EVALUATION OF CELLULOLYTIC ENZYME PRODUCTION BY *Trichoderma asperellum* VAYING THE CARBON SOURCE WITH CARBOXYMETHYL CELLULOSE

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ABSTRACT

Trichoderma asperellum is a filamentous fungus widely employed in biotechnology, particularly in the production of cellulolytic enzymes and the control of phytopathogens. The objective of this study was to produce FPase enzymes and investigate two different culture media: one containing only carboxymethyl cellulose (CMC) and the other containing both CMC and glucose. The microorganism was incubated for 96 hours, with samples collected every 24 hours. The results demonstrated that the presence of glucose in the culture medium affected the production of Fpase by *Trichoderma Asperellum*, leading to a reduction in enzymatic activity compared to the medium containing only CMC. Thus, the use of CMC as a carbon source holds promise for cellulase production and can contribute to the application and production of biotechnological products.

Keywords: *Thichoderma asperellum*. CarboxymethyL cellulose. Glucose. Fpase activity.

1 INTRODUCTION

Trichoderma asperellum is a filamentous fungus known for its diverse applications, including the production of cellulolytic enzymes in the food, pharmaceutical, and agricultural industries for the control of phytopathogens [1,2]. Recently, it has gained prominence in biofuel production, through the production of hydrolytic enzymes [3,4]. Cellulases are used in the biofuel production process due to the hydrolyze cellulose when lignocellulosic residues are used to produce bioethanol [5]. These enzymes are composed of three main types: endocellulase, exocellulase, and beta-glucosidase, which work synergistically in the hydrolysis of cellulose [6]. Studies have assessed the total cellulase activity, known as FPase, using filter paper as a substrate [7,8].

Research aims to increase cellulase production through submerged fermentation. In this context, the induction of cellulase production has been evaluated using carboxymethyl cellulose (CMC) in comparison with glucose as a carbon source. CMC, used as a carbon source, consists of complex molecules of β-D-glucose and 2-O-carboxymethyl-β-D-glucopyranose chains linked by β-1,4-glycosidic bonds [9]. In contrast, glucose has a simpler carbon chain structure and is easily consumed by the microorganism [10]. The objective of this work is to evaluate the production of FPase activity by *T. asperellum* and to observe the microorganism's behavior in the utilization of CMC and glucose in the culture medium for enzyme production.

2 MATERIAL & METHODS

The fungal strain of *T. asperellum* was cultivated at Potato Dextrose Agar (PDA) plates and maintained at a temperature of 25 °C with a photoperiod of 7 days. A 9 mm disc was inoculated into a 125 mL Erlenmeyer flask containing 50 mL of culture medium. The composition of the culture medium consisted of (g L^{-1}): CMC (10); KH_2PO_4 (2); $(NH_4)2SO_4$ (5); $MgSO_4.7H_2O$ (0.3); peptone (1) and 1 mL L^{-1} of trace element solution. The second cultivation medium consisted of replacing CMC (10) with CMC (5) and glucose (5). Samples were taken every 24 h for subsequent quantification of FPase.

The trace element solution was consisted of (g L^{-1}): KI (0.1), $FeSO_4.7H_2O$ (0.3), $CuSO_4.5H_2O$ (0.3), $CoCl_2.6H_2O$ (0.3), $MnCl_2.4H_2O$ (1.0), $ZnSO_4.7H_2O$ (4.5), $CaCl_2.2H_2O$ (4.5), and $Na_2EDTA.2H_2O$ (15.0) [11]. All material used was sterilized at 121 °C for 20 min. After inoculation, the Erlenmeyer was incubated in an orbital shaker at 28°C, 180 rpm for 96h.

The Fpase activity was assessed by employing a 1.0 x 6.0 cm segment of Whatman No. 1 filter paper and employing the 3,5-dinitro salicylic acid reaction method. Initially, a test tube was prepared by placing the filter paper followed by adding 1 mL of enzymatic broth and 2 mL of sodium acetate buffer. The mixture was allowed to react for 60 minutes at 50°C. Subsequently, a 1 mL sample was withdrawn and combined with 1 mL of water and 3 mL of DNS solution. The resulting mixture was then heated at 100°C for 5 minutes. After cooling to room temperature, spectrophotometric readings were obtained at 540 nm using a UV-Vis spectrophotometer (Shimadzu UV-2600)[12]. To calibrate the analysis, the enzymatic broth was substituted with water.

