MICROBIOLOGY

β-glucosidase from thermophilic fungus *Thermoascus crustaceus*: production and industrial potential

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Abstract: Microbial β-glucosidases can be used in several industrial processes, including production of biofuels, functional foods, juices, and beverages. In the present work, production of β-glucosidase by solid state cultivation of the fungus *Thermoascus crustaceus* in a low-cost cultivation medium (comprising agroindustrial residues) was evaluated. The highest production of β-glucosidase, about 415.1 U/g substrate (or 41.51 U/mL), was obtained by cultivating the fungus in wheat bran with 70% humidity, during 96 h at 40°C. The enzymatic activity was optimum at pH 4.5 and 65°C. β-Glucosidase maintained its catalytic activity when incubated at a pH range of 4.0-8.0 and temperature of 30-55°C. The enzyme was strongly inhibited by glucose; even when the substrate and glucose concentrations were equal, the inhibition was not reversed, suggesting a non-competitive inhibition. In the presence of up to 10% ethanol, β-glucosidase maintained its catalytic activity. In addition to β-glucosidase, the enzymatic extract showed activity of 36 U/g for endoglucanase, 256.2 U/g for xylanase, and 18.2 U/g for β-xylosidase. The results allow to conclude that the fungus *T. crustaceus* has considerable potential for production of β-glucosidase and xylanase when cultivated in agroindustrial residues, thereby reducing the cost of these biocatalysts.

Key words: Cellulases, filamentous fungus, industrial enzymes, solid state cultivation, xylanases.

INTRODUCTION

The economic dependence on fossil fuels and their imminent depletion in the near future has escalated the search for renewable sources of energy. Lignocellulosic biomass, found in abundance in nature, is mainly composed of cellulose, hemicellulose, and lignin, wherein cellulose is of great interest for obtaining fermentable sugars, aiming at ethanol production (Singhania et al. 2010).

Cellulases are enzymes capable of acting on cellulose, promoting their hydrolysis, which can be performed using three enzymes synergistically: endoglucanases (EC 3.2.1.4), which internally cleave the cellulose chains; exoglucanases (EC 3.2.1.91), which act at the ends of chains, releasing mainly cellobiose, and lastly, β-glucosidases (EC 3.2.1.21), which terminate hydrolysis, converting cellobiose to glucose (Bansal et al. 2012). β-Glucosidase is used in various industrial processes, such as the manufacture of functional foods derived from soybeans and also in the juice and beverage industry improving its aromatic quality (Leite et al. 2008). The main industrial sectors that use cellulases are textile, food, detergent, agriculture, and livestock industries. In these
industrial processes, enzymes may be exposed
to extreme pH and temperature conditions. Thus,
it is essential that biocatalysts used industrially
must have high structural stability (Pellegrini et
al. 2015).

Despite the wide applicability of
β-glucosidases, their large-scale use remains a
problem owing to the high cost of production. An
alternative to reducing the cost of these enzymes
would be the use of hyper-producing fungal
strains, associated with the use of low value
cultivation media, e.g. agroindustrial residues
that are employed as substrates in solid state
cultivation. This type of cultivation has several
advantages for enzymatic production because
it uses less energy, does not require strict
control of cultivation parameters, produces
less wastewater, has lower processing costs,
and is similar to the natural environment of
filamentous fungi (Santos et al. 2016, Garcia et
al. 2015, Almeida et al. 2018).

Our research group has been working on the
description of new biocatalysts from enzyme-
producing filamentous fungi isolated from the Midwest region of Brazil, with appreciable
biotechnological characteristics such as low
production cost, high structural stability, and
broad industrial applicability (Cavalheiro et
al. 2017, Costa et al. 2019, Garcia et al. 2015,
2018, Morais et al. 2018). Among the strains
recently isolated by our team, the thermophilic
filamentous fungus Thermoascus crustaceus
stood out for the production of the enzyme
β-glucosidase. Considering the small number
of studies that use this microorganism for the
production of industrial enzymes, this study
aimed to optimize the solid state cultivation
of T. crustaceus aiming at the production of
β-glucosidase and to evaluate the catalytic
properties of this enzyme and the enzymatic
extract, both obtained under optimal conditions.

