Appl Biochem Biotechnol (2010) 162:2111–2122
DOI 10.1007/s12010-010-8986-0

# *Trichoderma harzianum* IOC-4038: A Promising Strain for the Production of a Cellulolytic Complex with Significant β-Glucosidase Activity from Sugarcane Bagasse Cellulignin

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Received: 28 February 2010 / Accepted: 26 April 2010 /
Published online: 9 May 2010


**Abstract** Sugarcane bagasse is an agroindustrial residue generated in large amounts in Brazil. This biomass can be used for the production of cellulases, aiming at their use in second-generation processes for bioethanol production. Therefore, this work reports the ability of a fungal strain, *Trichoderma harzianum* IOC-4038, to produce cellulases on a novel material, xylan free and cellulose rich, generated from sugarcane bagasse, named partially delignified cellulignin. The extract produced by *T. harzianum* under submerged conditions reached 745, 97, and 559 U $L^{-1}$ of β-glucosidase, FPase, and endoglucanase activities, respectively. The partial characterization of this enzyme complex indicated, using a dual analysis, that the optimal pH values for the biocatalysis ranged from 4.9 to 5.2 and optimal temperatures were between 47 and 54 °C, depending on the activity studied. Thermal stability analyses revealed no significant decrease in activity at 37 °C during 23 h of incubation. When compared to model strains, *Aspergillus niger* ATCC-16404 and *Trichoderma reesei* RutC30, *T. harzianum* fermentation was faster and its extract showed a better balanced enzyme complex, with adequate characteristics for its application in simultaneous saccharification and fermentation processes.

**Keywords** Sugarcane bagasse · Cellulase · β-glucosidase · Cellulignin · Endoglucanase · *Trichoderma harzianum* · *Aspergillus niger* · *Trichoderma reesei*

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## Introduction

Brazil is the main sugarcane producer worldwide [1]. One of the most abundant cellulose source in the country is sugarcane bagasse. In 2008, 37% of the world's entire sugarcane production (1.74 billion tons) was harvested in Brazil, generating 97 million dry tons of bagasse, which is partially used for energy co-generation at mills [2, 3].

Due to its crystallinity and complexity, cellulose hydrolysis requires the synergism of cellulases, which together promote higher activity than the sum of the activities of the enzymes, when used separately [4]. The cellulolytic complex involves three main groups of enzymes: the β-1,4-exoglucanase, which are represented by the cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase, CBH, EC 3.2.1.91), and glucanohydrolase (1,4-β-D-glucan glucohydrolase, EC 3.2.1.74). These enzymes split both cellobiose and glucose units from the reducing (CBH type I) and non-reducing (CBH type II) ends of cellulosic fibrils and are generally inhibited by their hydrolysis products. The second group involves the endoglucanase (4-β-D-glucan 4-glucanohydrolase, EG, EC 3.2.1.4), which break randomly internal glucosidic linkages of the amorphous region of cellulose, liberating oligosaccharides with different degrees of polymerization. Finally, the third group is composed of β-glucosidase (β-D-glucoside glucohydrolase, BG, EC 3.2.1.21), which hydrolyze cellobiose and soluble oligosaccharides into glucose [5].

Cellulases can be produced by several microorganisms and plants. The genera *Aspergillus* and *Trichoderma* are some of the most reported among the filamentous fungi genera. *T. reesei* RutC30 is known as an excellent cellulases producer, but the low content of β-glucosidase in its extract is pointed out as a disadvantage for the complete cellulose hydrolysis [6], which is required for the release of glucose and its further fermentation to ethanol and other chemicals [5]. On the other hand, *A. niger* strains have been studied due to their ability to produce high levels of β-glucosidase, although the production of endo-active enzymes is deficient [7, 8]. Aiming at to produce well-balanced extracts, mixed cultures from the two genera are employed [9], but the synergism between the different groups of cellulases produced by pure cultures is often better than the observed for the extracts from co-cultures [10].

*T. harzianum* strains are seldom employed for production of cellulases. Castro [11] studied the potential of filamentous fungi for production of enzymes from the cellulolytic complex and observed the outstanding performance of *T. harzianum* IOC-4038 to produce high levels of β-glucosidase and, simultaneously, significant amounts of endoglucanases. Therefore, the objective of this work is to evaluate the production of cellulases by *T. harzianum* IOC-4038 during submerged fermentation (SmF) using sugarcane bagasse cellulignin and compare their properties with two model fungal strains.

