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ORIGINAL PAPER

A new approach for *Chrysoporthe cubensis* cellulolytic cocktail production using solid and submerged-state fermentation

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Abstract

The lignocellulosic material bioconversion to bioproducts has received significant attention in recent years. Cellulases and hemicellulases catalyze the hydrolysis of lignocellulosic materials into fermentable sugars that are afterward converted to bioproducts by microorganisms. *Chrysoporthe cubensis* grown under solid-state fermentation (SSF) has produced more effective enzymatic extracts for sugarcane bagasse saccharification than commercial cellulolytic preparations. However, the investigation of new approaches for enzyme production by this fungus is still lacking. In this work, an enzyme cocktail (SSF-SmF-cocktail) was produced by extracting enzymes of *C. cubensis* grown under SSF using the extract produced by the same fungus under submerged fermentation (SmF). The total cellulase (FPase), carboxymethylcellulase (CMCase), cellobiohydrolase (CBH), β-glucosidase, xylanase, β-xylosidase, β-galactosidase, α-galactosidase, and α-arabinofuranosidase activities were evaluated in crude extracts obtained from *C. cubensis* cultivation under SSF, SmF, and also in the SSF-SmF-cocktail. The *C. cubensis* protein profiles cultivated under SSF and SmF were compared by SDS-PAGE. Extract produced by *C. cubensis* grown under SmF presented proteins with estimated molecular weights of 10.7, 29.3, 38.6, 46.0, and 170.0 kDa, respectively, but not in that produced by this fungus under SSF. When cultivated under SSF, *C. cubensis* produced an extract with greater protein diversity between 13 and 51 kDa than that obtained by this fungus under SmF. The 83.0 and 95.3 kDa protein bands were present in both *C. cubensis* cultures. The *C. cubensis* SSF-SmF-cocktail presented better efficiency in glucose release after 48 h of the alkali-pretreated sugarcane bagasse hydrolysis when compared to those produced by this fungus under either SSF or SmF. This extract showed the highest xylananase/FPase rate and the second highest CMCase/FPase and b-glucosidase/FPase rates among the evaluated extracts, suggesting that these enzymes are the main determinants of this cocktail the efficiency on the alkali pretreated sugarcane bagasse sacchariffication process. These results demonstrated that the enzymes produced by *C. cubensis* cultivated under SSF and SmF are complementary for the alkali-pretreated sugarcane bagasse enzymatic hydrolysis, since the SSF-SmF-cocktail was more efficient than other extracts produced by this fungus and that the commercial Accellerase®. Therefore, the SSF-SmF-cocktail is a promising alternative for industrial applications.