MATERIALS AND METHODS

Microorganism

The present work used the thermophilic
filamentous fungus Thermoascus crustaceus,
isolated from sugarcane bagasse from São
Fernando Açúcar e Álcool Ltda., Dourados -
MS, Brazil. The microorganism was identified
by Fungi Ecology and Systematics Laboratory,
Institute of Biosciences, São Paulo State
University (UNESP), Rio Claro – SP, Brazil. This
strain was maintained in Sabouraud Dextrose
Agar at 4°C, at the Laboratory of Enzymology
and Fermentative Processes - LEPFER, Faculty of
Biological and Environmental Sciences - FCBA,
Federal University of Grande Dourados – UFGD.

Inoculum

The fungus was grown in 250 mL Erlenmeyer
flasks containing 40 mL of inclined Sabouraud
Dextrose Agar, maintained for 96 h at 45°C.
The microbial suspension was obtained by
adding 25 mL of nutrient solution consisting of
ammonium sulfate, magnesium heptahydrate,
and ammonium nitrate (0.1% w/v, each one),
followed by smooth scraping of the medium
surface. The microorganism was inoculated by
transferring 5 mL of the microbial suspension
(10⁵ spores/g dry substrate) to flasks containing
the agroindustrial residues (Garcia et al. 2015).

β-glucosidase production by solid state
cultivation

The enzyme was produced by cultivating the
fungus in 250 mL Erlenmeyer flasks containing 5
g of agroindustrial residues, previously washed
and dried at 60°C for 24 h. Several residues,
namely soybean meal, rice husk, corn husk,
corn cob, sugarcane bagasse, orange bagasse,
barley, and wheat bran, were used. All materials
were previously autoclaved for 20 min at 121°C.
The substrate that promoted the best enzyme
Production was adopted for the evaluation of other fermentative parameters, such as initial humidity (50–80%), temperature (30–50°C), and cultivation time (24–144 h) in subsequent trials. The optimized parameter in each step was adopted in subsequent cultivation. All assays were performed in triplicate, and the described values represent the respective averages (Morais et al. 2018).

**Enzyme extraction**

The enzymatic extract was obtained by the addition of 50 mL of distilled water in the Erlenmeyer flasks containing the fermented residues. The flasks were stirred for 1 hour at 150 rpm. The medium was then filtered using a synthetic cloth (nylon) and centrifuged at 3000xg for 5 minutes at 10°C. The supernatant was considered the enzymatic extract and used in subsequent assays (Garcia et al. 2015).

**Determination of β-glucosidase activity**

β-glucosidase activity was determined with 50 μL enzymatic extract, 250 μL sodium acetate buffer (0.1 M, pH 4.5) and 250 μL p-nitrophenyl-pD-glucopyranoside (4 mM, Sigma), and keeping the mixture for 10 minutes at 50°C. The enzymatic reaction was stopped using 2 mL of sodium carbonate (2 M) and the released product was quantified in a spectrophotometer at 410 nm. One unit of enzymatic activity was defined as the amount of enzyme required to release 1 μmol of nitrophenol per minute of reaction (Palma-Fernandez et al. 2002).

**Effect of pH and temperature on β-glucosidase activity**

Optimal pH was determined by measuring the activity at 50°C at different pH values (3.0–8.0) using McIlvaine 0.1 M buffer. Optimum temperature was determined by measuring the enzymatic activity at different temperatures (30–80°C), at the respective optimum pH. The pH stability was determined by incubating the enzymes for 24h at 25°C at different values of pH, using the following buffer solutions: 0.1 M McIlvaine (3.0–8.0) and 0.1 M Glycine - NaOH (8.5–10.5). Thermostability was determined by incubating the enzyme for 1 h at different temperatures, which ranged from 30 to 80°C. Residual activity was determined under the respective optimum pH and temperature conditions (Martins et al. 2012).

**Effect of glucose and ethanol on enzymatic activity**

Enzyme activity was quantified by the addition of glucose or ethanol at different concentrations in the reaction mixture (0–100 mM glucose; 0–40% ethanol). The reversibility of the glucose inhibitory effect on enzyme activity was assessed by measuring catalytic activity with substrate addition at concentrations twice more than the inhibitor concentration.

