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Improvement of the hydrolytic enzymes production by *Chrysoporthe cubensis* from wheat bran and beet flour

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ABSTRACT

The phytopatogen fungus Chrysoporthe cubensis produces a set of enzymes capable of efficiently degrading plant cell wall, making it a promising means to increase the efficiency of lignocellulosic biomass saccharification. Because wheat bran (WB) and beet flour (BF) are rich in cellulose, hemicellulose and pectin, the aim of this study was to evaluate the effect of the mixture of these two substrates in the enzyme extract composition produced by Chrysoporthe cubensis. WB was an efficient substrate, while FB induced almost no enzyme. However, the best enzyme extract was obtained from the 1:1 ratio (BF:WB), it showed a significant increase of all enzymatic activities tested if compared to extract produced only with WB. Moreover, this mixture also increased by > 80% the specific activity of most enzymes. The results showed that the mixture of different substrates may improve their ability to induce enzymes and alter the extract composition.

Keywords: biomass saccharification; hydrolytic enzymes; *Chrysoporthe cubensis*

INTRODUCTION

There is currently great interest in the degradation of lignocellulosic materials to monomeric sugars, since they can serve as raw materials for the production of valuable products such as ethanol (Falkoski et al., 2013). For complete biomass lignocellulosic degradation, the synergistic action of the cellulases and hemicelullases is very important (Van Dyk and Pletschke, 2012).

The application of enzymatic extracts rich in cellulases and hemicellulases allows for the conversion of hemicellulose in sugars and facilitates the access of cellulases to cellulose because of the increase on fiber porosity, which improves the saccharification yields (Gao and Tao, 2011). According to Albuquerque et al. (2015), the structure and composition of the carbon source used in the microorganism cultivation may determine the need for it to produce different types of enzymes, and the wheat bran is the one of the best carbon sources to cultivate *Chrysoporthe cubensis*. Beetroot, in turn, is rich in pectins, which may contain sugars as rhamnose, arabinose and galactose (Kliemann, 2006). Therefore, beet flour can be a good inducer of hemicellulases and pectinases. The present study evaluates the hydrolytic enzymes production by fungus *Chrysoporthe cubensis* when grown in beet flour and wheat bran, pure or mixed.

MATERIAL AND METHODS

The microorganism and inoculum preparation were performed according to the description by Visser et al. (2013), as well as grown under solid state fermentation (SSF). The carbon sources were beet flour and wheat bran, pure and mixed in different proportions (1:0; 1:1 and 3:1).

All enzymatic assays were performed 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 carboxymethilcellulose as substrates respectively, according to Ghose (1987). The total reducing sugar released during the enzymatic assays were quantified by the dinitrosalicylic acid (DNS) method (Miller, 1959) using glucose as standard. Xylanase activity was determined using xylan from beechwood (1.25% w/v at final concentration) as substrate. The enzymatic reactions were performed as described by Albuquerque et al. (2015) and the total reducing sugar released was determined via the DNS method using xylose as standard. β-glucosidase, β-xylosidase, α-mannosidase, β-galactosidase, β-cellobiohydrolase and α-arabinofuranosidase activities were measured using ρPNGlc, ρNPXyl, ρNPMan, ρNPGal, ρNPCel and ρNPAra as substrates, respectively. The reaction mixtures and absorbance reading were performed according to Albuquerque et al. (2015) and the amount of ρ-nitrophenol released was estimated by a standard curve. For all activities, one unit of enzymatic activity (U) was defined as the amount of enzyme that liberated 1 µmol of the corresponding product per minute, under the assay condition used.

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

RESULTS AND DISCUSSION

According to Figure 1, unlike the wheat bran which alone was capable of inducing fungus to produce various cellulases and hemicellulases, the beet flour was not good inducer of hydrolytic enzymes when it was used as sole carbon source. For mixtures, the maximal activities of α-arabinofuranosidase (0.81 ± 0.05 $U.mL^{-1}$), β-cellobiohydrolase (0.81 ± 0.01 $U.mL^{-1}$), β-galactosidase (0.15 ± 0.01 $U.mL^{-1}$), α-mannosidase (0.02 ± 0.00 $U.mL^{-1}$), β-xylosidase (0.13 ± 0.00 $U.mL^{-1}$) and β-glucosidase (3.93 ± 0.03 U.mL-1) were obtained from the 1:1 ratio (BF:WB). The highest endoglucanase (2.41 ± 0.10 $U.mL^{-1}$) and xylanase (36.00 ± 3.50 U.mL-1) activities were detected with the 1:3 ratio (BF:WB), while the maximal FPase activity (0.65 ± 0.02 $U.mL^{-1}$) was observed in the 3:1 ratio (BF:WB).

Thus, the extract obtained from the 1:1 ratio (BF:WB) was considered the best because it showed greater increase in activity on most enzymes tested. Compared to the extract produced only with WB, the increase was 50 times for the enzyme β-galactosidase, 5 times for α-mannosidase, 9 times for β-cellobiohydrolase, 6.5 times for β-xylosidase, 4.5 times for α-arabinofuranosidase and 2.3 times for β-glucosidase. The increase was smaller for FPase (1.8 times), endoglucanase (1.5 times) and xylanase (1.3 times).

Given the significant increase in hemicellulolytic activity provided by mixing BF and WB (1:1), we can infer that this enzyme cocktail would be more effective in the lignocellulose biomass saccharification, because the hemicellulose present in the biomass

would be degraded more easily, making the cellulose more accessible to the action of the cellulases, which also have their activity enhanced by mixing the two substrates.