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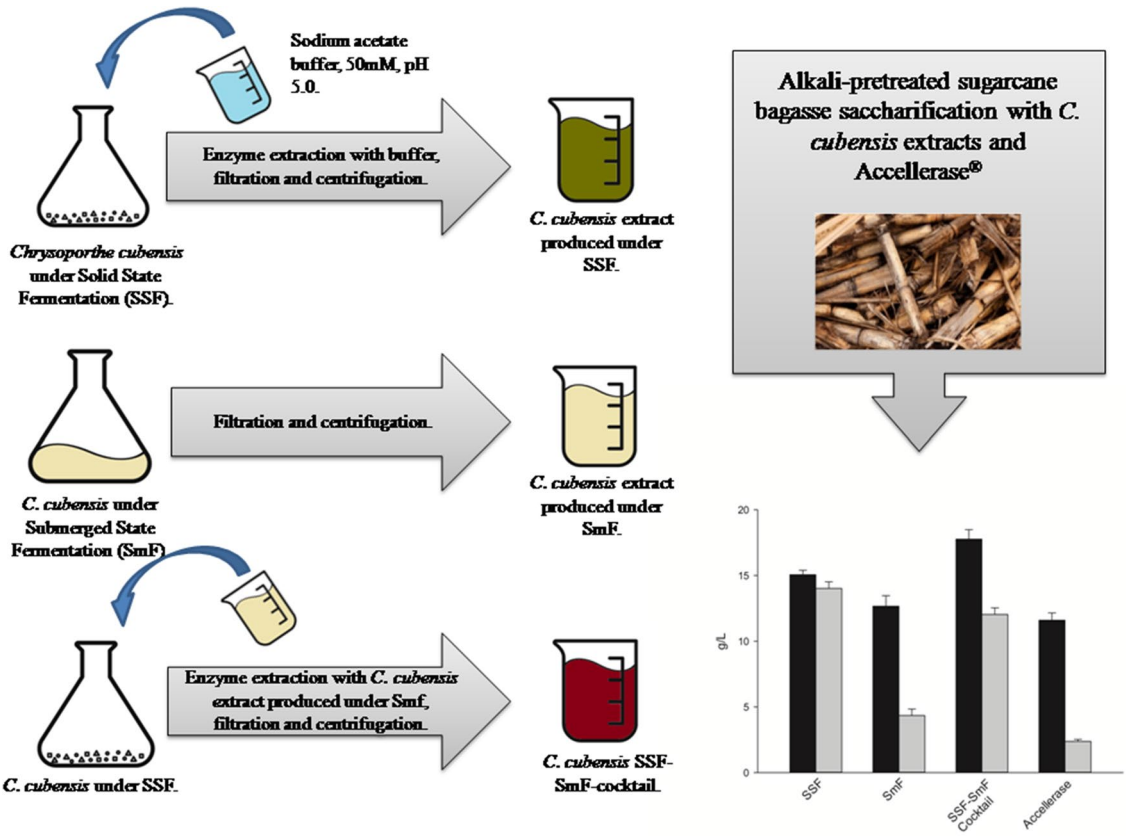
Graphical abstract



Keywords *Chrysoporthe cubensis* · Solid-state fermentation · Submerged fermentation · Saccharification

Introduction

Cellulases and hemicellulases are the main enzymes in the degradation of lignocellulosic polysaccharides to simple monomeric sugars, which are then converted to biofuels or other value-added products through microbial fermentation processes (Zhang et al. 2011).

Cellulose is degraded by the synergistic action of three types of enzymes: exoglucanase or cellobiohydrolase (EC 3.2.1.91), endoglucanase (EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21) (Champreda et al. 2019; Olsen et al. 2016). The hemicellulose fraction, a more complex polymer, requires a more diverse enzyme group, such as β-1,4-endoxylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37), and auxiliary enzymes, such as α-arabinofuranosidase (EC 3.2.1:55), α-glucuronidase (EC 3.2.1.139), α-galactosidase (EC 3.2.1.22), acetylxylan esterase (EC 3.1.1.72) and ferulic acid esterase (Visser et al. 2013).

Since enzymatic saccharification is one of the most important bottlenecks to lignocellulosic bioconversion technologies consolidation, new approaches have been made to produce low-cost and efficient cellulolytic preparations (Vicari et al. 2012).

Solid-state fermentation (SSF) and submerged fermentation (SmF) are the main approaches for cellulase production by microorganisms. In the SSF, there is an absence or near absence of free water, while the SmF is carried out in an aqueous medium (Barrios-González 2012).

SSF presents advantages such as yielding concentrated products with high stability, lower contamination risks, and lower costs than SmF (Holker et al. 2004; de Almeida et al. 2013). Moreover, the production of enzymes in the SSF currently gains more attention due to its advantages such as higher volumetric productivity, less water usage, and the use of natural products, such as agro-industrial wastes (Mendonça et al. 2020).

SmF is mainly used in large-scale processes, where bioreactors are required (Fanaei and Vaziri 2009). The industrial cellulase production is mainly done under SmF conditions (Subramaniyam and Vimala 2004). Fungi cultivation under

SSF presents some disadvantages including the build-up of gradients of temperature, pH, moisture, substrate concentration or pO_2 during cultivation, which are difficult to control under limited water availability (Hölker et al. 2004).

The ascomycete fungus, Eucalyptus pathogen, *Chrysoporthe cubensis* grown under SSF conditions produced more efficient enzymatic extracts for sugarcane bagasse saccharification than commercial cellulolytic preparations as Multifect CL®, Multifect XL® and Accellerase® (Falkoski et al. 2013; Maitan-Alfenas et al. 2015; Dutra et al. 2017; de Albuquerque et al. 2021; Tavares et al. 2021). However, little is known about the hydrolytic capacity of the extracts produced by this fungus under submerged-state fermentation or by the extracts mixtures of SSF and SmF *C. cubensis* secreted enzymes.