**Catalytic potential of crude enzymatic extract**

Endoglucanase (CMCase), exoglucanase (avicelase), and xylanase activities were quantified using 3% carboxymethylcellulose (Sigma, C5678), 1% avicel (Sigma) and 1% xylan (Sigma, Beechwood), respectively, as substrates. The reducing sugar released was quantified by the DNS method described by Miller (1959). The activity of β-xylosidase was measured with the synthetic substrate p-nitrophenyl-p-D-xylopyranoside (4 mM, Sigma), following the methodology described previously for β-glucosidase. One unit of enzymatic activity was defined as the amount of enzyme required to release 1 μmol of product per minute of reaction (Garcia et al. 2015).
Statistical analysis

The experiments were performed in triplicates and data were expressed as the mean value of the three independent assays. Statistical analysis of the data was performed by ANOVA with significance level of 1%. Tukey test was applied to all data.

RESULTS AND DISCUSSION

β-glucosidase production by solid state cultivation

*T. crustaceus* was able to grow on all substrates used (visual evaluation), but the highest production of β-glucosidase (410.93 U/g substrate or 41.09 U/mL) was obtained by cultivating the fungus in wheat bran (Table I). According to El-Shishtawy et al. (2014), cultivations in wheat bran promote higher enzymatic production owing to their nutritional value, large surface area, better air circulation, and efficient penetration of mycelium from filamentous fungi. Previous work confirms wheat bran as an excellent substrate for β-glucosidase production by filamentous fungi (Santos et al. 2016, Garcia et al. 2018, Morais et al. 2018).

Considering the results presented in Table I, wheat bran was selected as substrate for optimization of parameters that affect enzyme production by solid state cultivation, such as: initial moisture, temperature, and cultivation time.

Among the evaluated moisture values, the highest enzyme production was obtained in wheat bran containing 70% of initial moisture; however, there was no significant difference in enzyme production between cultivation with 65 and 70% of moisture (Figure 1a). The moisture of 70% was adopted for subsequent assays because it has a higher absolute value of β-glucosidase activity, when compared to the other results. Zimbardi et al. (2013) obtained higher β-glucosidase production by the fungus *Colletotrichum graminicola* with substrate moisture values close to those described in the present work.

Among the factors that influence the enzymatic production by solid state cultivation, water content is recognized as one of the most critical. Ideal moisture varies by microorganism and substrate used, affecting microbial growth and enzyme production. In cultivations containing low moisture content, solubility and nutrient diffusion are reduced, thereby impairing the growth of the microorganism. In contrast, high levels of moisture increase the risk of bacterial contamination and limit gas exchange, reducing enzyme production (Leite et al. 2008, Bansal et al. 2012, Delabona et al. 2013).

Regarding the influence of temperature, the highest β-glucosidase production by *T. crustaceus* (411.6 U/g of substrate or 41.16 U/mL) was obtained at 40°C (Figure 1b). *T. crustaceus* is a thermophilic fungus, adapted to grow at higher temperatures. According to Gomes et al. (2007), the ideal temperature for the cultivation of thermophilic fungi is between 40 and 50°C. This corroborates the results described in the present work.
Considerable production of the enzyme of interest could be observed in cultivations maintained at 30 to 45°C; however, when the temperature was raised to 50°C, a drastic reduction in β-glucosidase production was observed. High temperatures may cause degradation of membrane structures as well as denaturation of structural proteins and enzymes (Rajoka et al. 2004).

The last parameter evaluated at this stage of the work was the cultivation time. The highest enzyme production was obtained in 96 h of cultivation at 40°C, using wheat bran as substrate with 70% initial moisture, reaching about 415.1 U/g substrate (or 41.51 U/mL). After this period, enzyme production dropped considerably (Figure 1c).

When compared to other fungal species, the optimal cultivation time for the production of β-glucosidase by T. crustaceus was lower (or equal) and the enzyme activity was considerably higher than those obtained by the authors cited (Table II).

Microbial cultivation time is a factor that influences enzymatic production and the cost of obtaining the biocatalyst. Incubation for a short period may not result in maximum enzyme production, as the microorganism may still be adapting to the environmental conditions. On the other hand, prolonging cultivation can lead to nutrient depletion, thereby causing a decline in microorganism growth and enzyme production, increasing the production cost of the enzyme (Haq et al. 2006).

