Using Amazon forest fungi and agricultural residues as a strategy to produce cellulytic enzymes

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ABSTRACT

The successful strategy to produce cellulytic enzymes includes both microorganism selection and improved fermentation process conditions. This work describes the isolation, screening and selection of biomass-degrading fungi species from the Amazon forest and analyzes the enzymatic complex produced by a selected strain of Aspergillus fumigatus cultivated using different agro-industrial residues (wheat bran, sugarcane bagasse, soybean bran, and orange peel) as substrate in solid state fermentation (SSF). The profile of endoglucanase (CMCase), FPase, β-glucosidase and xylanase enzymatic activities obtained during 120 h of cultivation is presented. Enzyme activities up 160.1 IU g⁻¹ for CMCase, 5.0 FPU g⁻¹ for FPase, 105.82 IU g⁻¹ for β-glucosidase and 1055.62 IU g⁻¹ for xylanase were achieved. The enzymatic extract with higher CMCase activity was used to run a zymogram analysis that showed 3 bands of endoglucanase activity. Characterization studies of this extract showed that the CMCase was most active at either 65 °C or pH 3–3.5, indicating that this microorganism produces a thermophilic and acid endoglucanase. These data demonstrate that the fungal isolates from the Amazon forest are a potential source of cellulases and xylanases, providing support to further studies related to the use of these microorganisms to obtain the enzymes needed for biomass conversion.

1. Introduction

Cellulosic ethanol has attracted attention as a potential alternative renewable transportation fuel. One of the most promising routes for the conversion of cellulosic materials into ethanol is the enzymatic hydrolysis followed by fermentation [1,2]. The biomass-degrading enzymes needed in this process, the cellulases, are multi-enzyme systems composed of several enzymes, which act in synergy [3,4]. Cellulases are the third largest industrial enzyme product worldwide, by dollar volume, but will certainly become the largest volume industrial enzyme upon its use for converting biomass into biofuels [5]. However, the major bottleneck for a broader application of industrial cellulases is their high cost. The successful strategy to produce cellulases can be achieved through microorganism selection and improved fermentation process conditions. These include screening for new enzyme activities and developing pre-treatments that alter the cellulose lattice structure, increasing enzyme accessibility.

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It is therefore necessary to search for microorganisms that have a high rate of cellulase production. Prospecting for cellulose-producing fungi is one of the possible strategies to obtain the necessary enzyme complex to hydrolyze plant biomass [6,7]. Fungal strains that produce cellulases include Aspergillus, Fusarium, Penicillium and Trichoderma genera. One of the most reported microorganisms for cellulase production in the literature is the Rut C30 strain mutant of the filamentous fungus Trichoderma reesei [8,9]. However, it is known that the cultivation of T. reesei has some limitations including product inhibition of cellulase activity and lower cellubiose activities compared to other cellulolytic strains [10]. To reduce these limitations many studies have used genetic engineering methods. However, the avoidance of genetic modified organisms (GMOs) for large scale industrial use may facilitate site approval and avoid capital costs associated with their containment. In this context, Brazil’s high biodiversity level stands out as a potential environment to be explored in the search for microorganisms that have the desired characteristics for such application. The Amazon biome occupies nearly half of the Brazilian territory and is the world’s largest reserve of biological diversity, presenting special soil and climate characteristics for microorganism growth [11].

Regarding fermentation processing conditions, the current enzyme production technologies are conducted either in a liquid phase, known as submerged fermentation (SF), or using solid substrates termed solid state fermentation (SSF). Approximately 90% of all industrial enzymes are produced by SF, often using genetically modified microorganisms [12]. However, most of these enzymes could be produced by SSF using wild-type microorganisms. Though there are reports on cellulase production through SSF, the large scale commercial processes are still using SF [13,14]. However, growth of filamentous fungi by SSF is considered advantageous since the solid medium simulates its natural habitat [15]. This benefit is extended to the production of enzymes, yielding greater productivity when compared to SF [12]. Another advantage of SSF is the use of agro-industrial residues as a solid substrate, acting both as carbon and energy source.

