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PRODUCTION OF CELLULOLYTIC AND HEMICELLULOLYTIC ENZYMES BY *Aspergillus niger* 3T5B8 MUTANTS AND PARENTAL STRAINS IN SOLID-STATE FERMENTATION

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ABSTRACT

Several studies have sought to increase the efficiency of the enzymatic hydrolysis of lignocellulosic biomass. In this context, the improvement of strains producing cellulolytic and hemicellulolytic enzymes may result in more efficient enzymatic mixtures. The parent strain of Aspergillus niger 3T5B8, referred as a polygalacturonase producer, was used for genetic improvement in order to increase the production of cellulases and hemicellulases. The production of the enzymes CMCase, xylanase, beta-glucosidase and polygalacturonase by solid-state fermentation using two mutant strains P49 and P83 was evaluated and compared with the parental strain. The results showed a prominence for the P83 strain with an increase in the production of 174% for xylanase and 402% for polygalacturonase.

1. INTRODUCTION

Plant biomass is the most abundant source of renewable energy in the world. Due to the complexity of its structure, it presents differences in the degree of digestibility that result mainly from the composition variations of cellulose, hemicellulose and lignin and the proportions and interactions between the biomass components. Fungi play a vital role in the hydrolysis of biomass through the synthesis of hydrolytic enzymes, such as cellulases and hemicellulases. These hydrolytic enzymes are crucial for the bioconversion of the cellulose and hemicellulose fraction into simple sugars for

subsequent fermentation to biofuel molecules. However, the efficiency and cost of the enzymatic hydrolysis process are major obstacles in the production of biofuels (Peplow, 2014). Among the filamentous fungi, those of the *Aspergillus* genus are the most studied for the production of cellulolytic and hemicellulolytic enzymes. The fungus *Aspergillus niger* has received special attention due to its great fermentation capacity and high levels of enzyme production, mainly enzymes used in the hydrolysis of plant cell wall polysaccharides and in the food industry.

The strain of *A. niger* which was previously selected aimed the increasing of polygalacturonase production (Couri & Farias, 1995). In order to increase the production of cellulases and hemicellulases, two highlighted strains were P49 and P83 (Fávaro & Polleto, 2013). The objective of this work was to compare the production of the enzymes CMCase, xylanase, beta-glucosidase and polygalacturonase by the mutant strains and their parent by submerged fermentation using wheat bran as carbon source.

2. MATERIAL AND METHODS

2.1. Microorganisms and Enzyme production

The parental strain *A. niger* 3T5B8 from the collection of Embrapa Agroindústria de Alimentos and the mutant strains P49 and P83 were used to produce the enzymes CMCase, xylanase, beta-glucosidase and polygalacturonase by solid-state fermentation. The selected mutants were obtained from two cycles of mutagenesis (first with ultraviolet and the second with ethylmethanesulfonate). The experiments were carried out in cylindrical columns immersed in a thermostatic bath at 32°C for 96 hours. Wheat bran was used as a source of carbon and ammonium sulphate as a source of nitrogen with a C/N ratio of 14.

2.2. Determination of enzymatic activities and extracellular protein

The determination of beta-glucosidase activity was performed using 2% cellobiose as substrate diluted in 15 mM in sodium citrate / 0.1 M citric acid buffer, pH 5.0. The reaction occurred at 50°C for 30 minutes. After the incubation period, the amount of glucose released was quantified using a commercial kit (GOD-POD brand Doles®).

The determination of the xylanase, CMCase and polygalacturonase activities in the crude extract was performed using as substrates: 1% xylan solution (0.1 M acetate buffer, pH 5), 4% carboxymethylcellulose-CMC (50 mM sodium citrate buffer, pH 4.8) and 0.25% polygalacturonic acid (0.2 M acetate buffer, pH 4.5). Reactions were conducted at 50 ° C for 10 minutes for xylanase and

30 minutes for CMCase and disrupted by the addition of DNS. For polygalacturonase the reaction was conducted for 30 minutes at 35 ° C and also interrupted by the addition of DNS. The resulting reducing sugars were determined by the DNS method (Muller, 1959). The activity was calculated in IU / mL by determining the concentration of reducing sugars released during the degradation of the respective substrates. Protein concentration was determined according to Lowry et al. (1951).

3. RESULTS AND DISCUSSION

The strains were grown in solid-state medium with wheat bran as the main source of carbon. The values of extracellular protein (g/L) were: 5.86 ± 0.15; 6.75 ± 0.32 and 7.66 ± 0.36 for the parental strain 3T5B8 and for the mutants P49 and P83, respectively. The results obtained for the production of the enzymes CMCase, Xylanase, β-glucosidase and polygalacturonase are shown in Figure 1.

