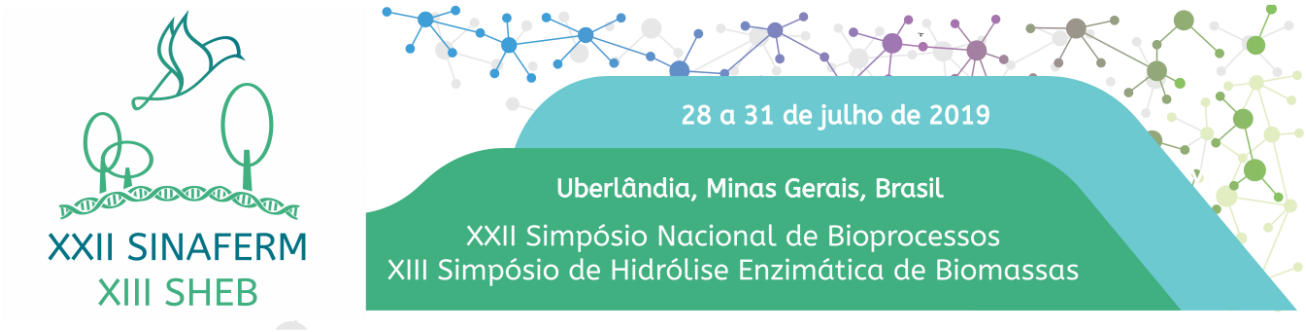

CELLULOLYTIC ENZYMES PRODUCTION BY *Talaromyces pinophilus* AND ITS APPLICATION IN BIOMASS DECONSTRUCTION

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ABSTRACT

Lignocellulosic materials are valuable energy source that can be used for reducing dependence on fossil fuels. Enzymatic cocktails containing cell wall-degrading are applied to disrupt lignocellulosic structure, converting it in monomer sugars of interest. Based in their several industrial applications, microbial cellulases have become strategic biocatalysts. This work selected best conditions of FPase production by Talaromyces pinophilus AR155 and applying this enzymatic extract in hydrolyses of pretreated by different processes. T. pinophillus AR155 cultivated in PB-6 medium, at pH 6.0 and 32°C, reached FPase production of 1.0 FPU/mL. In addition, the results of hydrolyses reinforce the importance of T. pinophillus AR155 as a potential source of holo-cellulolytic enzymes, since the crude extract produces by this wild strain presents performance comparable to an optimized commercial cocktail.

1. INTRODUCTION

The complete hydrolyses of lignocellulose substrate requires a variety of enzymes. This process has emerged as the most prominent technology for the conversion of biomass into monomer sugars, which are further used for subsequent fermentation into several products, as bioethanol (Van Dyk and Pletschke, 2012). A major technical challenge in the commercialization of cellulose-based processes is achieving rapid and complete enzymatic hydrolysis of lignocellulosic biomass at low protein loadings (Arantes and Saddler, 2011). Biomass pretreatment has been used to increase the yield of glucose from lignocellulosic materials during enzymatic hydrolysis. The crystalline structure of cellulose can be disrupted by pretreatments, which act disrupting the lignocellulosic complex, to make it more susceptible to the enzymatic attack.

A large diversity of microorganisms, including fungi and bacteria, is able to produce cellulases and others cell wall-degrading enzymes (Kuhad et al., 2011). The aim of this work is to select best cultivation conditions for FPase (total cellulases) production by *T.pinophilus* AR155 and to evaluate the enzymatic extract in hydrolysis of pretreated sugarcane bagasses.

2. MATERIAL AND METHODS

2.1. Selection of variables, optimization of cultivation and characterization of FPAse produced

Selection of variables: in order to evaluate the significant variables that impact cellulases production, a Plackett & Burmann design was applied, considering the reagents of classic Mandels and Weber medium (MWM) for cellulases production, and other reagents that could improve cellulase production, and chosen after literature search (Table 1).

Total cellulases (FPU) determination: Total cellulase activity on filter paper (FPase) was determined according to Xiao et al. (2004) in miniaturized assay. The glucose-equivalents released from filter paper hydrolysis was quantified by 3,5-Dinitrosalicylic acid (DNS) method. One unit of enzyme activity was defined as the amount of enzyme that releases one µmol of glucose per min.

Cultivation of the AR155 in different conditions: the strain was evaluated for cellulases production when grown in PB-6 (Table 1) media with initial pH of 4.0 or 6.0. The flasks were incubated at 28°C or 32°C during 5 days, and the crude extracts obtained were evaluated for FPAse activity.

2.2. Enzymatic hydrolysis of pretreated sugar cane bagasse using *T. pinophillus* AR155 cellulases

The performance of the enzymatic extract produced by *T. pinophillus* AR155 was evaluated against a sugarcane bagasse by several processes and results were compared with the commercial cocktail.

Pretreatments: Sugarcane bagasse were pretreated by (i) acid pretreatment: solid:liquid ratio of 1:10 (w/m) with 1.5% H_2SO_4 (v/v) at 121°C for 56 min; (ii) autohydrolysis: 1:10 (w/v), at 180°C for 1 h (iii) alkaline pretreatment: 1:8 (w/v) with 14% NaOH at 170°C for 40 min; (iv) steam explosion: 1:1 (w/v), under 15 kgf.cm^{-2} pressure for 8 min; and (v) organosolv: 1:6 (w/v), at 180°C for 2 h.