In this work, cellulolytic cocktails were produced by *C. cubensis* cultivated under SSF and under SmF. Besides that, it was produced an enzyme cocktail by the extraction of the enzymes produced by *C. cubensis* under SSF cultivation with the extract produced by the same fungus under SmF condition (SSF-SmF-Cocktail). The *C. cubensis* cocktails efficiency was evaluated for the alkali-pretreated sugarcane bagasse hydrolysis capacity.

Methods

Microorganism

The fungus *C. cubensis* LPF-1 used in this study was obtained from the mycological collection of the Forest Pathology Laboratory of the Universidade Federal de Viçosa in Viçosa, Minas Gerais State, Brazil. The fungus was maintained on PDA (potato dextrose agar) plates at 28 °C and subcultured every 15 days.

Chrysoporthe cubensis enzyme production

All undermentioned extracts were produced with the same final volume (50 mL) for comparison in terms of enzymatic activity per volume (U mL^{-1}). Wheat bran was used as the sole carbon source for cultivation conditions, since it is described as a strong inducer of lignocellulolytic enzyme production by *C. cubensis* (Falkoski et al. 2013; Maitan-Alfenas et al. 2015).

Submerged fermentation (SmF)

The *C. cubensis* cultivation under SmF was conducted in 125 mL Erlenmeyer flasks with 50 mL of culture medium composed of (g L^{-1}): $(NH_4)_2SO_4$, 1.4; urea, 0.3; KH_2PO_4, 2.0; $MgSO_4$ $7H_2O$, 0.3; $CaCl_2$, 0.3; and yeast extract, 2.0. The wheat bran was added to the medium at the final concentration of 10 g L^{-1}. The trace elements $FeSO_4$ $7H_2O$ (1.0 mg L^{-1}), $ZnCl_2$ (3.5 mg L^{-1}), $MnSO_4$ H_2O (1.0 mg L^{-1}), CoCl $6H_2O$ (1.0 mg L^{-1}), $CuSO_4 5H_2O$ (0.5 mg L^{-1}) and $20MoO_3$ $2H_3PO_4$ $48H_2O$ (0.02 mg L^{-1}) were also added. The flasks were autoclaved at 120 °C for 20 min, inoculated with 0.5 mL of a spore suspension (2.2×10^6 spores mL^{-1}), and placed in a shaker for seven days at 180 rpm, and 28 °C. The samples were centrifuged at 10,000×*g* for 20 min and the supernatants were used as enzyme extracts.

Inoculum preparation for solid-state fermentation (SSF)

The inoculum was prepared by growing the fungus under SmF in 250 mL Erlenmeyer flasks containing 100 mL of medium with the following composition, in g L^{-1}: glucose, 10.0; NH_4NO_3, 1.0; KH_2PO_4, 1.0; $MgSO_4$, 0.5 and yeast extract, 2.0. Each flask was inoculated with 1.0 mL agar plugs cut from a 5-day-old colony of *C. cubensis* grown on PDA plates and incubated in a rotary shaker for 5 days, at 150 rpm and 28 °C. The culture obtained was used to inoculate the solid culture media.

Solid-state fermentation (SSF)

