as source of crude extracellular activity. The mycelia pads obtained were ground in a mortar with 2 volumes of glass beads, at 0 °C, and extracted with sodium acetate buffer 100 mM, pH 5.0. The slurry was centrifuged at 12,000xg for 15 min, at 4 °C. The supernatant was the source of crude intracellular enzyme.

Enzymatic assays and protein determination

The quantification of β-xylosidase and arabinanase activities were performed using the method described by Kersters-Hilderson *et al.* (1982) and by Miller (1959), respectively. For the first method it was used 1% *p*-nitrophenyl-β-D-xylopyranoside (*p*NP-xyl, Sigma N2132) in 100 mM sodium succinate, pH 5.0 as substrate. The reaction mixture was incubated at 60 °C, for 5 min, and the *p*-nitrophenol), formed was quantified by spectrophotometry at 405 nm. The method was previously standardized by a curve of *p*-nitrophenol (PNP, Sigma 1041) (0.09 to 0.54 μmols). The activity unit was defined as the amount of enzyme capable of liberating 1 μmol of *p*-nitrophenol/min/mL. In order to determine the arabinanase activity the assay was carried out at 60 °C with a mixture containing 250 μL of a solution of 1% debranched arabinan, Megazyme P-DBAR, in 100 mM sodium succinate, Sigma 14160, pH 5.0, and 250 μL of diluted enzyme. Aliquots (100 μL) were withdrawn after different time intervals (15 and 30 min), and the assay tubes covered with parafilm and boiled for 5 min. The tubes were then chilled and 1 mL of distilled water was added. The blanks consisted of 100 μL of reaction mixture with the immediate addition of 100 μL of DNS. The control was performed by incubating the substrates in 100 mM sodium succinate, pH 5.0, for 30 min at 60 °C, and the reducing sugars were detected. The absorbance readings were made at 540 nm using a microplate. Arabinose, Sigma A3256, was used as the standard (0.1 to 1.0 $mg.mL^{-1}$), and the activity unit (U) was defined as the amount of enzyme capable of liberating 1 μmol of reducing sugar formed per minute per mL, under the assay conditions. Protein was determined at 595 nm according to Bradford (1976), using bovine serum albumin, Sigma 05470, as standard. The unit was defined as mg protein/mL intra or extracellular sample.

Reproducibility of results

All results are the average of at least three (n = 3) independent experiments. The standard deviation for the arabinose curve was 0.0031, $p < 0.0001$, and for the p-nitrophenol curve was 0.02808, $p < 0.0001$.

Results and discussion

Analysis of the morphological characteristics of filamentous fungi

Aiming to select good fungi producers that degrade plant cell wall, twelve microorganisms were obtained from our fungi collection and analyzed in relation to their morphological characters (Figure 1). Our observations confirmed the genera of the fungi previously identified, as described in Methods.

Effect of the temperature on the grown of the isolated fungi

Thermostable enzymes are of interest because elevation of the reaction temperature (up to a certain limit) generally increases the reaction rate and reduces the risk of microbial contamination (Collins *et al.*, 2005). Among the twelve species examined most of them (*Aspergilli* and *Paecilomyces*) achieved maximum growth at 35 °C. Furthermore, it was found that nine species grew at 45 °C, and *A. thermomutatus* anamorph of *Neosartorya*