The choice of these lignocellulosic materials should be based on their abundance and cost, as well as their physical-chemical characteristics. In Brazil, sugarcane is one of the largest agricultural monocultures, supplying an enormous amount of residues. Because of its low ash content, bagasse is a large scale agricultural residue that have the desired characteristics for such application. The Amazon biome occupies nearly half of the Brazilian territory and is the world’s largest reserve of biological diversity, presenting special soil and climate characteristics for microorganism growth [11].

2. Materials and methods

2.1. Fungi isolation and identification

The fungal strains used in this study were isolated by screening 100 soil and decomposed wood samples from the natural Amazon forest reserve of Embrapa Eastern Amazon (Brazilian company for Agricultural Research), located in Belém, Pará - Brazil. Approximately 5 g of soil or pieces of decomposed wood (length of 10–20 mm) were transferred to tubes containing 5 mL of a nutrient medium [19]. After 72 h of incubation at 37 °C, the samples were transferred to potato dextrose agar and to a media with 30 g L−1 of malt-agar. Purified fungi were transferred to plates that contained (g L−1): 2.0 NaNO3, 1.0 K2HPO4, 0.5 MgSO4, 0.5 KCl, 2.0 Avicel, 0.2 peptone, 17 agar and 0.25 chloramphenicol. Fungal identification was carried out using morphological markers according to classical taxonomical keys [20–22]. Selected strains are maintained at Embrapa culture collection (São Carlos, SP, Brazil).

2.2. Initial screening

Initial screening for cellulose degrading strains were carried out in Petri plates using a synthetic agar medium composed of (g L−1) 3.0 NaNO3, 0.5 MgSO4, 0.5 KCl, 0.01 FeSO4.7H2O, 1.0 KH2PO4 and 30.0 agar supplemented with 5.0 g L−1 of crystalline cellulose (Avicel) (Sigma, St. Louis, USA) as the only source of carbon. Fungal isolates were inoculated and plates were incubated at 37 °C during 96 h. Selection was based on fungal growth under this condition.

2.3. Agro-industrial residues

The sugarcane bagasse used as solid substrate was kindly provided by Edra Eco Sistemas (Ipêna, SP, Brazil). It was used without any pre-treatment and a particle size between 1 and 2 mm was selected. Exploded sugarcane bagasse was kindly provided by a local sugarcane mill (Usina Nardini, Vista Alegre do Alto, SP, Brazil). Soybean and wheat bran where purchased from a local store. Orange peel was kindly provided by a local orange processing industry (Citrosuco, Matão, SP, Brazil). These three materials were thoroughly washed with water, dried at room temperature, milled with a knife mill and classified to particle size between 1 and 2 mm. All processed material was stored in sealed plastic bags and maintained refrigerated at 4 °C until experimentation.

2.4. Enzyme production

Solid state fermentation was used for fungal cultivation and evaluation of the enzymatic complex produced using different carbon sources. For preparation of fungal inoculums for fermentation, 2 mL of sterile distilled water containing 1.0 g L−1 of Tween 80 was introduced into the sporulated slants of each fungus and the spores were dislodged into the liquid by gentle pipetting. Fungi were cultured in cotton-plugged 250 mL Erlenmeyer flasks containing 5 g of substrate moistened with mineral salt medium [19] in order to...
achieve 60% moisture. Each flask was sterilized by autoclaving at 121 °C for 20 min before inoculation with spores at 10⁷ mL⁻¹, measured by the Neubauer chamber count method. For the second screening step, ten selected strains from the first screening on avicel were cultivated using only wheat bran as substrate. The selected fungus strain based on endoglucanase activity was then cultivated in different agro-industrial residues. Lignocellulosic substrates (wheat bran, exploded sugarcane bagasse, sugarcane bagasse and wheat bran (1:1), soybean, orange peel and orange peel and wheat bran (1:1)) were prepared as described above. The flasks were incubated at 37 °C for 5 days. After incubation period, 50 mL of citrate buffer (pH 5.0, 50 mmol L⁻¹), prepared as described by Gomori [23], was added to each flask. The suspensions were stirred at 2 Hz in an orbital shaker incubator (Solab, Brazil) at 35 °C for 30 min. The solids were separated by centrifugation at 10,000 g at 4 °C for 15 min and the enzyme activity of the supernatant was assayed.

