Production of cellulolytic and hemicellulolytic enzymes by the pectinolytic *Aspergillus niger* 3T5B8-C88-P83 mutant strain under different cultivation conditions

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Embrapa Agroenergia has been conducting genetic improvement of filamentous fungi to increase the production of cellulases and hemicellulases to deconstruct lignocellulosic biomass. In a conventional breeding program for *Aspergillus niger* 3T5B8 performed in 2015, after two rounds of mutagenesis (ultraviolet followed by ethyl methanesulfonate), the pectinolytic mutant strain 3T5B8-C88-P83 was selected, which presents genetic changes in the catabolite repression by glucose pathway and a significant increase in polygalacturonase production compared to the original 3T5B8 strain. This work aimed to evaluate the production of cellulolytic and hemicellulolytic enzymes of the 3T5B8-C88-P83 mutant under different cultivation conditions, seeking its future application in the enzymatic processing of cotton fabrics. Endoglucanase production by the 3T5B8-C88-P83 mutant strain was evaluated in 250 mL Erlenmeyer flasks containing different liquid and solid culture media (four repetitions). For submerged fermentation (50 mL), two salt compositions (S1 and S2) were evaluated in combination with six various carbon sources: (1) CMC 1%; (2) CMC 1% + D-(+)-cellobiose-octaacetate/COA 0.6%; (3) Cellulose 1%; (4) Cellulose 1% + COA 0.6%; (5) Sugarcane bagasse 0.5% + Wheat bran 0.5%; and (6) Sugarcane bagasse 0.5% + Wheat bran 0.5% + COA 0.6%. For solid-state fermentation, the culture media were FES1 (12 g of wheat bran + 7.5 mL of 1.18% (w/v) ammonium acetate solution with five inoculum mycelial discs), FES2 (12 g of wheat bran + 7.5 mL of 1.18% (w/v) ammonium acetate solution with ten inoculum mycelial discs), and FES3 (6 g of wheat bran + 6 g of sugarcane bagasse + 7.5 mL of 1.18% (m/v) ammonium acetate solution with ten inoculum mycelial discs). Incubation was carried out for five days at 30ºC. In the submerged fermentation condition, agitation was at 200 rpm, and after cultivation, the supernatant was collected to determine enzymatic activity. In the solid-state fermentation condition, after cultivation, enzymatic extraction was carried out with 0.2 M acetate buffer (50 mL), pH 4.5, at 32ºC under agitation at 180 rpm for 1 hour. A comparison was performed of the enzyme production profile of the original *A. niger* 3T5B8 strain with their respective mutants, the first selected after mutagenesis with ultraviolet (3T5B8-C88) and the second after the subsequent ethyl methanesulfonate treatment (3T5B8-C88-P83). In this case, the cultivation was carried out in solid-state fermentation using the FES2 culture medium under the conditions described previously (four replications). Solid media were the most efficient in inducing endoglucanase production by the 3T5B8-C88-P83 mutant strain. The FES2 culture medium produced the best result (1.4 U/mL). It was found that the enzymatic extract of the pectinolytic mutant strain 3T5B8-C88-P83 obtained from cultivation in FES2 medium presented significantly higher values than the original 3T5B8 strain not only for the polygalacturonase activity but also for the endoglucanase, xylanase, and β-glucosidase. The data confirm the previous genetic improvement of this strain and define a simple cultivation condition to produce enzymatic extract for its future application in the processing of cotton fabrics under laboratory conditions.

Key words: *Aspergillus niger*; Polygalacturonase; Mutant; Solid-State Fermentation.

Produção de enzimas celulolíticas e hemicelulolíticas pela linhagem mutante pectinolítica *Aspergillus niger* 3T5B8-C88-P83 sob diferentes condições de cultivo

Este trabalho teve como objetivo avaliar a produção de enzimas celulolíticas e hemicelulolíticas pela linhagem mutante pectinolítica *Aspergillus niger* 3T5B8-C88-P83 sob diferentes condições de cultivo. Verificou-se que o extrato enzimático da linhagem 3T5B8-C88-P83 obtido do cultivo no meio FES2 apresentou valores de atividade enzimática significativamente superiores ao da linhagem original 3T5B8 não apenas para poligalacturonase, mas também para endoglicanase, xilanase e β-glicosidase. Os dados confirmam o melhoramento genético prévio desta linhagem e definem uma condição simples de cultivo para sua aplicação futura no processamento de tecidos de algodão.

Palavras-chave: *Aspergillus niger*; Poligalacturonase; Mutante; Fermentação em Estado Sólido.

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