## Materials and Methods

### Sugarcane Bagasse Pretreatments

Cellulignin was obtained from sugarcane bagasse (Costa Pinto Mill, Piracibaca, SP, Brazil) using two sequential pretreatments (acid and alkaline, respectively) according to the methodology described by Castro et al. [12]. This material still contains lignin, and for this reason it was named partially delignified cellulignin (PDC).

## Microorganisms and Growth Conditions

*Trichoderma harzianum* IOC-4038 and *Aspergillus niger* ATCC-16404 were obtained from the culture collection of Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, RJ, Brazil. *T. reesei* RutC30 was kindly provided by Professor Fernando Araripe from the University of Brasilia. The strains were maintained in PDA plates (DIFCO, Franklin Lakes, NJ, USA) at 30 °C for 9–10 days before inoculation.

Resuspended spores from the strains ($5.33\times10^7$, total amount) were inoculated in 100 mL of adapted Mandels and Weber medium [13] in 500-mL conical flasks and incubated at 200 rpm at 30 °C. After 3 days, 10 mL of the medium containing grown cells (concentrations of 4.57, 2.63, and 2.68 g $L^{-1}$ for *A. niger*, *T. harzianum*, and *T. reesei* cultures, respectively) were transferred to 1-L conical flasks containing 200 mL of adapted Mandels and Weber medium two-fold concentrated (containing in grams per liter= $(NH_4)_2SO_4$, 5.6; $KH_2PO_4$, 4.0; $CaCl_2{\cdot}2H_2O$, 0.8; $MgSO_4{\cdot}7H_2O$, 0.6; peptone, 1.8; yeast extract, 0.5; and containing in milligrams per liter=$FeSO_4{\cdot}7H_2O$, 10.0; $MnSO_4{\cdot}4H_2O$, 3.2; $ZnSO_4{\cdot}7H_2O$, 2.8; $CoCl_2{\cdot}6H_2O$, 40.0) with 7.5 g $L^{-1}$ of PDC (instead of glucose) and was incubated at 200 rpm at 30 °C. Throughout the fermentation, aliquots were withdrawn, sonicated for 1 min for desorption of enzymes, and centrifuged at 20,000×*g* for 5 min for cell and residual substrate harvesting. Supernatants were stored frozen until the enzyme assays were carried out.

## Enzyme and Protein Assays

FPase, endoglucanase, and β-glucosidase activities were determined using Whatman no. 1 filter paper, carboxymethyl cellulose (CMC, medium viscosity), and cellobiose (Sigma, St. Louis, MO, USA) as substrates, respectively, according to standard conditions described by Ghose [14]. These protocols were set as standards for the subsequent analyses. Exoglucanase activity was determined as the procedure used for filter paper, but the 1× 6 cm filter paper strip was substituted by 50 mg of avicel (Merck, Rio de Janeiro, RJ, Brazil). Reducing sugars, expressed as glucose liberated during reactions on filter paper, avicel, and CMC were quantified by DNS method [15] using glucose as standard, and glucose released during β-glucosidase reaction was quantified using an analysis kit based on the enzymes glucose oxidase and peroxidase (Laborlab, São Paulo, SP, Brazil). Endoxylanase and protease activities were determined using Birchwood xylan and azocasein (Sigma) as substrates, according to Bailey et al. and Charney and Tomarelli [16, 17], respectively. For all enzyme activities, except protease, one enzyme unit (U) was defined as the amount of biocatalyst that releases 1 μmol of the correspondent monosaccharide (xylose for xylanase and glucose for the other groups of enzymes) per minute under the assay conditions. Regarding protease, unit (U) was defined as the enzyme quantity that promotes the increase of one absorbance unit per minute, under the assay conditions. CMC of different viscosities (Sigma) were also tested to measure activity of endoglucanases according to the same methodology cited above. Finally, *p*-nitrophenyl-glucopyranoside (pNPG, Sigma) in the concentration of 3 mM (citrate buffer, pH 4.8) was used as substrate for the determination of β-glucosidase activity (at 50 °C). The initial rate of hydrolysis was determined by on-line measurement of absorbance (405 nm). The enzyme unit was defined as the amount of biocatalyst that releases 1 μmol of *p*-nitrophenol per minute under the assay conditions. Total extracellular protein content was measured

using the Bio-Rad protein reagent (Bio-Rad Laboratories, Hercules, CA, USA) according to the Bradford method [18], and bovine serum albumin (Sigma) was used as standard. All analyses were done in triplicate in a temperature-controlled incubator (Dubnoff—Nova Técnica, São Paulo, SP, Brazil). Results were expressed as mean±1 SD.