2.5 Enzyme assays

Cellulase activity on filter (FPase), endoglucanase (CMCase), xylanase and β-glucosidase activities were determined. All enzymes were analyzed according to the standard procedure recommended by the Commission on Biotechnology, IUPAC [24] with some modifications. Filter paper activity was assayed by incubating the suitable diluted enzyme (0.1 mL) with 0.9 mL citrate buffer (50 mmol L⁻¹, pH 5.0) containing filter paper Whatman no. 1 (50 mg, 1 × 6 cm). The reaction mixture was incubated at 50 °C for 60 min. Endoglucanase activity was carried out by using 0.1 mL of suitably diluted enzyme and 0.9 mL of 40 g L⁻¹ carboxymethylcellulose (CMC) (Sigma, St. Louis, EUA) solution in citrate buffer (50 mmol L⁻¹, pH 5.0). This mixture was incubated at 50 °C for 15 min. Xylanase activity was determined under similar conditions as described above, except that 1% xylan birchwood (Sigma, St. Louis, EUA) solution was used as substrate. Finally, the β-glucosidase activity was determined by using cellobiose (Sigma, St. Louis, EUA) as substrate and quantifying the sugars released by using an enzymatic kit for glucose measurement (Laborlab, São Paulo, Brazil). All the enzyme assays were performed in duplicate. One unit of FPase (expressed as filter paper units - FPU) and endoglucanase activity corresponds to 1 μmol of glucose released per minute. One unit of xylanase activity corresponds to 1 μmol of xyllose released per minute. The quantification of the reducing groups was performed using the dinitrosalicylic acid (DNS) method [25]. Results were expressed as activity units per mass of initial dry solid substrate (IU g⁻¹ or FPU g⁻¹).

2.6 Partial crude enzyme characterization: influence of pH, temperature and thermal stability

The temperature profile for CMCase activity (supernatant corresponding to the optimal production condition on SSF) was determined by assaying the activity at different reaction temperatures (30, 35, 40, 45, 50, 55, 60, 65 and 70 °C) in 50 mmol L⁻¹ sodium citrate buffer (pH 5.0). Accordingly, CMCase activity was assayed in different reaction buffers (50 mmol L⁻¹ glycine-HCl for pH 3.0; 50 mmol L⁻¹ sodium citrate for pH 3.5–6.0; 50 mmol L⁻¹ citrate phosphate for pH 6.5–7.0; 50 mmol L⁻¹ phosphate for pH 7.5–8.0; 50 mmol L⁻¹ Tris–HCl for pH 8.5–10.0) at 50 °C in order to determine the pH profile. For thermal stability determination, the crude supernatant obtained in the optimal production condition (CMCase initial activity of 190 IU g⁻¹) was incubated at 60 °C for 1 h in the absence of substrate. The residual CMCase activity was measured at different time periods. At the end of incubations the test tubes containing the enzyme were immediately cooled down by placing them on ice and then kept at 4 °C overnight. After this period the samples were taken to test the enzyme activity under standards pH and temperature conditions.