Enzymatic hydrolysis: Enzymatic hydrolysis were performed in deep well plates containing 5% (m/v) pretreated biomass, 10 FPU/g of dry matter in pH 5.0 (100 mM citrate/citric acid buffer), shaking at 200 rpm at 50°C. After 24 hours, the liquid fraction was recovered and used to determine glucose, xylose and cellobiose concentration by HPLC-RID (Aminex HPX-87H). A commercial cocktail was included, in the same loading, as a control of the experiments.

3. Results

After the evaluation of several variables using a Plackett & Burmann design, it was possible to obtain a new formulation with a smaller number of reagents, designed as PB-6, in which the fungal strain produces 0.7 FPU/mL, 40% more FPase compared to MWM (0.5 FPU/mL). The composition of both media is presented in Table 1. It is worthy to mention that the simplification of the media contributes to reduce the cost of enzyme production.

Table 1. Components of the medium Mandels & Weber and PB-6 media for *T.pinophillus* AR155 cultivation at pH 6, 28°C,180 rpm, for 5 days.

Component	Concentration	MWM	PB-6
Urea	g/L	0.30	-
Peptone	g/L	0.75	0.50
Yeast Extract	g/L	0.25	0.50
$(NH_4)_2SO_4$	g/L	1.40	0.50
K_2HPO_4	g/L	2.00	1.00
$CaCl_2$	g/L	0.40	-
$MgSO_4.7H_2O$	g/L	0.30	-
$NaNO_3$	g/L	-	1.00
$CuSO_4.5H_2O$	mg/L	-	2.00
$ZnSO_4.7H_2O$	mg/L	1.40	-
$CoCl_2.6H_2O$	mg/L	2.00	-
$MnSO_4.H_2O$	mg/L	1.04	2.00
$FeSO_4.7H_2O$	mg/L	5.00	10.00
PEG6000	g/L	1.00	1.00
Steam explosion pre-treated bagasse	g/L	20.00	20.0
Wheat bran	g/L	5.00	10.00

Results of FPAse obtained after AR155 cultivation in PB-6, with initial pH of 4.0 or 6.0, and at 28°C or 32°C showed that this strain produced higher titles of FPAse when cultivated at 32°C in both the values of pH evaluated. The higher FPAse activity (1.01 ±0.02) was obtained when strain was cultivated at 32 °C and pH 6. Under these conditions, double the FPAse activity was reached compared to initial conditions (MWM, pH 6, at 28 °C). Then, the crude extract containing 1 FPU/mL was used for hydrolysis of pretreated sugarcane bagasses.

The efficiency of the crude extract was very similar to commercial preparation in pretreated bagasses saccharification, with exception of bagasse pretreated by organosolv (Figure 1). Results are also similar for xylose and cellobiose (data not shown).

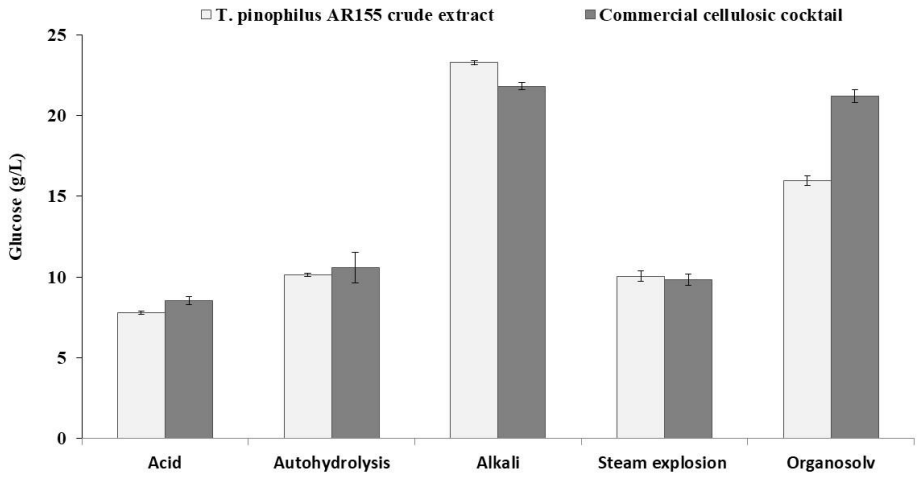

Figure 1. Hydrolysis of sugarcane bagasse pre-treated with different strategies, using the crude extract of T. pinophillus AR155 in PB-6 medium pH 6.0 at 32°C (a) and the commercial cellulosic cocktail (b).

These results corroborate the importance of *T. pinophillus* AR155 as a potential source of holocellulolytic enzymes, since the crude extract produced by a wild strain presents performance comparable to an optimized commercial cocktail. The efforts of optimizing culture conditions for maximizing enzymes production, as well as scale-up to controlled conditions in bioreactors will be carried out in the future.

4. ACKNOWLEDGEMENTS

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