PRODUCTION OF GLUCOAMYLASES BY ASPERGILLUS STRAINS FOR INDUSTRIAL APPLICATIONS

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ABSTRACT

Glucoamylases, enzymes with amylolytic activity, high-value proteins currently used in the hydrolysis of starchy agro-industrial waste. These enzymes play a crucial role in the breakdown of starch into short-chain monosaccharides, which can be further utilized in the production of various bioproducts such as biomass, probiotics, biopolymers and biofuels' production process. This study aimed to enhance glucoamylase production by focusing on fungi with accelerated metabolism and with easy accessibility. Ten strains of fungi belonging to the genus *Aspergillus* were maintained (4°C) on PDA agar, reactivated in the same medium to achieve maximum sporulation at 30°C in 7 days. Additionally, morphological identification of these fungi was conducted using the micro-culture technique (PDA, 30°C). The productivity of these fungi was evaluated in various liquid fermentative media to determine their maximum production potential (120 rpm, 30°C) over a 7-day fermentation period. The produced enzyme was separated from the biomass by centrifugation at 5000g for 10 minutes, culminating with the enzymatic activity analysis at pH 4.5 and 55°C using the DNS methodology to quantify glucose as total reducing sugar. *Aspergillus Oryzae* LPB1808 exhibited the highest enzymatic activity (14,37 U/mL) using a starchy fermentative medium. Process optimization and enzyme purification and formulation will be carried out aiming its application,.

Keywords: Glucoamylases. Aspergillus. Fermentative media. Enzymatic activity. DNS.

1 INTRODUCTION

Fungi are easily accessible microorganisms with low requirements for their development; many of them produce highly commercially valuable amylolytic enzymes, such as glucoamylases. These enzymes act as catalysts in chemical reactions, breaking down starchy compounds into glucose. They are used for the growth of other microorganisms or for the biorefinery of reusable organic matter. The enzyme market has experienced exponential growth in recent years. A report was published showing that the enzyme market is projected to reach $4.28 trillion by 2029. Due to their reusability and wide applications, methodologies for optimizing their production processes are constantly under research to increase profitability[1,2]. The main objective is the identification, evaluation, and isolation of glucoamylases, a crucial enzyme in the digestion process of long-chain sugars[3].

Glucoamylase (1,4-alpha-D-glucan glucohydrolase, EC 3.2.1.3) is a hydrolytic enzyme belonging to the amylase group, also known as amylo-glucosidase. It can hydrolyze the 1,4-alpha-D-glucose bonds in starch or other polysaccharides, releasing glucose molecules from their non-reducing ends, with potential to hydrolyze 1,6-alpha-glucosidic bonds but with lower efficiency[4].

Glucoamylases have been discovered in various microorganisms, including bactérias like Bacillus stearothermoplhillus[5], *Flavobacterium sp*.[6],*Halobacterium sodamense*[7],*Clostridium sp*.[8],*C. thermosaccharolyticum*[9],*C. acetobutylicum*[10] and *C. thermohydrosulfuricum*[11], some yeast *Endomyces sp.* [11], Saccharomyces diastaticus[12] and fungi like *Aspergillus* y *Rhizopus*, *Aspergillus sp*.[13], *A. awamori*.[14], *A. candidus*[15], *A. niger*[16], *A. oryzae*[17] and *A. phoenicus*[18], Rhizopus family fungi such as *Rhizopus sp*.[19], *R. niveus*[20] and *Therrnornyces lanuginosus*[21] a thermophilic fungus producer of a thermostable glucoamylase.

The production of enzymes using bioreactors is a way to increase the production of commercial bio-compounds and assess future expectations. These require promising initial studies to be taken to an industrial level. Considering that glucoamylase is an exogenous enzyme released into the fermentative medium, the value this enzyme can achieve is directly proportional to its enzymatic activity, purity, and operational conditions[18,19,21]. Lastly, the cleaning process of materials when working with fungi is straightforward; however, sterilization should be considered before disposal to extinguish their viability and prevent environmental colonization and contamination[22,23]. The main objective was to evaluate, among the 10 strains of Aspergillus and select one for optimization and scaling processes.