### Characterization of Enzyme Complexes

In order to investigate the dual influence of pH and temperature in the catalytic power of cellulases, these factors were manipulated simultaneously. Temperatures evaluated were in the range of 30–80 °C and the pH values were from 3.0 to 6.0. All analyses were done at standard conditions as described previously. Data were fitted to Gaussian and Lorentzian models and optimal pH and temperature were estimated using the SigmaPlot 9.0 software (Systat Software Inc., San Jose, CA, USA).

The stabilities at three different temperatures, 37, 50, and 60 °C, were determined by incubating the crude extracts in a temperature-controlled incubator for 23 h. Enzyme quantifications were done at standard conditions.

For the estimation of the kinetic parameters of β-glucosidase and endoglucanase, solutions containing 1–40 mM cellobiose and 1–20 g $L^{-1}$ of CMC of medium viscosity were considered, respectively. Reactions were carried out at 50 °C, pH 4.8 for 5 to 30 min. Parameters were estimated using the Lineweaver–Burk plot.

Finally, reactions were performed on different substrates in order to determine the catalytic specificity of the enzyme complexes from *T. harzianum* and the model strains. In this set of experiments, the extracts produced by SmF were compared to a commercial product, GC-220®, provided by Genencor International Inc. (Palo Alto, CA, USA).

## Results and Discussion

### Cellulase Production from PDC

*T. harzianum* as well as the model fungi *A. niger* and *T. reesei* were cultured in conical flasks in the presence of PDC, which was custom-generated in order to induce high production of cellulases. As reported by Castro et al. [12], this pretreated material contained 68.8% of glucan and 9.3% of lignin, but xylan was absent. During SmF, aliquots were drawn and analyzed according their FPase (the global synergistic activity), primarily endoglucanase (on CMC) and β-glucosidase (using cellobiose) activities. Kinetics of cellulases production by *T. harzianum* (Fig. 1) showed high endoglucanase and β-glucosidase production yields (559±32 and 745±18 U $L^{-1}$, after 97 and 166 h, respectively), when compared to FPase activity (97±7 U $L^{-1}$ after 166 h), thus indicating the production of well-balanced complexes for both substrate depolymerization and saccharification to fermentable sugars throughout the fermentations. The β-glucosidase activity produced by *T. harzianum* IOC-4038 in the present work was a little higher than the one reported by Ahmed et al. [19] after the study of the cultivation of *T. harzianum* E-58 (629 IU $L^{-1}$). Regarding the model strains, the profiles of cellulase production when *A. niger* was cultured in PDC revealed a predominant β-glucosidase activity (reaching 423± 10 U $L^{-1}$ after the same time of cultivation when the highest activity was observed for the extract produced by *T. harzianum*) relatively to endoglucanase and FPase activities (328± 22 U $L^{-1}$ and 28±12 U $L^{-1}$ after 166 h, respectively). After a long time of process (290 h), *A. niger* produced the highest β-glucosidase activity (1,627 U $L^{-1}$) between the three