The quantification of FPase is described by Equation 1:

$$UI = \frac{DxCxVt}{TxVe} \quad (1)$$

Which "D" is the dilution (performed when it was necessary to dilute the enzymatic solution); "C" is the concentration determined by the DNS method (μmol mL^{-1}); "Vt" is the total volume of the reaction (mL); "t" is the reaction time (min); "Ve" is the volume of the enzymatic solution (mL).

3 RESULTS & DISCUSSION

The results of Fpase (UI mL^{-1}) enzymatic analysis for CMC in were 0.000; 0,133; 0.134; 0,150 e 0.156. For CMC + Glucose, the values were 0.000; 0.049; 0.072; 0.071, and 0.067, respectively, for the corresponding hours: 0, 24, 48, 72, and 96 for both. The results are expressed graphically in Figure 1.

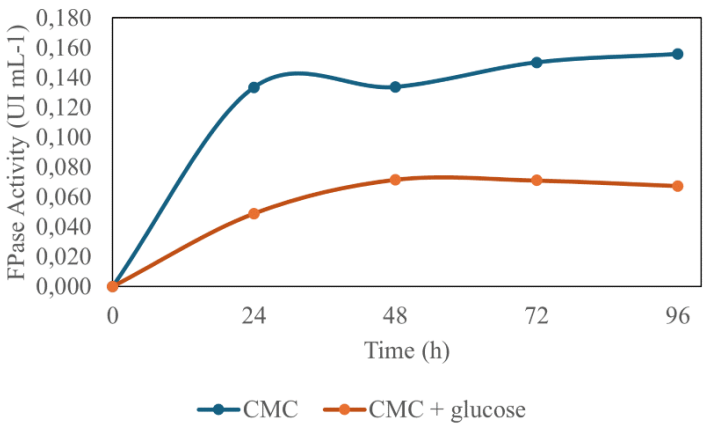

Figure 1 - Fpase activity for CMC and CMC and glucose at 96 hours of incubation.

Considerable enzymatic activity was observed when using CMC as a substrate than in comparison to the growing medium containing CMC + glucose. With induction using CMC medium, a maximum FPase production of 0.156 IU ml-1 in 96 hours was achieved. In the second test, where CMC and glucose were added, lower values of enzymatic activity were detected. This discrepancy suggests that the presence of glucose in the culture medium influenced enzyme production, leading to reduced Fpase activity compared to medium containing only CMC, possibly caused by the induction of CMC and the microorganism's need to produce enzymes.

Compared to previous studies, the production of Fpase by different strains of Trichoderma under different cultivation conditions (150 rpm, 28ºC) resulted in a maximum Fpase concentration of 0.130 U/g after 216 hours of fermentation [13]. However, there are also results of Fpase production of 0.1 U/mL in 96 hours of cultivation at 200 rpm and pH 5.0 [14]. The *T. harzanium* EUA20 achieved production of 14.19 U/mL of Fpase in 6 days [7]. The maximum production of 8.70 IU/mL of Fpase occurred after 96 hours of fermentation at 120 rpm and 30 ºC although there are studies that obtained Fpase concentrations between 2.6 and 112 U/mL in 5 days of fermentation [15]. These results highlight the importance of different cultivation conditions and Trichoderma strains in Fpase production, emphasizing different optimization conditions to maximize enzyme production efficiency.

Carboxymethylcellulose serves as a stimulant for cellulase production in fungal cultures like *Trichoderma* when compared to less complex substrates such as glucose. These findings align with literature, as cultivation conditions demonstrate enhanced cellulase production with CMC as the substrate compared to molecules with simpler carbon chains [16]. Microbial growth is likewise influenced by the cultivation medium, typically accelerating when utilizing easily metabolizable substrates [17]. However, in environments where microorganisms must produce enzymes to break down structures for consumption, microbial growth tends to be slower due to the challenges associated with substrate consumption [18].

4 CONCLUSION

The presence of glucose in the culture medium significantly impacted enzyme production, leading to a decrease in Fpase activity compared to the medium containing only CMC. To further optimize the process, it is recommended to explore different parameters such as temperature, pH and composition of the culture medium, as well as testing various concentrations of CMC and glucose. Furthermore, the use of CMC as a carbon source shows promise for the production of cellulase and, consequently, the generation of bioproducts.

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