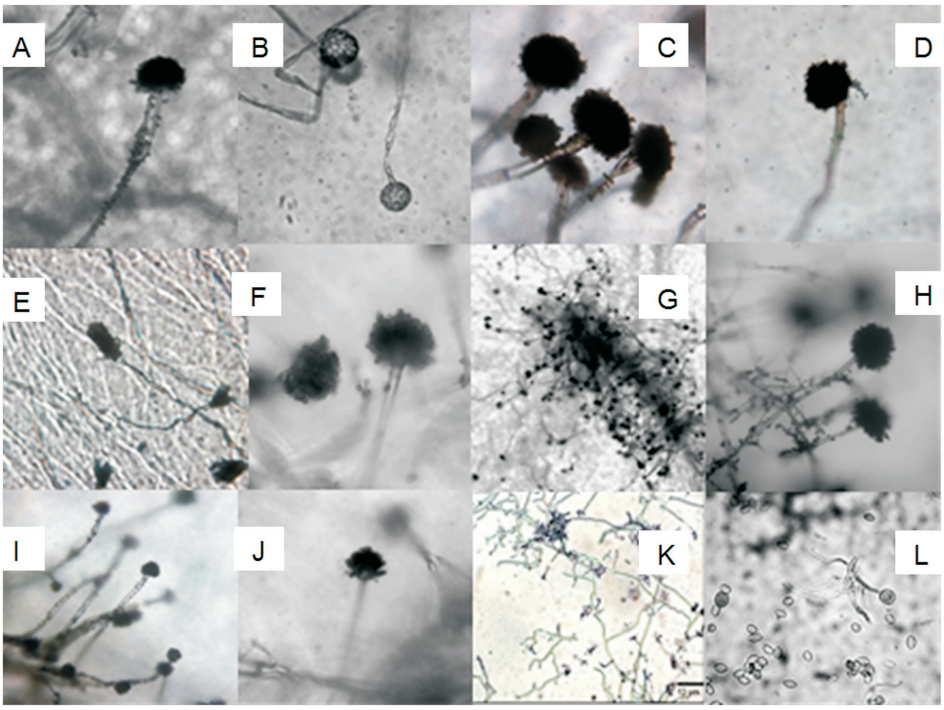

Figure 1 - Microscopic morphology obtained from Light Microscopy of filamentous fungi, 400x. A- *A. niger* var. *awamori*; B- *M. racemosus*; C- *A. niger*; D- *A. caespitosus*; E- *P. purpurogenum*; F- *A. parasiticus*; G- *A. thermomutatus*; H- *A. terreus*; I- *A. phoenicis*; J- *A. ochraceus*; K- *B. brongniartii*; L- *P. variotii*.

pseudofischeri reached its maximum development at 40 °C-45 °C, having a 4.0 cm halo after 48 h, featuring an increase of approximately 0.083 cm/h. It could be observed that *A. thermomutatus* as well as *A. parasiticus* were the only fungi that grew at 50 °C, with a mycelial halo of 0.5 cm after 48 h (Table 1). *B. brongniartii* and *M. racemosus* had better growth at 30 °C, on the other hand, *P. purpurogenum* showed optimum growth at both temperatures (30 °C-35 °C). Then, these three fungi showed mesophilic character compared to the *Aspergilli* species studied. These results corroborate the reports of Pedersen *et al.* (2007), where it was verified that among the termophilic fungi, the commercial production of xylanases is highlighted in the genera *Aspergillus* and *Trichoderma*, due to its potential thermotolerance and production of thermostable enzymes.

Screening of β-xylosidase produced by filamentous fungi

Enzymes produced by microorganisms are excellent for industrial use, once that the production is fast; lower production costs; possibility of large-scale production in industrial fermentors; wide range of physical and chemical characteristics; possibility of genetic manipulation; it is seasonal independent; etc. However, the preference is given to the extracellular form, *i.e.*, secreted from the culture medium due to the ease of enzyme extraction. These characteristics are typical of filamentous fungi, but the same is not observed for bacteria and yeasts (Polizeli *et al.*, 2005). In this context, the production of extra and intracellular β-xylosidases, and arabinanase from various fungi was analyzed correlating them with the growth temperature (Figure 2).

The extracellular activity was higher to *A. niger*, *A. phoenicis*, *P. variotii*, *A. ochraceus*, *A. niger* var. *awamori* (about 0.723 U/mL, Figure 2A), when the fungi were cultivated at 30 °C. However, the activity decreased about 31% (compared to the temperature of 30 °C - average of 0.223 U/mL) when the microorganisms were cultivated at higher temperature (35-40 °C, Figures 2B, C), and at 45 °C this activity was reduced to very low levels (Figure 2D).