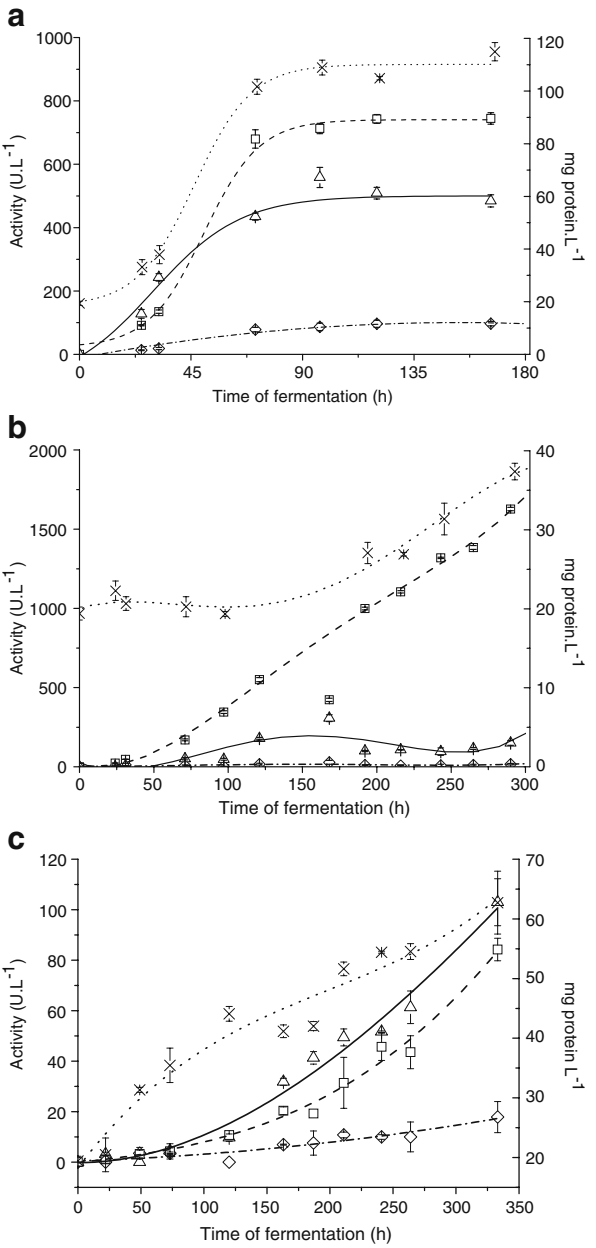


**Fig. 1** Kinetic profiles of cellulases production by **a** *T. harzianum*, **b** *A. niger*, and **c** *T. reesei*. FPase activity (*lozenges*, *dash dotted line*); β-glucosidase activity (*squares*, *dashed line*); endoglucanase activity (*triangles*, *solid line*); protein content (*multiplication symbols*, *dotted line*)

strains evaluated, and this result is corroborated by literature data, which reports the great ability of species from this genus [7, 8]. *T. reesei* showed a long acclimation period (120 h), reaching its highest β-glucosidase (84±4 U $L^{-1}$), endoglucanase (103±9 U $L^{-1}$), and FPase (18±6 U $L^{-1}$) activities only after 333 h of process.

In the present work, one important difference among the profile observed for *T. harzianum* and the profiles observed during the cultivation of *A. niger* and *T. reesei* is the faster kinetics that the former presented when incubated with PDC. Such distinction is pointed out when the volumetric productivities are compared. According to Table 1, *T. harzianum* showed productivities between 2.1 and 22 times higher than those obtained using *A. niger* or *T. reesei*. High specific activity for β-glucosidase was observed when *A. niger* was cultured, but only after a long time of fermentation (265 h). According to Wingren et al. [20], the cost of cellulases is one of the major economical bottlenecks in the process of bioethanol production. Therefore, the use of strains that are able to produce these enzymes in short periods (high productivity) would represent a positive influence in the profitability of a plant which use this process.

In the present study, focus was also given on the cost-effectiveness of the process. So, for the determination of cellulase properties, the enzyme extracts were not submitted to downstream processing steps, such as purification or concentration.

## Temperature and pH Influence in the Cellulolytic Activity

The effects of temperature in the range of 30–80 °C and pH values in the range of 3.0–6.0 in FPase, endoglucanase, and β-glucosidase activities were determined using a factorial design with six levels. Three-dimensional graphs showing the dual effect of these parameters in the enzymes produced by *T. harzianum* are presented in Fig. 2. The optimal temperatures determined for cellulases from *T. harzianum* (48–53 °C) were lower than those determined for the enzymes produced by *A. niger* (54–57 °C) and *T. reesei* (52–57 °C). Concerning optimum pH range, cellulases from *T. reesei* acted better in pH between 5.1 and 5.3. However, optimum pH range for the enzymes produced by *A. niger* was 5.0–5.5, broader than that observed for the enzymes produced by *T. harzianum* (5.0–5.2).

To act best at lower temperatures is an advantage of the cellulolytic complex produced by *T. harzianum*, comparatively to the complexes produced by *A. niger* and *T. reesei*, due to the higher suitability of the enzymes in relatively mild conditions, thus allowing the simultaneous fermentation of the released sugars by mesophilic strains. Such characteristic allows the crude enzyme extract produced by *T. harzianum* to be used in simultaneous saccharification and fermentation (SSF) processes, which are commonly conducted at 37 °C and pH 5.0–5.5 [21, 22]. In these conditions, the relative FPase, endoglucanase, and β-glucosidase activities (compared to the maximum correspondent activities observed during

**Table 1** Maximum values of volumetric productivity and specific activity observed for production of cellulases by *T. harzianum* and the model fungi, *A. niger* and *T. reesei*[a].