2 MATERIAL & METHODS

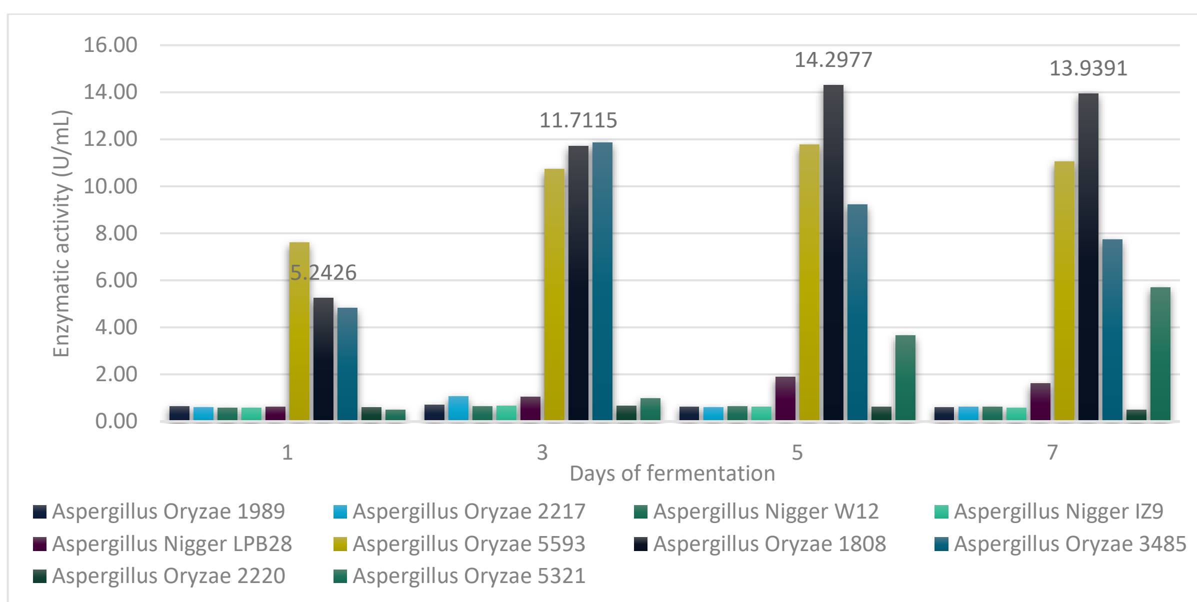
Strains of *Aspergillus* from the DEBB strain bank were used. *Aspergillus oryzae* LPB1989, LPB2217, LPB5593, LPB1808, LPB3485, LPB2220, LPB5321, *Aspergillus niger* LPBW12, LPBIZ9, LPB28, which were preserved in PDA at 4°C, were cultivated in 50 mL of PDA to produce spores[24], Spore suspension was prepared with a 1% Tween 80 and agitation with a magnetic stir bar[25]. The spore count per milliliter of solution was performed as reported by Pereira de Souza[26] in a Neubauer chamber The composition of the liquid fermentative medium was extracted from Gomes' work.[27], which reported glucoamylase production by *Aspergillus flavus*, using a medium composed of 1.0% carbon source, 0.14% $(NH_4)_2SO_4$, 0.60% K_2HPO_4, 0.20% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.50% yeast extract, 0.20% peptone, 0.20% beef extract, at pH 5.0, inoculated with a spore suspension (1×10^6 spores/mL), in a rotary shaker at 100 rpm for 96 h at 28°C. Enzymatic activity determination was performed in triplicate to avoid statistical errors, following the methodology of Zaghetto de Almeida[4], detecting the presence of reducing sugars by DNS.

3 RESULTS & DISCUSSION

During the analysis of *Aspergillus* strains, it was confirmed that all of them exogenously present glucoamylase activity during Lugol's analysis. The minimum spore concentration per mL was $3.01*10^8$, which allowed a inoculum volume of 10% (v/v) of the total medium, avoiding the percentage alteration of other compounds.

Among the 10 strains evaluated through fermentation of the starchy liquid medium, the strain *Aspergillus oryzae* LPB 1808 achieved the highest yield reaching its maximum activity on the 5th day with a productivity of 14.29U/mL. The kinetics profile of glucoamylases' production by the different strains are presented in Figure 1.

Figure 1 Enzymatic activity over fermentation days.



The glucoamylase from *Aspergillus oryzae* 1808 exhibited better activity (14.29U/mL) than that reported for *Aspergillus awamori*, 8.3 U/mL [14], *Aspergillus flavus*, 3.5 U/mL[27], and *Thermomyces lanuginosus*, U/mL[21]. The kinetics was carried out for 7 days considering the growth and maturation time of fungal spores, as shown in Figure 2. This fungus will be considered for the subsequent phases of optimization of glucoamylases production and process scale-up.

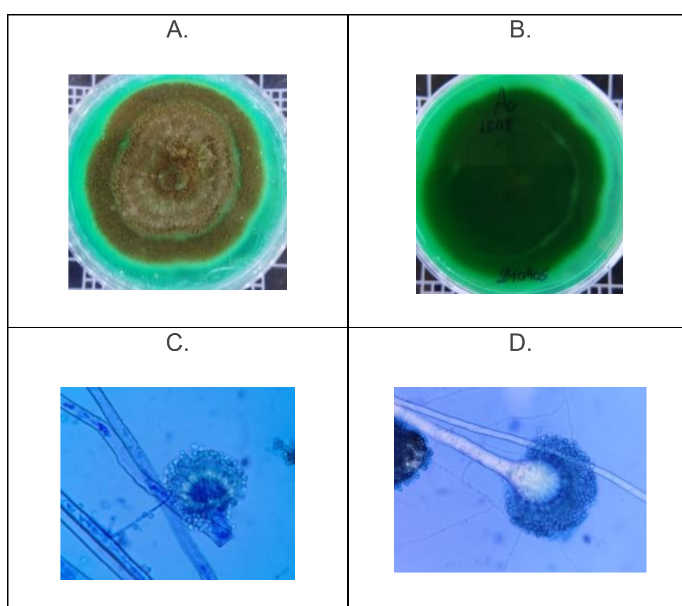


Figure 2: *Aspergillus Oryzae* 1808. (A-B); Macroscopic growth of the strain on Petri dish, (C-D); observation of the strain under microscope using microculture technique.

4 CONCLUSION

The glucoamylase enzyme was successfully produced with Aspergillus oryzae LPB 1808 using a starchy medium, achieving the highest activity among all the strains evaluated, even better than in some studies where the same production technology was used. Based on the reported data, scaling up with this fungus for glucoamylase production is proposed. The next steps of the work will include statistical analysis for the optimization of the fermentative medium, process conditions, enzyme purification, and recovery.

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