The intracellular β-xylosidase activity was higher in cultures at 30 °C, for the same microorganisms mentioned above (0.279 U/mL average levels, Figure 2A). Nevertheless, this activity reduced approximately 50% at 35 °C-40 °C and 77% at 45 °C (about 0.065). The fungi growth was accompanied by decreased activity (Table 2), but a drastic reduction was not observed. Then, by the correlation of activity/mycelial protein (specific activity) it was possible to suggest that the temperature of 45 °C did not favor the cell machinery to enzyme synthesis. On the other hand, it was observed that the intracellular enzyme levels were higher at 35 °C for *Aspergillus thermomutatus* followed by *A. caespitosus, A. parasiticus, A. terreus*, *M. racemosus and P. purpurogenum* (0.136 U/mL average activity levels, Figure 3B) compared at 30 °C and at 40 °C.

At 45 °C insignificant levels of intracellular activity were detected. In relation to the protein levels, it was veri-

Table 1 - Radius length of the mycelia halo analyzed of fungi culture.

Fungi	Radius of the mycelial halo (cm) - 48 h				
	30 °C	35 °C	40 °C	45 °C	50 °C
Aspergillus caespitosus	2.3 ± 0.017	3.0 ± 0.015	2.2 ± 0.014	1.5 ± 0.012	n.g.
Aspergillus niger var. awamori	2.7 ± 0.015	3.2 ± 0.014	2.0 ± 0.015	1.0 ± 0.014	n.g.
Aspergillus thermomutatus	1.7 ± 0.014	3.5 ± 0.016	3.5 ± 0.017	4.0 ± 0.017	0.5 ± 0.015
Aspergillus ochraceus	1.8 ± 0.017	2.2 ± 0.016	1.6 ± 0.015	1.0 ± 0.014	n.g.
Aspergillus phoenicis	3.7 ± 0.016	3.5 ± 0.017	2.0 ± 0.017	1.3 ± 0.017	n.g.
Aspergillus terreus	2.0 ± 0.020	2.7 ± 0.019	2.2 ± 0.015	1.8 ± 0.014	n.g.
Aspergillus parasiticus	2.8 ± 0.015	3.0 ± 0.015	1.6 ± 0.016	0.9 ± 0.009	0.5 ± 0.016
Aspergillus niger	2.0 ± 0.015	3.0 ± 0.020	1.0 ± 0.010	0.8 ± 0.016	n.g.
Beauveria brongniartii	1.2 ± 0.018	0.5 ± 0.010	n.g.	n.g.	n.g.
Mucor racemosus	5.0 ± 0.020	4.0 ± 0.014	1.0 ± 0.017	n.g.	n.g.
Paecilomyces variotii	1.7 ± 0.017	2.5 ± 0.017	1.5 ± 0.014	0.8 ± 0.017	n.g.
Penicillium purpurogenum	0.8 ± 0.012	0.8 ± 0.011	0.3 ± 0.009	n.g.	n.g.

n.g. - the fungi did not grow.