Chrysoporthe cubensis was cultured under SSF using wheat bran as the main carbon source. The fermentations were carried out in 125 mL Erlenmeyer flasks containing 5 g (dry weight) of the wheat bran moistened with culture media (final moisture of 60%), presenting the following composition, in g L^{-1}: NH_4NO_3, 1.0; KH_2PO_4, 1.5; $MgSO_4$, 0.5; $CuSO_4$, 0.25 and yeast extract, 2. Furthermore, $MnCl_2$ (0.1 mg L^{-1}), H_3BO_3 (0.075 mg L^{-1}), Na_2MoO_4 (0.02 mg L^{-1}), $FeCl_3$ (1.0 mg L^{-1}) and $ZnSO_4$ (3.5 mg L^{-1}) also were added to the medium as trace elements. The flasks were autoclaved at 120 °C for 20 min and then inoculated with 3 mL (containing 1.5×10^7 spores mL^{-1}) of the inoculum obtained as aforementioned. The flasks were maintained at 28 °C in a temperature-controlled chamber, and the enzymatic extraction was performed after seven days of fermentation. Enzymes secreted during SSF were extracted with sodium acetate buffer, 50 mM, pH 5, at a ratio of 10 mL from buffer to 1 g dry substrate, under the agitation of 150 rpm for 60 min at room temperature. Solids were separated by filtration through a nylon cloth followed by centrifugation at 15,000*g* for 10 min, and the clarified supernatants were frozen and stored for subsequent enzymatic analysis. Experiments were carried out with three replicates for each medium composition and each incubation time.

Chrysoporthe cubensis SSF-Smf-Cocktail production

For *C. cubensis* SSF-SmF-cocktail production this fungus was cultivated under solid state fermentation (SSF), as aforementioned, and the enzyme extraction was carried out using the the *C. cubensis* SmF extract (produced as previously described) instead of buffer solution at a ratio of 10 mL from the extract to 1 g dry substrate, under the agitation of 150 rpm for 60 min at room temperature. Solids were separated by filtration through a nylon cloth followed by centrifugation at 15,000*g* for 10 min, and the clarified supernatants were frozen and stored for subsequent enzymatic analysis. Experiments were carried out with three replicates for each medium composition and each incubation time.

Enzymatic assays

All enzymatic assays were carried out in sodium acetate buffer, 100 mM, pH 5, at 50 °C in triplicate, and the mean values were calculated. Relative standard deviations of measurements were below 5%. FPase and endoglucanase activities were determined using Whatman No. 1 filter paper and carboxymethylcellulose as substrates, respectively (Ghose 1987). The total reducing sugar liberated during the enzymatic assays was quantified by the dinitrosalicylic acid (DNS) method (Miller 1959) using glucose as a standard. Xylanase activity was determined using beechwood xylan (final concentration of 1% w/v) as substrate. The enzymatic reactions were initiated with the addition of 100 µL of enzyme extract diluted to 400 µL substrate solution with the polysaccharide prepared in a buffer. The reaction mixtures were incubated for 30 min and the amount of reducing sugars released was determined by the DNS method using glucose as standard. Cellobiohydrolase, β-galactosidase, β-glucosidase, β-xylosidase, α-galactosidase, and arabinofuranosidase activities were measured using 4-nitrophenyl β-D-cellobioside, 4-nitrophenyl β-D-galactopyranoside, 4-nitrophenyl β-D-glucopyranoside, 4-nitrophenyl β-D-xylopyranoside, 4-nitrophenyl α-d-galactopyranoside and 4-nitrophenyl α-L-arabinofuranoside as substrates, respectively. One enzyme activity unit (U) was defined as the amount of enzyme which released a µmol of the product (equivalent glucose and 4-nitrophenol) per minute under assay conditions used for all activities.

Protein quantification

Protein concentration in the enzymatic extracts was determined by the Coomassie Blue binding method using bovine serum albumin as the standard (Bradford 1976).

Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using a 12% (w/v) polyacrylamide gel with a 5% stacking gel and the Mini-Protean II system (BioRad) according to the method previously described (Laemmli 1970), with some modifications. The gel obtained on SDS-PAGE was stained with G-250 colloidal Coomassie blue according to the method described (Dyballa and Metzger 2009). The molecular mass of the proteins was determined by linear regression obtained by correlating the logarithm of the molecular weight marker with the distance covered in the SDS-PAGE. The molecular weight markers were purchased from GE Healthcare Life Sciences.

Sugarcane bagasse alkaline pretreatment and saccharification

The ground sugarcane bagasse, particle size less than 1 mm, was submitted to alkaline pretreatment with NaOH 1% (w/v) solution (Dutra et al. 2017).