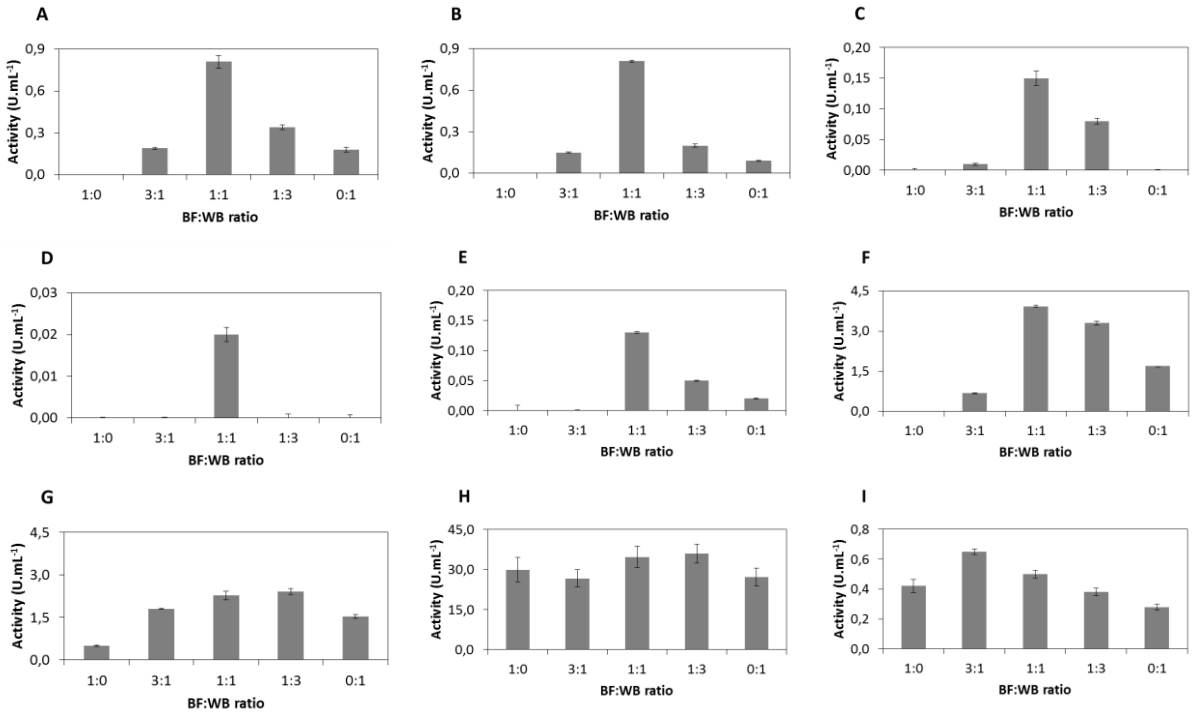

Figure 1: Effect of carbon source on the production of α-arabinofuranosidase (A), β-cellobiohydrolase (B), β-galactosidase (C), α-mannosidase (D), β-xylosidase (E), β-glucosidase (F), endoglucanase (G), xylanase (H) and FPase (I) by the fungus *Chrysoporthe cubensis* cultured under SSF.

The ideal enzyme cocktail should provide high enzyme activities of interest and a low concentration of proteins, because these two features result in high specific activity of a particular enzyme. The concentration of proteins present in the extracts was as high as the amount of wheat bran used as carbon source (Table 1).

Table 1: Comparative analysis of protein and specific enzymatic activity present in each extract produced. The specific enzyme activity was calculated dividing the enzyme activity by the protein concentration.

BF:WB ratio	**Protein (mg.mL^{-1})**	**Specific enzyme activity (U.mg^{-1})**								
		α-ara	β-cel	β-gal	α-man	β-xyl	β-glu	Endo	Xyl	FPase
1:0	0.04 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.6	816.2	n.d.
3:1	0.05 ± 0.02	3.5	2.8	0.2	n.d.	n.d.	12.7	34.0	501.4	12.3
1:1	0.15 ± 0.02	5.4	5.3	1.0	0.1	0.8	25.9	15.0	228.6	3.3
1:3	0.38 ± 0.01	0.9	0.5	0.2	n.d.	0.1	8.8	6.4	95.9	1.0
0:1	0.60 ± 0.02	0.3	0.2	n.d.	n.d.	n.d.	2.8	2.5	45.2	0.5

α-ara: α-arabinofuranosidase; β-cel: β-cellobiohydrolase; β-gal: β-galactosidase; α-man: α-mannosidase; β-xyl: β-xylosidase; β-glu: β-glucosidase; Endo: endoglucanase; Xyl: xylanase; n.d.: not detected.

Compared to wheat bran, the mixture BF and WB (1:1) induced an increase of about 18, 26 e 9 times on specific activity of enzymes α-arabinofuranosidase, β-cellobiohydrolase and β-glucosidase, respectively. It also made measurement of β-galactosidase, α-mannosidase and β-xylosidase specific activities. However, the enzyme extract yielded from the 1:0 ratio (BF:WB) showed the highest xylanase specific activity, while for most other enzymes the values were not measured due to being too low (Table 1). Due to the knowledge that a high specific enzyme activity indicates less contaminants in the extract, fewer purification steps and lower cost, the xylanase yielded from the 1:0 ratio (BF:WB) is almost completely free of cellulases and it can be applied in industrial processes such as pulp bleaching.

CONCLUSION

The beet flour by itself was not an effective carbon source to induce *Chrysoporthe cubensis* to produce hydrolytic enzymes. However, when mixed with wheat bran, especially in the 1:1 ratio, it was able to increase not only the variety and the amount of enzymes, but also their specific activity. We acknowledge CAPES, FAPEMIG and CNPq for the resources provided.

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