2.7 Zymogram

Crude enzyme preparations (70 μg of protein, measured according to the Bradford method [26]) were fractionated by native polyacrylamide gel electrophoresis (PAGE) using 10% acrylamide gel [27]. For developing the CMCase, the PAGE gel was incubated for 15 min in 0.05 mol L⁻¹ sodium citrate buffer (pH 5.0) and then overlaid with polyacrylamide gel containing CMC (0.5%, w/v) for 30 min at 50 °C. The overlay gel was removed and stained with 0.2% Congo Red solution (Sigma, St. Louis, EUA). Bands corresponding to CMCase appeared as clear zones against a dark background after destaining with 1M NaCl followed by treatment with 10% (v/v) acetic acid solution.

3 Results and discussion

3.1 Fungal isolation and screening studies

Screening studies were carried out in two steps. The first selection was based on the ability of fungal isolates to grow in a medium containing only crystalline cellulose (Avicel) as a carbon source. Selected strains from the first step were then grown in wheat bran under SSF and a second selection criterion was based on the highest CMCase activity produced after 5 days of incubation.

Of all 110 fungal strains isolated from the Amazon Forest, 46 isolates were able to grow in a nutrient medium containing Avicel as the only carbon source. Of this total, 10 fungal strains that showed a more expressive growth on Avicel plates were selected for cellulase production evaluation under SSF on wheat bran (Fig. 1). All 10 fungal strains evaluated were able to produce significant titers of endoglucanase. Higher endoglucanase activities were obtained by the species A. fumigatus P40M2 (43.65 IU g⁻¹), Aspergillus niger P47C3 (39.15 IU g⁻¹) and Trichoderma harzianum P34P9 (36.16 IU g⁻¹). Based on these results, the A. fumigatus P40M2 strain was selected for further studies on the influence of the carbon source on enzyme production by using different agro-industrial residues as substrates of SSF.

3.2 Influence of biomass on enzyme production by A. fumigatus strain P40M2

In order to evaluate the effect of the nature of the carbon source on enzyme production, the selected strain A. fumigatus P40M2 was cultivated by SSF using different agro-industrial residues...
as substrates (wheat bran, exploded sugarcane bagasse, sugarcane bagasse, sugarcane bagasse and wheat bran (1:1), soybean bran, orange peel and orange peel and wheat bran (1:1)). The effect of the carbon source on the enzymatic production was evaluated in terms of cellulase and xylanase activities. Cellulase is a complex of enzymes that contains: (1) endoglucanases (EG) which cleave internal bonds in the amorphous regions of the cellulose chains, (2) cellobiohydrolases (CBH) which act on the ends of the chains liberating cellobiose moieties, and (3) β-glucosidases which hydrolyze cellobiose to glucose [28]. Xylan is the principal type of hemicelluloses and represents a vast resource that can be used to produce fermentable sugars and fuels. The complete cleavage of xylan is carried out by the synergistic action of β-xylanase and its accessory enzymes, including β-xylosidase, α-arabinofuranosidase, α-glucuronidase and acetyl xylan esterase [29]. The enzyme production profile of endoglucanase, FPase, β-glucosidase and xylanase activities obtained during 120 h of fermentation were carried out and are presented as follows.

### 3.2.1. Endoglucanase production profile

Fig. 2 shows the endoglucanase production profile by *A. fumigatus P40M2* cultivated under SSF using different carbon sources. It can be observed that the nature of the carbon source in the culture medium had a significant influence on endoglucanase production. Soybean bran was found to result in maximal endoglucanase production (160.1 IU g⁻¹ of substrate), followed by wheat bran (122.8 IU g⁻¹) and a mixture of sugarcane bagasse and wheat bran (1:1) (112.6 IU g⁻¹). The highest production for all three substrates was achieved after 72 h of incubation. After this period, there was a decrease in endoglucanase activity, which was also observed when using wheat bran and a mixture of sugarcane bagasse and wheat bran (1:1) as substrates.