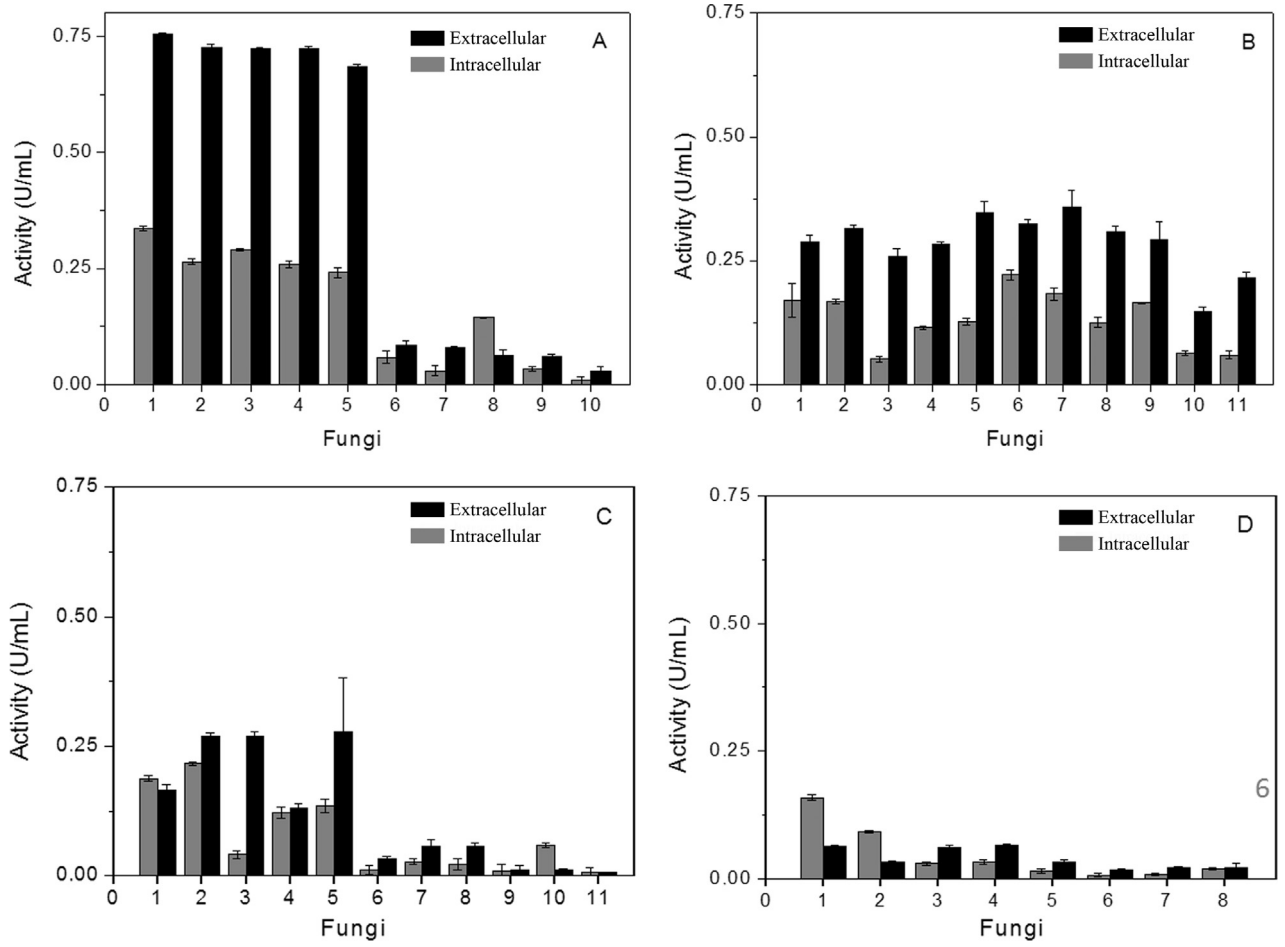

Figure 2 - Analysis of intracellular and extracellular β-xylosidase activities produced by fungi grown at different temperatures: (A) 30 °C, (B) 35 °C, (C) 40 °C and (D) 45 °C. Fungi: 1- *A. niger*, 2- *A. phoenicis*, 3- *P. variotii*, 4- *A. ochraceus*, 5- *A. niger* var. *awamori*, 6- *A. thermomutatus*, 7- *A. caespitosus*, 8- *A. terreus*, 9- *A. parasiticus*, 10- *M. racemosus*, 11- *P. purpurogenum*, 12- *B. brongniartii*.

Table 2 - The amount of protein produced by various microorganisms.