The enzymatic cocktails produced by *C. cubensis* and commercial Accellerase® 1500 purchased from Dupont/Genencor International Inc. (Rochester, NY, USA) were applied in a biomass saccharification experiment. The *C. cubensis* enzymatic cocktails were concentrated fivefold before the experiment using an Amicon Ultra-filtration system (Millipore Co. – Billerica, MA, USA) and a YM-10 (Cut-off Mr 10,000 Da) membrane filter. Enzymatic saccharification of alkali-treated sugarcane bagasse was performed in 25 mL Erlenmeyer flasks at an initial solid concentration of 2% dry matter (w/v) in 5.0 mL of 50 mM sodium acetate buffer at pH 4.5. Enzyme loading was specified as 10 FPase units per gram of biomass with the addition of sodium azide (10 mM) and tetracycline (40 µg mL^{-1}) to the reaction mixture to inhibit microbial contamination. The reaction was carried out in an orbital shaker at 250 rpm and 50 °C for different time intervals up to 72 h. These samples were immediately heated to 100 °C to denature the enzymes, cooled, and then centrifuged for 5 min at 15,000 *g*. Products of the saccharification assays were analyzed by High-Performance Liquid Chromatography (HPLC) with a Shimadzu series 10 A chromatography. The HPLC was equipped with an Aminex HPX-87P column (300 × 7.8 mm) and refractive index detectors. The column was eluted with water at a flow rate of 0.6 mL min^{-1} and 80 °C.

Statistical analysis

The values of *C. cubensis* activities on different condition of cultivation were analyzed using Assistat 7.7 software, performing analysis of variance (ANOVA) followed by Tukey's

test at a significance level of 5% (α = 0.05). The standard deviation was also calculated for all assays.

Results and discussion

Electrophoretic profiles from *C. cubensis* extracts produced under SSF and SmF

SDS-PAGE showed different protein profiles for *C. cubensis* extracts produced under SSF and SmF (Fig. 1). Extract produced by *C. cubensis* grown under SmF presented proteins with estimated molecular weights of 10.7, 29.3, 38.6, 46.0 and 170.0 kDa, respectively, but not under SSF. Extract produced by this fungus cultured under SSF showed greater protein diversity between 13 and 51 kDa than under SmF. The differential protein secretion by *C. cubensis* under SSF and SmF agreed with reports from *Aspergillus terreus* CM20 and *Aspergillus niger* showing differentiated electrophoretic proteins patterns secreted for SSF and SmF cultivation

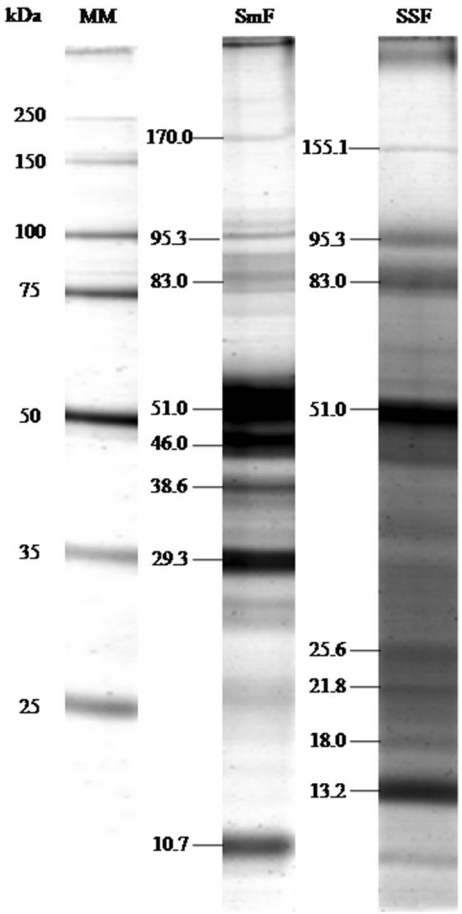


Fig. 1 SDS-PAGE from *Chrysoporthe cubensis* secreted proteins cultivated under submerged fermentation (SmF); and solid-state fermentation (SSF). *MM* Molecular marker

(Venegas et al. 2013; Saritha et al. 2016; Acuña-Argüelles et al. 1995). *Aspergillus terreus* CM20 secreted multiple isoforms of endo-β-glucanase, β-glucosidase, and xylanase under SSF, but not under SmF, which was demonstrated by zymogram analysis (Saritha et al. 2016). SSF cultivation provides higher oxygenation and lower sugar supply than SmF cultivation, promoting morphological and physiological differences leading to differential protein secretion by fungi (Viniegra-González et al. 2003), justifying the results obtained for *C. cubensis*.