| Strain | FPase | Endoglucanase | β-Glucosidase |
|---|---|---|---|
| Volumetric productivity (U $L^{-1}$ $h^{-1}$) | | | |
| *T. harzianum* IOC-4038 | 1.10±0.10 (71) | 7.60±0.36 (32) | 9.57±0.41 (71) |
| *A. niger* ATCC-16404 | 0.39±0.32 (32) | 1.48±0.05 (166) | 4.54±0.10 (120) |
| *T. reesei* RutC30 | 0.05±0.02 (333) | 0.31±0.03 (333) | 0.25±0.01 (333) |
| Specific activity (U mg $protein^{-1}$) | | | |
| *T. harzianum* IOC-4038 | 0.92±0.11 (120) | 6.42±0.31 (32) | 7.09±0.13 (120) |
| *A. niger* ATCC-16404 | 0.58±0.48 (32) | 4.42±0.15 (121) | 52.91±1.00 (265) |
| *T. reesei* RutC30 | 0.28±0.10 (333) | 1.64±0.15 (333) | 1.34±0.07 (333) |

[a] Values in parentheses correspond to time of fermentation (h) when the maximum results were observed

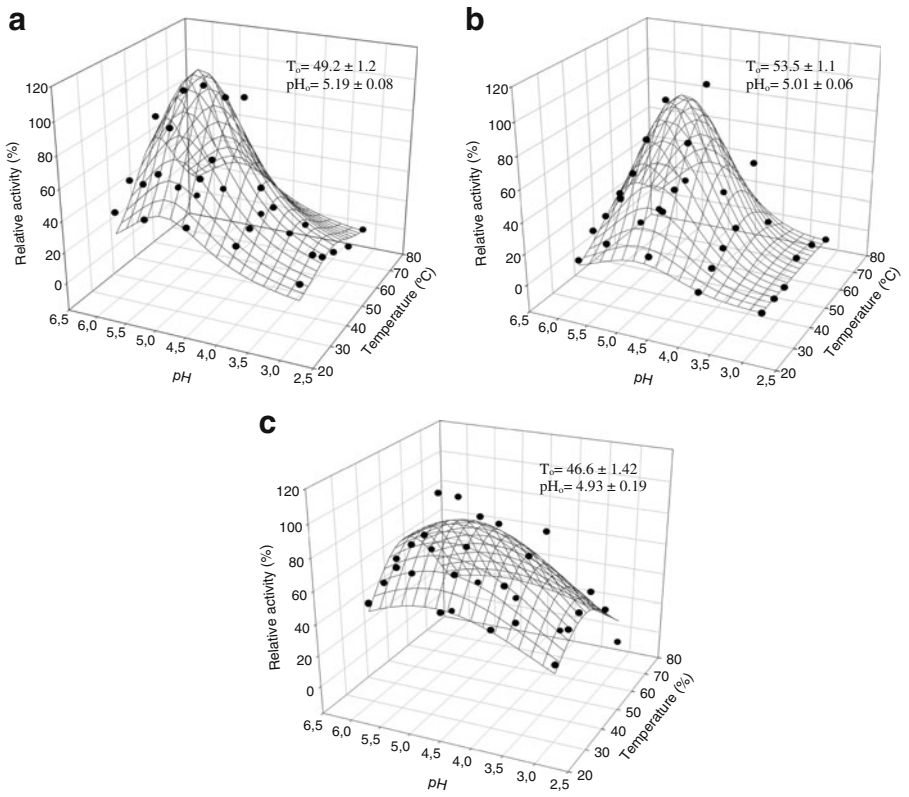


**Fig. 2** The effect of pH and temperature on enzyme activity of **a** FPase, **b** β-glucosidase, and **c** endoglucanase from *T. harzianum*

dual analysis) of the enzyme complex produced by *T. harzianum* were 59.3±6.1%, 79.3± 4.8%, and 44.5±1.3%, respectively, while for the same group of enzymes produced by *A. niger* were 55.5±3.0%, 82.6±9.0%, and 41.5±1.3%, respectively. Cellulases produced by *T. reesei* showed 25.2±5.8%, 53.2±2.7%, and 31.9±4.3% of their maximum FPase, endoglucanase, and β-glucosidase activities, when quantified under the SSF conditions. These results show that the extract produced by *T. harzianum* presents great potential for application to ethanol and other chemical production using processes that require complete cellulose hydrolysis at mild conditions.