	Protein (mg/mL)							
	Intracellular				Extracellular			
	30 °C	35 °C	40 °C	45 °C	30 °C	35 °C	40 °C	45 °C
A. niger	0.142	0.171	0.156	0.138	0.256	0.289	0.275	0.116
A. phoenicis	0.170	0.168	0.155	0.132	0.262	0.315	0.294	0.152
P. variotii	0.031	0.022	0.038	0.023	0.300	0.259	0.237	0.171
A. ochraceus	0.103	0.095	0.106	0.075	0.307	0.284	0.203	0.125
A. niger var. awamori	0.105	0.127	0.111	0.089	0.300	0.346	0.207	0.115
A. thermomutatus	0.122	0.135	0.188	0.192	0.289	0.335	0.247	0.197
A. caespitosus	0.157	0.183	0.146	0.133	0.256	0.359	0.103	0.089
A. terreus	0.086	0.125	0.120	0.056	0.310	0.308	0.155	0.049
A. parasiticus	0.168	0.175	0.131	0.115	0.326	0.293	0.123	n.g.
M. racemosus	0.074	0.093	0.047	n.g.	0.320	0.148	0.100	n.g.
P. purpurogenum	0.061	0.060	0.037	n.g.	0.152	0.215	0.156	n.g.
B. brongniartii	0.113	0.099	n.g.	n.g.	0.235	0.118	n.g.	n.g.

n.g. - the fungi did not grow.
Standard deviation: 0.004.

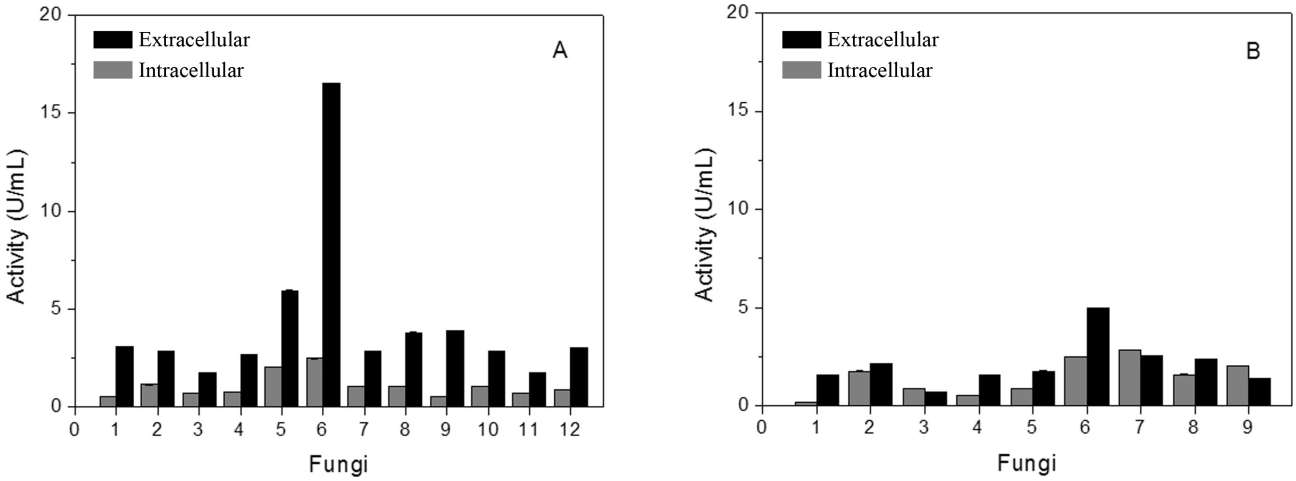

Figure 3 - Intracellular and extracellular arabinanase activities produced by fungi grown at different temperatures: (A) 30 °C and (B) 40 °C. The microorganisms were cultured on Czapek medium, initial pH 6.0, supplemented with 1% wheat fiber under static condition, during five days. Fungi: 1- *A. niger*, 2- *A. phoenicis*, 3- *P. variotii*, 4- *A. ochraceus*, 5- *A. niger* var. *awamori*, 6- *A. thermomutatus*, 7- *A. caespitosus*, 8- *A. terreus*, 9- *A. parasiticus*, 10- *M. racemosus*, 11- *P. purpurogenum*, 12- *B. brongniartii*.

fied that there was not considerable variation as previously detected to the other *Aspergilli* cited (Table 2). The filamentous fungus *B. brongniartii* that grew at temperatures of 30 °C and 35 °C (Table 2, average 0.106 mg of mycelia protein/mL) but did not produce β-xylosidase in any of the studied temperatures. From the biotechnological point of view, this obtained data are interesting since there are few reports on extracellular β-xylosidases and their action mechanism on xylooligosaccharides.