Chrysoporthe cubensis secreting 170.0 and 155.1 kDa proteins under SmF and SSF, respectively, agreed with the report of this fungus secreting some high molecular weight proteins (from 120 to 200 kDa), although the most predicted carbohydrate-active enzymes showed molecular weights from 15 to 120 kDa (Tavares et al., 2020). The 83.0 and 95.3 kDa protein bands were observed for both *C. cubensis* cultures. The production of 83.0 kDa protein by *Chrysoporthe cubensis* agreed with the report of this fungus secreting a glycosidase (85 kDa) able to improving sugarcane bagasse saccharification when added in cellulolytic cocktails as a supplement (Andrade et al. 2017).

Enzymatic production by *C. cubensis*

The enzymatic cocktail obtained by extracting the *C. cubensis* enzymes produced under SSF with the extract produced by the same fungus under SmF (SSF-SmF-Cocktail) showed the highest activity of FPase, endoglucanase, cellobiohydrolase, xylanase, and β-xylosidase, respectively (Table 1). This result was expected since this cocktail is composed of *C. cubensis* enzymes produced under both conditions. SSF

Table 1 Cellulase and hemicellulase activities produced by *Chrysoporthe cubensis* under submerged fermentation (SmF) and solid-state fermentation (SSF) with wheat bran as carbon source

Enzyme	Activity (U mL^{-1})		
	SmF	SSF	SSF-SmF
FPase	0.13 ± 0.004^{b}	0.12 ± 0.008^{b}	0.18 ± 0.013^{a}
CMCase	4.84 ± 0.074^{b}	3.44 ± 0.038^{c}	6.35 ± 0.202^{a}
β-Glu	0.13 ± 0.032^{b}	1.30 ± 0.044^{a}	1.09 ± 0.164^{a}
CBH	0.11 ± 0.004^{a}	0.26 ± 0.035^{b}	0.37 ± 0.009^{a}
Xylanase	13.2 ± 0.485^{c}	14.9 ± 0.599^{b}	25.95 ± 0.463^{a}
β-Xyl	0.04 ± 0.000^{a}	0.04 ± 0.000^{a}	0.05 ± 0.000^{a}
β-Gal	0.22 ± 0.009^{a}	ND	0.02 ± 0.001^{b}
α-Gal	ND	0.06 ± 0.005^{a}	0.03 ± 0.003^{b}
α-Ara	ND	0.02 ± 0.001^{a}	0.01 ± 0.002^{b}

The averages followed by the same letter do not differ significantly by the Tukey test at 5% of significance

ND not detected, *β-Glu* β-Glucosidase, *CBH* cellobiohydrolase, *β-xyl* β-xylosidase, *β-Gal* β-galactosidase, *α-Gal* α-galactosidase, *α-Ara* α-arabinofuranosidase

Table 2 Comparative analysis of cellulase and hemicellulase activities of the extracts produced by *Chrysoporthe cubensis* under SSF or SmF and the SSF-SmF-Cocktail compared to the Accellerase cocktail

Units of enzymatic activity/Units of FPase activity				
Enzyme	SmF	SSF	SSF-SmF	Accellerase
CMCase	37.2	28.71	35.27	19.85
β-Glu	1.02	10.85	6.07	1.23
CBH	0.87	2.21	2.07	0.2
Xylanase	101.3	124.5	144.17	1.14
β-Xyl	0.35	0.34	0.27	0.02
β-Gal	1.73	ND	0.09	ND
α-Gal	0.005	0.48	0.16	ND
α-Ara	ND	0.08	0.03	ND

The values displayed were obtained by dividing each total enzymatic activity by the total FPase activity found in each cellulolytic extract

ND not detected, *β-Glu* β-Glucosidase, *CBH* cellobiohydrolase, *β-xyl* β-xylosidase, *β-Gal* β-galactosidase, *α-Gal* α-galactosidase, *α-Ara* α-arabinofuranosidase

enzyme extraction with the extract obtained under SmF has been described as a strategy for more concentrated enzymatic cocktail production than those obtained by simple culture under SSF or SmF (Visser et al 2013). It is expected that cocktails produced by this approach will be much more complete than those obtained under SSF or SmF, since they contain enzymes secreted under the two cultivation conditions (Visser et al. 2015). This is interesting for lignocellulosic biomass saccharification, which requires high hydrolases diversity for an efficient hydrolysis process (Dutra et al. 2017).