Many *Aspergillus* species are described in the literature as good producers of endoglucanase. Soni et al. [30] reported a maximal production of endoglucanase (98.5 IU g⁻¹ of substrate) in rice straw when comparing different sources of carbon during cultivation of *A. fumigatus* under SSF. Wheat bran has also been reported to induce high levels of endoglucanase in *Aspergillus* sp. Thygesen et al. [31] observed endoglucanase activity of 66.6 IU g⁻¹ in SSF using wheat bran as substrate for the fungus *A. niger*.

Sugarcane bagasse resulted in lower levels of endoglucanase production (16.71 IU g⁻¹) after 96 h of fermentation, when compared to the other carbon sources. Soni et al. [30] reported endoglucanase production of 29.2 IU g⁻¹ when using bagasse as substrate during *A. fumigatus* growth. Grigorevski-Lima et al. [32] obtained endoglucanase activity up to 21.06 IU g⁻¹ in solid state using sugarcane bagasse as substrate. Gupte and Madamwar [33] found an endoglucanase activity of 14.55 IU g⁻¹ when using sugarcane bagasse, in comparison to other substrates during cocultivation of *Aspergillus* *ellipticus* and *A. fumigatus*.

In the present work, endoglucanase activity was 18.42 IU g⁻¹ when orange peel was used as substrate, and this value was increased to 32.92 IU g⁻¹ when orange peel was mixed with wheat bran (1:1 ratio). It was verified that the use of wheat bran in combination with other substrates was very effective in increasing enzyme production. Further productivity improvements could be achieved by optimizing the SSF relevant parameters, such as temperature, moisture content, aeration, supplementation of the medium and pre-treatment of the substrate. Mamma, Kourtoglou and Christakopoulos [18] were able to increase endoglucanase activity from 9.2 to 60.5 IU g⁻¹ in solid state using orange peel as substrate and fungus *A. niger* after optimizing the initial moisture content of the solid medium. Even though it is difficult to compare enzyme production values given the different cultivation conditions and microorganisms employed in each study, the values reported here using fungal isolates from the Amazon forest were able to demonstrate that the selected strain of *A. fumigatus* can efficiently produce endoglucanases when growing in agro-industrial residues.

### 3.2.2. FPase production profile

Fig. 3 shows the effect of carbon source on the FPase production profile by *A. fumigatus*. Higher productions of FPase were obtained when wheat bran as well as a mixture of
sugarcane bagasse and wheat bran (1:1) were used as solid substrates, reaching values of up to 5.0 FPU g⁻¹. This result is rather interesting when compared to the other substrates evaluated, since soybean bran resulted in FPase activity values that were less than half of those obtained with wheat bran (2.4 FPU g⁻¹). The other substrates did not result in significant FPase activities. The maximum activity of FPase occurred after 96 h incubation.

Grigorevski-Lima et al. [32] also had maximum FPase activity (5.35 FPU g⁻¹) when using wheat bran as substrate, followed by sugarcane bagasse (4.4 IU/g). Soni et al. [30] obtained FPase activities of 2.11 and 3.37 FPU g⁻¹ when using wheat bran and rice straw, respectively, as solid substrates and a strain of A. fumigatus. Although wheat bran is considered as an inducer of FPase for fermentation with Aspergillus sp. [34], the addition of wheat bran to orange peel did not induce FPase activity significantly. The observed difference in FPase activities can be attributed to the structural heterogeneity of the cellulosic substrates, as well as to the differences in culture conditions during fermentation [35]. Sukumaran et al. [36] obtained 4.55 FPU g⁻¹ when growing A. niger MTCC 7956 in wheat bran as substrate and Gupte and Madamwar [33] obtained 3.75 FPU g⁻¹ during cocultivation of A. ellipticus and A. fumigatus using sugarcane bagasse as substrate and 2.1 FPU g⁻¹ when A. ellipticus was used alone. The values reported here using fungal isolates from the Amazon forest were in the same order of magnitude of the values reported in the literature, some of the values achieved only after SSF process optimization.