**Biochemical characterization of β-glucosidase produced by T. crustaceus**

The β-glucosidase produced by T. crustaceus showed optimum activity at pH 4.5 and 65°C (Figures 2a and 2b). The characteristics of the enzyme produced by T. crustaceus are close to those described in the literature for fungal β-glucosidases. According to Santos et al. (2016), β-glucosidase produced by the fungus Gongronella butleri showed optimal activity at pH 4.5 and 65°C. Pereira et al. (2015) demonstrated...
that *Thermomucor indicae-seudatica* N31 β-glucosidase showed maximum activity at pH 4.5 and temperatures of 65–75°C. The optimal pH and temperature values of β-glucosidase produced by *Lichtheimia corymbifera* described by Morais et al. (2018) resemble the results obtained in this work.

The β-glucosidase produced by the fungus *T. crustaceus* maintained its catalytic activity after 24 h of incubation between pH 4.0 and 9.0, and retained around 50% of the initial activity at pH 10.0 (Figure 2c). As for thermal stability, the enzyme remained stable at 30–55°C after 1 h of incubation, presenting about 60% of residual activity at 60°C (Figure 2d). The results described confirm the structural stability of the β-glucosidase produced by *T. crustaceus*. Delabona et al. (2013) described the stability of β-glucosidase produced by *Aspergillus fumigatus* P40M2; this enzyme maintained its catalytic properties at pH 4.0-5.5 and temperatures of 40-60°C. The results obtained by Fusco et al. (2018) demonstrated that *Dictyoglomus turgidum* β-glucosidase is reasonably stable in the pH range of 5.0-8.0, maintaining over 90% of its activity.

Enzymes produced by thermophilic microorganisms generally have high structural stability, favoring their application in several industrial processes. This fact justifies the constant search for thermophilic strains. Thermostability is an important characteristic for industrial enzymes, since the substrate conversion rate increases at high temperatures and under these conditions the risk of contamination is also reduced (Gomes et al. 2007, Leghlimi et al. 2013, Morais et al. 2018).

### Effect of ethanol and glucose on β-glucosidase activity

The study of the effect of ethanol on β-glucosidases activity is very important, because these enzymes are generally exposed to several industrial process containing alcohol (Garcia et al. 2015).

Assays to evaluate the effect of ethanol on enzymatic activity were performed at 50°C to prevent alcoholic evaporation and to ensure that the reaction mixture contains the desired ethanol concentration. At initial ethanol concentrations, the enzyme increased its catalytic activity up to 35%, and the catalytic activity reduced in the assays performed with ethanol concentration equal to or higher than 25% (Figure 3a).

The increased catalytic potential of β-glucosidase may be related to the enzyme glycosyltransferase activity. In this case, ethanol acts as the preferential acceptor of intermediate glycosyl cation during substrate hydrolysis,
thereby increasing the rate of enzymatic reaction (Leite et al. 2008, Krisch et al. 2012, Garcia et al. 2015).

Considering that fermentative processes using *Saccharomyces cerevisiae* are carried out at temperatures below 50°C and that concentrations above 10% of ethanol are harmful to the fermenting microorganism itself (Gu et al. 2001), it can be stated that the β-glucosidase produced by *T. crustaceus* exhibits greater stability than is necessary for use in these processes.

Another important parameter evaluated was the effect of glucose on β-glucosidase activity. Enzymatic activity decreased as glucose concentration increased in the reaction mixture, remaining 30% of the initial activity in the assay performed with 100 mM glucose (Figure 3b).

According to the literature, most fungal β-glucosidases are strongly inhibited by glucose. Garcia et al. (2018) reported that the β-glucosidase produced by *Lichtheimia ramosa* was inhibited by this monosaccharide, and 50% of the initial activity was recovered in solutions containing 50 mM glucose. Santos et al. (2016) reported similar results for the β-glucosidase produced by *Gongronella butleri*. The β-glucosidases of *Lichtheimia corymbifera* and *Byssochlamys spectabilis* were strongly inhibited by glucose, and approximately 50% of their activities were recovered at 50 and 30 mM glucose, respectively (Morais et al. 2018).

The type of inhibition exerted by glucose on enzyme activity was evaluated by adding substrate to the reaction mixture at concentrations twice more than the inhibitor. Despite the high substrate concentration, the reversibility of inhibition was not observed, which is a non-competitive inhibition characteristic (Table III).