Some studies in the literature consist of β-xylosidase secretion (Kiss and Kiss, 2000; Rizzatti *et al.*, 2001; Saha , 2001; Lenartovicz *et al.*, 2003; Guerfali *et al.*, 2008; Yan *et al.*, 2008; Michelin *et al.*, 2012a and 2012b), but others have demonstrated that β-xylosidases remain associated to the mycelium (Kumar and Ramón, 1996; Ito *et al.*, 2003; Katapodis *et al.*, 2006; Lembo *et al.*, 2006; Ohta *et al.*, 2010). The induction of the xylanolytic enzymes initially occurs by the physical contact between cell and the inducer source; which suggests the existence of some recognition site on the cell surface. Constitutive xylanases are supposed to be responsible for the initial hydrolysis of xylan, producing small β-D-xylopyranosyl oligosaccharides such as xylobiose and xylotriose. β-Xyloside permeases mediate the transport of these oligosaccharides into the cell, where

they trigger the expression of the xylanolytic system genes. So, β-xylosidases are expressed to hydrolyze xylooligocchacarides to xylose (Polizeli *et al.*, 2005) and they are mostly expressed at the intracellular level and not secreted to the external environment.

Identification of filamentous fungi good producers of arabinanase

The arabinanase activity was studied at 30 °C and 40 °C. It was verified that the filamentous fungus *A. thermomutatus* exhibited the best extracellular arabinanase activity at 30 °C compared to other fungi. Some studies have demonstrated arabinanase activities higher than our findings, with temperatures ranging from 35 °C to 75 °C (Hong *et al.*, 2009; Squina *et al.*, 2010; Seo *et al.*, 2010; Inacio and de Sá-Nogueira, 2008). Furthermore, the highest enzymatic activity on debranched arabinan found in this study was similar to that found by Hong *et al.* (2009), but it was much lower compared to other studies (Wong *et al.*, 2008; Skjøt *et al.*, 2001).

These enzymatic levels were 2.75-fold higher in relation to *A. niger* var. *awamori,* the second best producer. It was verified in these two microorganisms that there was a predominance of extracellular forms (85% and 66%, respectively). As far as we know, these two fungi have not been used for the arabinanase production, which makes them strong candidates for the industrial production of this enzyme.

Indeed, most of the fungi cultivated at 40 °C diminished the extracellular arabinanase activity, but, in contrast, the intracellular activity was higher in cultures at 40 °C for most microorganisms tested constituting a rate of 50% for extracellular activity/intracellular activity (Figure 3). An interesting data correlating arabinanase synthesis and fungi growth temperature was the fact that at 40 °C the intracellular levels produced by *A. phoenicis*, *P. variotti*, *A. caespitosus* and *A. parasiticus* were close, similar or slightly higher than the extracellular activity. Then, it is possible to conclude that the fungi growth temperature affects the arabinanase secretion, but how this process occurs is obscure and the elucidation of this phenomenon deserves further investigation.

Studies on hemicellulases have advanced, mainly due to industrial interests, but unlike the majority of components of the arabinases group, arabinanases and arabinofuranosidases, are related enzymes whose action mechanisms are being unveiled in recent years. But the great importance of these enzymes as an important player of the plant cell wall deconstruction mechanism makes this research a valuable screening for the identification of fungal species in Brazil with the potential to produce ancillary hemicellulases (Takao *et al.*, 2002; Hong *et al.*, 2009).

Conclusion

With these results it is possible to conclude that of twenty Brazilian fungal species analyzed of large mycology collection library, the most proved to developed at temperatures around 30 °C-35 °C, however two microorganism grown at 50 °C, as the results demonstrated that specific species of fungi are promising for production of hemicellulases (β-xylosidases and arabinanase), that have an important role in the cell wall deconstruction and thus have a promising biotechnological potential.

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