## Thermal Stability of the Enzyme Complexes

The investigation of the stability of the cellulolytic complex produced by *T. harzianum* as well as those complexes produced by *A. niger* and *T. reesei* was performed for 23 h at three different temperatures. Profiles of the residual activities in the extracts produced by *T. harzianum* and *A. niger* are presented in Fig. 3. The relative standard deviations for FPase, β-glucosidase, and endoglucanase activities regarding the extract produced by *T. harzianum* were 6.9%, 2.3%, and 5.0%, respectively.

As expected, the higher the temperature, the higher the loss in activity. At 60 °C, all groups of enzymes produced by *T. harzianum* showed fast thermal denaturation,

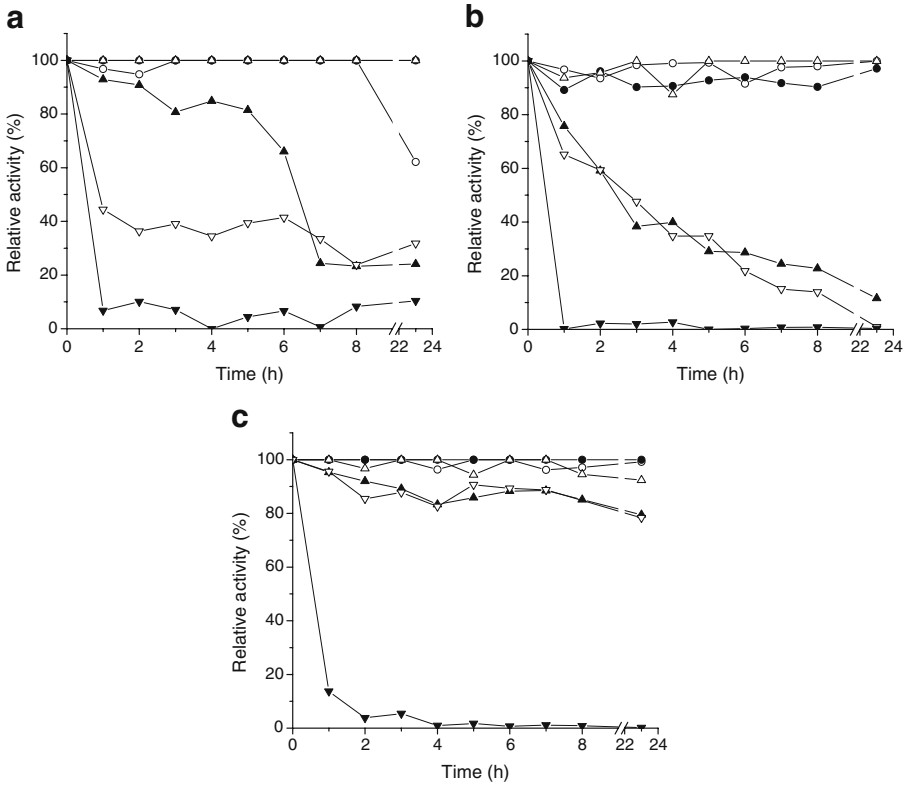


**Fig. 3** Thermal stability of cellulases produced by *T. harzianum* (*closed symbols*) and *A. niger* (*open symbols*) at 37 °C (*circles*), 50 °C (*upside triangles*), and 60 °C (*downside triangles*). **a** FPase; **b** β-glucosidase; **c** endoglucanase

corresponding to a half-life time lower than 1 h. In contrast, endoglucanase activity of the enzyme extract from *A. niger* proved to be very stable, keeping its activity above the 80% line during all the periods evaluated (Fig. 3). The higher stability of cellulases produced by *Aspergillus* strains was previously associated to the possible excretion of soluble polysaccharides [23]. At 37 °C, all enzyme activities, except FPase from *A. niger* and β-glucosidase from *T. reesei* (data not shown), maintained at least 80% of their initial activity after 23 h of incubation.

From a general perspective, the results observed in the present study indicate that the cellulolytic complex produced by *T. harzianum* presents potential for application to the complete hydrolysis of cellulose, especially at mild temperature, such as that used in SSF processes [21, 22], while the high thermal stability of endoglucanase from *A. niger* suggests that this group of enzymes can be used in processes which require high temperatures, such as those found in the textile industry [24].