In this type of inhibition, the inhibitor and substrate do not compete for the same binding site (active site); the inhibitor binds to another region of the enzyme, decreasing the product generation rate. In this case, increasing the substrate concentration in the reaction mixture does not reverse the inhibition (Leite et al. 2008, Santos et al. 2016).
Glucose inhibition of β-glucosidase is a serious problem for obtaining fermentable sugars from cellulose hydrolysis present in vegetable biomass (Pereira et al. 2015, Garcia et al. 2018). An alternative to overcome this problem is to perform the process called saccharification and simultaneous fermentation. In this process, the saccharification of cellulose is performed simultaneously with the alcoholic fermentation. Thus, glucose released by cellulose hydrolysis is converted to ethanol by the fermenting microorganism, reducing the inhibitory effect of this monosaccharide on enzyme activity. However, the enzymes employed in the process must be tolerant to the presence of ethanol in the reaction mixture (Singhania et al. 2013, Pereira et al. 2015, Garcia et al. 2018, Morais et al. 2018).

The results described in the present work confirm the ethanol stability of the β-glucosidase produced by T. crustaceus, allowing the inference that this enzyme has potential to be applied in saccharification and simultaneous fermentation processes.

**Catalytic potential of the enzymatic extract obtained under optimized conditions**

The catalytic potential of the enzymatic extract produced by T. crustaceus under optimized cultivation conditions was evaluated (Table IV). Enzymatic extract showed no considerable cellulases activities, with 36 U/g for endoglucanase and 3.3 U/g for exoglucanase. The values described are below the values obtained by microorganisms described as producers of these enzymes. Hansen et al. (2015) reported that the cellulase production by solid state cultivation of the fungi Fusarium chlamydosporum and Trichoderma reesei (QM9414) yielded 281.8 and 94.2 U/g of endoglucanase and 182.4 and 99.7 U/g exoglucanase, respectively.

Regarding hemicellulases, T. crustaceus showed potential for production of xylanase (256.2 U/g substrate) and a small amount of β-xylosidase (18.2 U/g substrate), under the optimized culture conditions. The catalytic properties described for the enzymatic extract are favorable for application in the pulp and paper industry. The presence of xylanases and the low activities of endo and exocellulases make the enzymatic extract suitable for application in the pulp and paper industry, in the paper bleaching process, as they ensure that the cellulose fibers of the paper are not degraded by cellulolytic enzymes (Brienzo et al. 2012).

The characteristics described are difficult to observe in enzymatic cocktails obtained by...
microbiological cultivations in complex media, such as wheat bran, thereby motivating the production of xylanolytic enzymes by submerged cultivation containing commercial xylan as exclusive carbon source, which increases the cost of producing these enzymes, making impractical the use of these biocatalysts in the pulp and paper industry (Costa et al. 2016).

### CONCLUSIONS

The results show that the use of agroindustrial residues in the solid state cultivation process is viable for production of β-glucosidase by *Thermoascus crustaceus*, especially when cultivated in wheat bran. The β-glucosidase produced has wide industrial applications, justified by its characteristics and high production in low value culture media, and can be used in processes to obtain second-generation ethanol, as well as in the juice and beverage industry. The enzymatic extract has the potential to be applied in pulp and paper bleaching processes owing to its high xylanase activity, low cellulolytic activity, and low production cost.

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### REFERENCES

ALMEIDA PH, OLIVEIRA ACC, SOUZA GPN, FRIEDRICH JC, LINDE GA, COLAUTO NB & VALLE JS. 2018. Decolorization of remazol brilliant blue R with laccase from *Lentinus crinitus* grown in agro-industrial by-products. An Acad Bras Cienc 90: 3463-3473.

BANSAL N, TEWARI R, SONI R & SONI SK. 2012. Production of cellulases from *Aspergillus niger* NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manag 32: 1341-1346.

BRIENZO M, MONTE JR & MILAGRES AMF. 2012. Induction of cellulase and hemicellulase activities of *Thermoascus aurantiacus* by xylan hydrolyzed products. World J Microbiol Biotechnol 28: 113-119.

CAVALHEIRO GF, SANGUINE IS, SANTOS FR, COSTA AC, FERNANDES M, PAZ MF, FONSECA GG & LEITE RSR. 2017. Catalytic properties of amylolytic enzymes produced by *Gongronella butleri* using agroindustrial residues on solid-state fermentation. Biomed Res Int 2017: 1-8.