3.2.3. β-glucosidase production profile
Wheat bran also resulted in maximal production of β-glucosidase (105.82 IU g⁻¹), followed by a mixture of sugarcane bagasse and wheat bran (1:1) (38.04 IU g⁻¹) (Fig. 4). The highest activity occurred after 96 h of incubation. It is difficult to compare the results described in the literature, since the conditions to measure β-glucosidase activity and enzyme production were different. Soni et al. [30] obtained β-glucosidase activity of 99.4 IU g⁻¹ by using wheat bran as solid substrate and a maximum activity of 250.9 IU g⁻¹ by using wheat straw as solid substrate.

There was an increase in β-glucosidase activity when sugarcane bagasse was supplemented with wheat bran, since when sugarcane bagasse was used as the sole substrate, no significant enzyme production was observed (Fig. 4). The literature suggests that β-glucosidase induction requires specific operational conditions and wheat bran, mainly due to its mineral composition, favors the induction of β-glucosidase in the fermentation medium [37]. The fungus T. reesei, one of the most studied microorganisms for the production of cellulases, produces a relatively low amount of β-glucosidase, which is a disadvantage in terms of biomass saccharification process [38]. Brijwani et al. [39] reported that the deficit of β-glucosidase was overcome when T. reesei was co-cultured with Aspergillus oryzae, allowing a high β-glucosidase activity after 96 h incubation (10.71 IU g⁻¹) by using soybean hulls and wheat bran. Kang et al. [40] related a maximum β-glucosidase activity of 100.0 IU g⁻¹ by using rice straw as substrate by A. niger KK2 and Gao et al. [41] related 119 IU g⁻¹ using corn stover as substrate by Aspergillus terreus M11. Regarding β-glucosidase activity, the values reported here were mostly in the same order of magnitude of the values reported in the literature.

3.2.4. Xylanase production profile
Xylanase enzymes are responsible for xylan hydrolysis, which is the main polysaccharide component of hemicelluloses. Xylans account for 20–35% of the total dry weight of hardwoods and herbaceous plants and represent a vast resource that can be used for the production of fermentable sugars and fuels [42]. In SSF cultures, wheat bran and rice are used as xylanases inducers. Alternative substrates for enzyme production have also been reported, such as sugarcane bagasse, rice husks and wood pulp [43].

Concerning xylanase activity, higher yields were also obtained using wheat bran as substrate (1055.62 IU g⁻¹), followed by sugarcane bagasse and wheat bran (1:1) (821.52 IU g⁻¹), soybean (484.21 IU g⁻¹) and orange peel and wheat bran (1:1) (437 IU g⁻¹) (Fig. 5). All of the other substrates did not result in significant activities. Soni et al. [30] obtained xylanase activity of 1722 IU g⁻¹ by using wheat bran as solid substrate and a maximum activity of 2782 IU g⁻¹ by using wheat straw as solid substrate.

![Fig. 3](image1.png) Effect of different carbon sources on the production of FPase by A. fumigatus under solid state fermentation (SSF).

![Fig. 4](image2.png) Effect of different carbon sources on the production of β-glucosidase by A. fumigatus under solid state fermentation (SSF).
solid substrate. Senthilkumar et al. [44] related a maximum activity of 1.024 IU g$^{-1}$ using wheat bran as substrate by Aspergillus fischeri. When the fungus was cultivated on cane bagasse and orange peel, the maximum activity was of 68.10 and 43.05 IU g$^{-1}$, respectively. Lemos et al. [45] obtained a maximum activity of 2.50 IU g$^{-1}$ by Aspergillus awamori on cane bagasse and Mamma, Kourtoglou and Christakopoulos [18] related a maximum activity of 77.1 IU g$^{-1}$ by A. niger BTL on orange peel as the sole carbon source. The values reported here for xylanase activity were mostly superior to the values reported in the literature.