## Estimation of Kinetic Parameters

Estimation of the apparent kinetic parameters, $K_M$ and $V_{max}$, were done for the endoglucanase and β-glucosidase activities in the enzyme extract produced by SmF using PDC as raw material. For the former group of enzymes produced by *T. harzianum*, $K_M$ and

$V_{max}$ were 22.314±0.078 g $L^{-1}$ and 0.120±0.000 μmol $U^{-1}$ $min^{-1}$, respectively. Regarding β-glucosidase, these same parameters were estimated as 1.549±0.022 mM and 0.129±0.002 μmol $U^{-1}$ $min^{-1}$, respectively. Although *T. harzianum* fermentations have demonstrated higher endoglucanase production when compared to the profiles of the two model strains, the kinetic analysis showed that the same group of enzymes produced by *A. niger* has faster action towards medium viscosity CMC, which was evidenced by the higher $V_{max}$ value observed for the action of endoglucanase from this latter strain (1.040±0.022 μmol $U^{-1}$ $min^{-1}$). *T. harzianum* extracts, however, demonstrated higher affinity for medium viscosity CMC when compared to the extracts produced by *A. niger*, of which the $K_M$ was 45.614±0.949 g $L^{-1}$. Regarding β-glucosidase, the $V_{max}$ value of the enzyme produced by *A. niger* (0.108±0.002 μmol $U^{-1}$ $min^{-1}$) was in the same order of magnitude of that found for the enzyme produced by *T. harzianum*, and the $K_M$ found for the β-glucosidase from the former strain was 3.284±0.057 mM. No inhibition was observed by the enzyme complexes when incubated with cellobiose and CMC with concentrations up to 14.4 and 20 g $L^{-1}$, respectively.

## Catalytic Specificity Towards Several Substrates

Crude extracts from *T. harzianum*, as well as those obtained from *A. niger* and *T. reesei*, were incubated with several substrates in order to determine their specificity towards different structures. The values observed for the extracts from the three strains evaluated in this work (*T. harzianum* IOC-4038, *A. niger* ATCC-16404, and *T. reesei* RutC30) are presented in Table 2, and, for comparison, the values determined at the same conditions for the commercial product GC-220® are shown.

As can be observed in Table 2, only the enzyme extract produced by *A. niger* showed proteolytic action under azocasein, although this did not seem to damage the stability of cellulases. Similar behavior was reported by Akiba et al. [25] when an endoglucanase from *A. niger* IFO 31125 resistant to proteases was studied. The authors attributed this resistance to the protein glycosylation, which contributed to a carbohydrate content of 9% (m/m) in

**Table 2** Substrate specificity of the extract from *T. harzianum* and its comparison with the extracts from *A. niger* and *T. reesei* and with the commercial product GC-220®.

| Substrate | Activity (U $L^{-1}$) | | | |
|---|---|---|---|---|
| | *T. reesei* | *A. niger* | *T. harzianum* | GC-220®[a] |
| Avicel | 35.6±1.4 | 88.4±3.0 | 134.7±8.7 | 6.5±5.9 |
| Azocasein | ND | 2.3±0.4 | ND | ND |
| Cellobiose | 84.2±4.4 | 957.3±19.2 | 638.1±14.2 | 13.7±1.0 |
| HV CMC | 135.1±5.8 | 235.4±14.2 | 330.4±8.3 | 190.4±9.9 |
| MV CMC | 183.9±16.3 | 462.2±18.0 | 531.7±45.8 | 215.5±20.6 |
| ULV CMC | 323.9±16.1 | 565.6±44.4 | 637.0±22.6 | 361.2±6.0 |
| Filter paper | 17.8±6.1 | 36.2±4.6 | 87.8±4.6 | 22.4±3.4 |
| pNPG | 82.0±0.7 | 508.0±4.2 | 1,144.0±19.1 | 30.0±0.4 |
| Birchwood xylan | 537.4±14.9 | 1,510.3±53.0 | 421.5±119.2 | 110.4±1.1 |

[a] Real values of activity are achieved multiplying by $10^4$

*ND* not detected, *HV* high viscosity, *CMC* carboxymethyl cellulose, *MV* medium viscosity, *ULV* ultra low viscosity

the whole structure. In the present work, besides proteases, the extract produced by *A. niger* also showed high endoxylanase activity observed when the enzyme complex was incubated with Birchwood xylan. This result possibly suggests a constitutive synthesis of endoxylanases since the material used for the fermentations did not contain xylan [12]. Another possibility is that the observed xylan hydrolysis might have been caused by action of cellulases. Vries and Visser [26] reported that both endoglucanase and β-glucosidase produced by *Aspergillus* species are able to degrade the backbone of xyloglucan. Concerning the three distinct CMC solutions used in this evaluation, it can be observed that the higher the viscosity of the solutions, the lower the activity, which is expected due to mass transfer limitations promoted by diffusivity problems.