COSTA AC, CAVALHEIRO GF, VIEIRA ERQ, SANDRA JR, GOES RHTB, PAZ MF, FONSECA GG & LEITE RSR. 2019. Catalytic properties of xylanases produced by *Trichoderma piluliferum* and *Trichoderma viride* and their application as additives in bovine feeding. Biocatal Agric Biotechnol 19: 101161.

COSTA AC, SCALABRINI RP, SILVESTRE MA, RODRIGUES A, PAZ MF, FONSECA GG & LEITE RSR. 2016. Production of xylanase by a new strain of *Thermoascus aurantiacus*: obtainment

Table III. Residual activity at equal substrate (pNPβG) and inhibitor concentrations.

| Enzyme     | Res. Activity (%) PNPG – 2 mM | Res. Activity (%) PNPG – 50 mM Glucose – 25 mM | Inhibition type |
|------------|-------------------------------|-----------------------------------------------|----------------|
| T. crustaceus | 100                           | 56                                            | Non-competitive |

Table IV. Catalytic potential of enzymatic extract obtained by solid state cultivation of the fungus *T. crustaceus* in wheat bran containing 70% of initial moisture, at 40°C and 96 h.

| Enzyme       | U/mL | U/g substrate |
|--------------|------|---------------|
| Endoglucanase| 3.6  | 36.0          |
| Exoglucanase | 0.3  | 3.0           |
| β-glucosidase| 41.5 | 415.0         |
| Xylanase     | 25.6 | 256.0         |
| β-xylosidase | 1.8  | 18.0          |
of enzymatic extract with reduced cellulolytic activity for application in pulp and paper industries. Biosci J 32: 1040-1048.

DELABONA PS, PIROTA RDPB, CODIMA CA, TREMACOLDIC CR, RODRIGUES A & FARINAS CS. 2013. Effect of initial moisture content on two Amazon rainforest Aspergillus strains cultivated on agro-industrial residues: biomass-degrading enzymes production and characterization. Ind Crop Prod 42: 236-242.

EL-SHISHTAWY RM, MOHAMED SA, ASIRI AM, GOMAA AM, IBRAHIM IM & AL-TALHI HA. 2014. Solid fermentation of wheat bran for hydrolytic enzymes production and saccharification content by a local isolate Bacillus megatherium. BMC Biotechnol 14: 29.

FUSCO FA, FIORENTINO G, PEDONE E, CONTURSI P, BARTOLOCCI S & LIMAUDO D. 2018. Biochemical characterization of a novel thermostable β-glucosidase from Dictyoglomus turgidum. Int J Biol Macromol 113: 783-791.

GARCIA NFL, SANTOS FR, BOCCHINI DA, PAZ MF, FONSECA GG & LEITE RSR. 2015. Production of β-glucosidase on solid state fermentation by Lichtheimia ramosa: potential for sugarcane bagasse saccharification. Ind Crop Prod 122: 49-56.

GARCIA NFL, SANTOS FR, GONÇALVES FA, PAZ MF, FONSECA GG & LEITE RSR. 2015. Production of β-glucosidase on solid state fermentation by Lichtheimia ramosa on agroindustrial waste: characterization and catalytic properties of the enzymatic extract. Electron J Biotech 18: 314-319.

GOMES E, GUEZ MAU, MARTIN N & SILVA R. 2007. Enzimas termoestáveis: fontes, produção e aplicação industrial. Quim Nova 30: 136-145.

GU Y, QIAO M, ZHOU Q, ZHOU Z & CHEN G. 2001. Hyperproduction of alcohol using yeast fermentation in highly concentrated molasses medium. Tsinghua Sci Technol 6: 225-230.

HANSEN GH, LÜBECK M, FRISVAD JC, LÜBECK PS & ANDERSEN B. 2015. Production of cellulolytic enzymes from ascomycetes: Comparison of solid state and submerged fermentation. Process Biochem 50: 1327-1341.

HAQ I, JAVED MM & KHAN TS. 2006. An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of Aspergillus niger MSK-1 and Trichoderma viride MSK-10. Afr J Biotechnol 5: 609-614.