Chrysoporthe cubensis cultivated under SSF produced higher β-glucosidase and cellobiohydrolase activities (1.30 and 0.26 U mL^{-1}, respectively) than those obtained by SmF (0.13 and 0.11 U mL^{-1}, respectively). The extract produced by this fungus under SmF showed higher FPase, endoglucanase, and β-galactosidase activities under SmF (0.13, 4.84, and 0.22 U mL^{-1}, respectively) than those obtained under SSF (0.12, 3.44 U mL-1 and not detected, respectively). These results are in agreement with those reported for the mutant *Penicillium janthinellum* NCIM 1171 that differentially secreted cellulolytic enzymes under SSF and SmF. *Penicillium janthinellum* NCIM 1171 secreted two β-glucosidases isoforms under SmF, but only one isoform under SSF, with wheat bran and Avicel as carbon sources (Singhvi et al. 2011).

Chrysoporthe cubensis enzyme profile and enzymatic saccharification

Enzymatic extracts produced by *C. cubensis* under SSF or SmF, and also the SSF-SmF-cocktail, were applied in the alkali-pretreated sugarcane bagasse saccharification process and compared with a commercial enzymatic cocktail.

To compare the extracts applied in saccharification assays, the enzymatic activities were normalized relative to FPase activity, which is the total cellulase activity of the enzymatic complexes (Table 2). The CMCase/FPase, β-glucosidase/FPase, cellobiohydrolase/FPase, xylanase/FPase, β-xylosidase/FPase, β-galactosidase/FPase, β-galactosidase/FPase, and β-arabinofuranosidase / FPase ratios of *C. cubensis* extracts produced under SSF or SmF, and in SSF-SmF-cocktail were higher than those found for the commercial extract Accellerase®. *Chrysoporthe cubensis* extract produced under SSF and the SSF-SmF-cocktail showed higher β-glucosidase/FPase ratios (10.85 and 6.07, respectively) than the extract produced by this fungus under SmF and the commercial extract (1.02 and 1.23, respectively). The highest CMCase/FPase and β-galactosidase/FPase ratios were observed in *C. cubensis* extract cultivated under SmF (37.20 and 1.73, respectively), whereas the highest ratios of β-glucosidase/FPase, cellobiohydrolase/FPase, α-galactosidase/FPase, and α-arabinofuranosidase/FPase were obtained for the extract produced by this fungus under SSF (10.85, 2.21, 0.48 and 0.08, respectively). The *C. cubensis* SSF-SmF-cocktail showed the highest xylanase/PFase rate (144.17). These results agree with *C. cubensis* previous reports, which also showed that this fungus produces much more complete cellulolytic extracts than many commercials preparations (Maitan-Alfenas et al. 2015; Dutra et al. 2017).