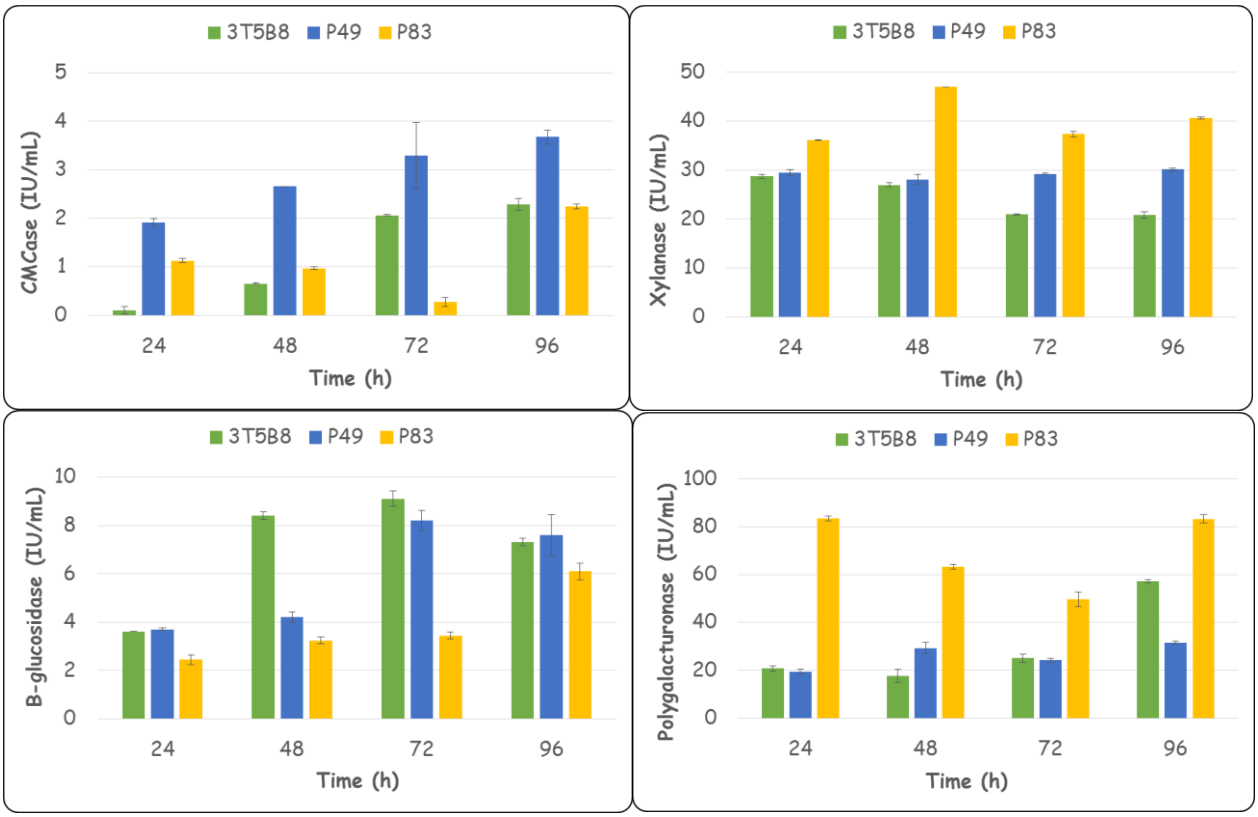

Figure 1: Production profile of the enzymes CMCase, Xylanase, B-glucosidase and polygalacturonase for the strains of *Aspergillus niger* parental 3T5B8 and mutants P83 and P49.

The values found for CMCase are low since the *Aspergillus* fungus is not a good producer of this enzyme (Wen et al., 2005). The maximum production for CMCase was 3.7 IU/mL for strain P49, followed by 2.2 IU/mL for both strains P83 and 3T5B8. Maximum yield occurred in 96 hours for all strains evaluated. The concentration of CMCase using mutant strains was 61% higher for P49 strain compared to parental strain 3T5B8.

Aspergillus spp. is known as an excellent producer of β-glucosidase, xylanase and polygalacturonase and its enzymes can supplement the cellulolytic enzyme extract of *Trichoderma* spp. in the enzymatic hydrolysis of lignocellulosic biomass (Gottschalk et al., 2013). In relation to xylanase and polygalacturonase production, the mutant strain P83 provided the highest production with activity levels of xylanase of 46.9 IU/mL in 48 hours and polygalacturanse of 83.6 IU/mL in 24 hours (174% and 402 % higher than the parental strain 3T5B8). As the *A. niger* 3T5B8 parent strain was selected for increased polygalacturonase production (Couri & Farias, 1995), the results showed that the new strain P83 increased 4 times the production and also the productivity. In relation to β-glucosidase activity, the mutants do not shown any improvement. This new enzymatic complex may be more efficient in the enzymatic hydrolysis of lignocellulosic biomass. In this way, Embrapa Agroenergia intends to obtain new mutants using genetic engineering.

4. REFERENCES

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