### 3.3. Effect of temperature, pH and thermostability analysis

CMCases obtained from A. fumigatus P40M2 grown in soybean under SSF conditions presented maximal activity at 65 $^\circ$C (Fig. 6). The activity decreased when incubated at 70 $^\circ$C, but still retained about 60% after one hour incubation. Our results strongly suggest that cellulases being produced seem to be thermophilic, which is considered ideal for many biotechnological processes. Grigorevski-Lima et al. [32] related a maximum activity of the enzyme by the fungus A. fumigatus at 65 $^\circ$C and when incubated at 80 $^\circ$C the enzyme still remained 30% of its activity. Saqib et al. [46] also obtained an optimum temperature of 65 $^\circ$C under SSF by the fungus A. fumigatus. The pH profiles (Fig. 7) showed more than 100% activity (187.0 IU g$^{-1}$) in the acidic pH (3.0), according to Gao et al. [41] this remarkable characteristic was also detected in a thermoacidophilic fungus, A. terreus M11. Grigorevski-Lima et al. [32] obtained a maximum activity of the enzyme CMCase obtained by A. fumigatus at pH 2.0. When CMCase was incubated at alkaline pH the activity was very low, decreasing about 25%. This biochemical characteristic is very interesting for processes that require acid conditions. Besides, acid
stability is also important in order to reduce microbial contamination. Considering the optimal temperature, strain P40M2 can be considered as producer of a thermoacidophilic endoglucanase.

Thermostability of the enzyme refers to its resistance to unfolding upon heating, i.e., to the thermal denaturation. Various parameters are used to determine the thermostability of enzymes, including the half life of the enzyme [47]. The A. fumigatus P40M2 supernatant was able to retain 100% of the residual activity at 60 °C for 50 min and 91% after 1 h incubation (172.0 IU g⁻¹) (Fig. 8). Saqib et al. [46] evaluated the production of endoglucanase in supernatant obtained by the fungus A. fumigatus under SSF using rice bran as substrate and found 50% of the original CMCase activity at 75 °C after 1 h of incubation. Grigorevski-Lima et al. [32] obtained similar results, the supernatant obtained from A. fumigatus FBSPE was able to retain 100% of the residual CMCase activity at 50 °C for 2 h of incubation. These results were similar to the results obtained in the literature and showed the potential of the enzymes produced, since crude extracts were evaluated, which were not subject to preliminary purification.

3.4. Detection of endoglucanase activity by zymogram staining

The extracts obtained by culturing A. fumigatus P40M2 on wheat bran and soybean, showed the expression of three forms of endoglucanases (Ia, Ib and Ic) and in the bagasse only two EG isoforms on native PAGE (Fig. 9). Soni et al. [30] related four isoforms in the presence of corn cob and in the bagasse only two EG isoforms. Grigorevski-Lima et al. [32] showed the expression of six EG isoforms in the presence of sugarcane bagasse under submerged culture. The observed differential expression of cellulases can be attributed to a structural heterogeneity of the cellulolytic substrates, as well as differences in culture conditions during cultivation [46]. Such multiple forms of endoglucanases have also been reported in different Aspergillus strains.

4. Conclusion

The fungal isolates from the Amazon forest were efficient producers of cellulase and xylanase. The use of agro-industrial residues as substrate for microbial growth also showed to be a promising alternative, with good perspectives for scaling up. The characterization of the crude enzyme has shown A. fumigatus P40M2 endoglucanase to be active in the acidic pH range 3–5, with maximal activity at either pH 3.0, or 65 °C, hence a promising organism for the production of acidophilic and thermophilic cellulases. It is important to point out that cultivations were carried at a specific condition and further productivity improvements can be achieved by optimizing the SSF relevant parameters. These data demonstrate that the fungal isolates from the Amazon forest are a potential source of cellulases and xylanases, providing support to further studies related to the use of these microorganisms to obtain the enzymes needed for biomass conversion.

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