KRISCH J, BENCSIK O, PAPP T, VÅGVOLGYI C & TÁKÓ M. 2012. Characterization of a β-Glucosidase with Trangalactosylation Capacity from the Zygomycete Rhizomucor miehei. Process Biochem 41: 555-560.

LEGHLIMI H, MERAHI Z, BOUKHALFA-LEZZAR H, COPINET E & DUCHIRON F. 2013. Production and characterization of cellulolytic activities produced by Trichoderma longibrachiatum (GHL). Afr J Biotechnol 12: 465-475.

LEITE RSR, ALVES-PRADO HF, CABRAL H, PAGNOCCA FC, GOMES E & SILVA R. 2008. Production and characteristics comparison of crude β-glucosidases produced by microorganisms Thermoascus aurantiacus e Aureobasidium pullulans in agricultural wastes. Enzyme Microb Technol 43: 391-395.

MARTINS ES, LEITE RSR, DA-SILVA R & GOMES E. 2012. Production and characterization of polygalacturonase from thermophilic Thermoascus aurantiacus on submerged fermentation. Ann Microbiol 62: 1199-1205.

MILLER GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31: 426-428.

MORAIS TB, BARBOSA PMG, GARCIA NFL, ROSA-GARZON NG, FONSECA GG, PAZ MF, CABRAL H & LEITE RSR. 2018. Catalytic and thermodynamic properties of β-glucosidases produced by Lichtheimia coromifera and Byssolchlamys spectabilis. Prep Biochem and Biotechnol 48: 777-786.

PALMA-FERNANDEZ ER, GOMES E & DA-SILVA R. 2002. Purification and characterization of two β-glucosidases from thermophilic fungus Thermoascus aurantiacus. Folia Microbiol 47: 685-690.

PELLLEGRINI VO, SERPA VI, GODOY AS, CAMILO CM, BERNARDES A, REZENDE CA & POLIKARPOV I. 2015. Recombinant Trichoderma harzianum endoglucanase I (Cel7B) is a highly acidic and promiscuous carbohydrate-active enzyme. Appl Microbiol Biotechnol 99: 9591-9604.

PEREIRA JC, MARQUES NP, RODRIGUES A, OLIVEIRA TB, BOSCOLO M, DA-SILVA R, GOMES E & BOCCHINI-MARTINS DA. 2015. Thermophilic fungi as new sources for production of cellulases and xylanases with potential use in sugarcane bagasse saccharification. J Appl Microbiol 118: 928-939.

RAJOKA MI, KHAN S, LATIF F & SHAHID R. 2004. Influence of carbon and nitrogen sources and temperature on hyperproduction of a thermotolerant β-glucosidase from synthetic medium by Kluyveromyces marxianus. Appl Biochem Biotechnol 117: 75-92.

SANTOS FR, GARCIA NFL, PAZ MF, FONSECA GG & LEITE RSR. 2016. Production and characterization of β-glucosidase from Gongronella butleri by solid-state fermentation. Afr J Biotechnol 15: 633-641.

SINGHANIA RR, PATEL AK, SUKUMARAN RK, LARROCHE C & PANDEY A. 2013. Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresour Technol 127: 500-507.
SINGHANIA RR, SUKUMARAN RK, PATEL AK, LARROCHE C & PANDEY A. 2010. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol 46: 541-549.

ZIMBARDI ALRL, SEHN C, MELEIRO LP, SOUZA FHM, MASUI DC, NOZAWA MSF, GUIMARÃES LHS, JORGE JA & FURRIEL RPM. 2013. Optimization of β-glucosidase, β-xylosidase and xylanase production by Colletotrichum graminicola under solid-state fermentation and application in raw sugarcane trash saccharification. Int J Mol Sci 14: 2875-2902.

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* In memoriam

Author contributions
Maria Alice Silvestre: collected samples, isolated and selected the fungus for β-glucosidase production; André Rodrigues: carried out the taxonomic identification of the microorganism; Andreza de Paula Garbin, Nayara Fernanda Lisboa Garcia and Gabriela Finoto Cavalheiro were responsible for the execution of all laboratory assays; Marcelo Fossa da Paz and Gustavo Graciano Fonseca were responsible for the orientation of the enzymatic production assays (Bioprocesses) and Rodrigo Simões Ribeiro Leite was responsible for the enzymatic analyzes. All authors discussed the results and contributed to the writing of the manuscript.