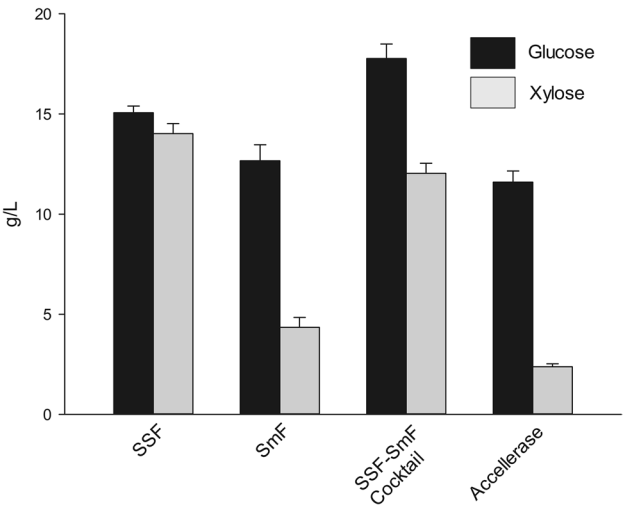


Fig. 2 Glucose and xylose production after 48 h of alkali pretreated sugarcane bagasse enzymatic saccharification using extracts produced by *Chrysoporthe cubensis* under solid-state fermentation (SSF) and submerged fermentation (SmF), cocktail produced by extracting of the enzymes from *C. cubensis* culture under SSF with the extract produced by this fungus under SmF (SSF-SmF-Cocktail) and the commercial extract Accellerase®

The *C. cubensis* SSF-SmF-cocktail released more glucose after 48 h of alkali-pretreated sugarcane bagasse saccharification (17.8 g L^{-1}) than those produced by this fungus under SSF or SmF, and the commercial extract Accellerase®, 15.1, 12.7, and 11.6 g L^{-1}, respectively (Fig. 2). These results demonstrated that the enzymes produced by this fungus under SSF and SmF complement themselves for a better alkali-pretreated sugarcane bagasse saccharification process. It can be justified by the fact of the sugarcane bagasse complexity, with cellulose, lignin, and hemicellulose in its composition, requiring a much more complete enzymatic cocktail for its efficient hydrolysis (Dutra et al. 2017). The low glucose release ratios in the alkali-pretreated sugarcane bagasse hydrolysis by the extract produced by *C. cubensis* under SmF and the commercial cocktail can be explained by the lower β-glucosidase/FPase ratios of these extracts compared to those found in the extract produced by this fungus under SSF and in the SSF-SmF-cocktail, respectively. Another factor that may justify the lower release of glucose by these extracts is their lower efficiency to hydrolyze the hemicellulosic sugarcane bagasse fraction, a barrier to the action of cellulases in the hydrolysis of cellulose observed by low levels of xylose release (Fig. 2).

Another crucial factor for the higher efficiency of the *C. cubensis* extract produced under SSF and the SmF-SSF-cocktail in the glucose release is the higher xylanase/FPase ratio in comparison to the extract produced by this fungus under SmF and the commercial extract. Xylanases catalyze xylans hydrolysis facilitating the cellulases access to cellulose (Hu et al. 2011).

The *C. cubensis* extract produced under SSF released more xylose (14.0 g L^{-1}) in alkali-pretreated sugarcane bagasse saccharification than the extract produced by this fungus under SmF, the SSF-SmF-cocktail, and the commercial extract, 12.0, 4.0, and 2.4 g L^{-1}, respectively (Fig. 2). The higher xylose release from alkali-pretreated sugarcane bagasse saccharification by the *C. cubensis* extract produced under SSF can be justified by its higher β-xylosidase/FPase ratio compared to those obtained for the extract produced by this fungus under SmF, the SSF-SmF-cocktail, and the commercial extract, respectively, since β-xylosidases hydrolyze xylobiose and xylotriose to xylose (Inoue et al. 2016). The *Chrysoporthe cubensis* extract produced under SSF would be indicated when xylose is desired as a final product, as in the case of the production of xylitol from sugarcane bagasse (Rao et al. 2006; Hernández-Pérez et al. 2016).

The *C. cubensis* SSF-SmF-cocktail was more efficient for glucose release from alkali-pretreated sugarcane bagasse hydrolysis than the extracts produced by the same fungus under SSF or SmF. These results demonstrated that the enzymes produced under the two culture conditions were complementary for the hydrolysis of the alkali-pretreated sugarcane bagasse.

Conclusion

Enzyme extracts produced by *C. cubensis* under SSF and SmF showed different secreted protein and enzyme profiles. The enzyme cocktail obtained by the extraction of *C. cubensis* enzymes produced under SFF with the extract produced by the same fungus under SmF (SSF-SmF-cocktail) was more efficient for the glucose release from alkali-pretreated sugarcane bagasse saccharification than the other extracts produced by *C. cubensis* and also the commercial cellulolytic extract.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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