Considering the three major groups of cellulases, β-glucosidase, endoglucanase, and exoglucanase, represented by their respective action in cellobiose, medium viscosity CMC, and avicel, results show that GC-220® has a preponderant standardized activity in CMC (91%) but very low specific activity under cellobiose and avicel (5.8% and 2.8%, respectively) (Fig. 4). The enzyme extract produced by *T. reesei* presented the most similar profile when compared to the commercial preparation GC-220®. The distribution of enzyme activities in the extract from *A. niger*, as indicated in Table 1, presented the highest relative β-glucosidase activity (63%). The extract from *T. harzianum* was the best, in terms of balance of the three types of cellulolytic activity evaluated in this work, with no preponderant activity. The proportion found in the enzyme complex produced by *T. harzianum* is interesting for the complete cellulose hydrolysis since it was found that the enzymes can catalyze the breakdown of both insoluble crystalline cellulose (avicel) and soluble cellulose (CMC), which occur during the earlier stages of hydrolysis, as well as release glucose from small molecules (cellobiose) at the latter stages of hydrolysis [5]. The crucial role of β-glucosidase activity for the increasing of cellulose conversion rate was previously reported [9, 27]. Studies concerning seven cellulolytic preparations from filamentous fungi showed that the higher the proportion of β-glucosidase activity, the higher the conversion of softwood samples into glucose, achieving 5.8% as the highest β-glucosidase proportion, when compared to endoglucanase and exoglucanase [25].

Therefore, this work presented the production of a cellulolytic complex comprising endoglucanases, exoglucanases, and β-glucosidases, among other accessory enzymes, by SmF using a low-cost material derived from sugarcane bagasse. The pretreatments applied to this agroindustrial residue allowed the generation of a tailored material, xylan free and

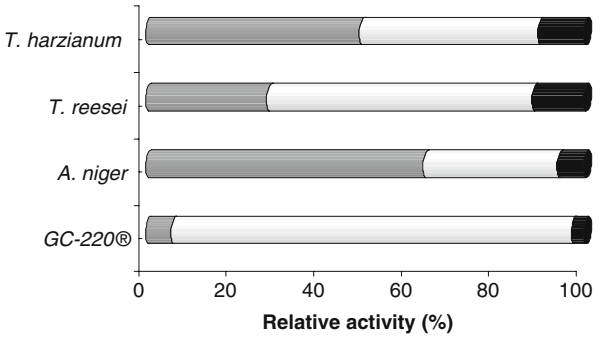


**Fig. 4** Distribution of β-glucosidase (*gray*), endoglucanase (*white*), and exoglucanase (*black*) activities in the crude extract from *T. harzianum* and its comparison to extracts from *A. niger*, *T. reesei*, and a commercial preparation

cellulose rich, which was named cellulignin. The filamentous fungus *T. harzianum* IOC-4038 was shown to be a potential producer of a well-balanced cellulolytic crude extract, which was optimally active at temperatures near 50 °C and in acidic pH values and highly thermostable at 37 °C for 23 h.

## Conclusions

The filamentous fungus *T. harzianum* IOC-4038 was revealed as a potential producer of a well-balanced cellulolytic complex, presenting fast kinetics for production of endoglucanases and β-glucosidases, achieving 559 and 745 U $L^{-1}$ as the highest activities, after 97 and 166 h of fermentation, respectively. The optimal activities of its extract were detected at pH values ranging from 4.9 to 5.2 and at temperatures between 47 and 54 °C, which were lower than those determined for *A. niger* ATCC-16404 and *T. reesei* RutC30, assumed as model strains. The crude extract from *T. harzianum* showed high thermal stability at 37 °C, which is a temperature commonly used in fermentation processes. Quantification of activities using different substrates showed that *T. harzianum* produced a cellulolytic complex with a β-glucosidase activity 76% higher than that observed using *T. reesei* and 8.4 times higher than that found in the commercial product GC-220®. Thus, *T. harzianum* IOC-4038 proved to be a promising fungal strain for production of cellulases from a sugarcane bagasse cellulignin, aiming at their application to processes where catalytic activities at mild conditions are desired.

**Acknowledgments** Thanks to Daniele F. Carvalho and Marcelle L. A. Carvalho for their technical support. The authors also thank PETROBRAS, the Brazilian Council for Research (CNPq), and the Rio de Janeiro State Foundation for Science and Technology (FAPERJ) for their financial support.

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