PRODUCTION OF AMYLASE BY AN ENDOPHYTIC *Penicillium sp.* ISOLATED FROM *Manihot esculenta*

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ABSTRACT

Amylases are a family of enzymes of great importance to biotechnology, with applications in various branches of industry. Face to their growing demand in industrial processes and the urgent necessity to find new sources of these enzymes, the aim of this work was to evaluate the secretion of α-amylase by an endophytic strain of *Penicillium sp.*, isolated from *Manihot esculenta* roots, in submerged fermentation. The fermentation test was carried out in Erlenmeyer flasks (30 ºC, 120 rpm) and evaluated each 24 hours to check α-amylase activity. The results indicated a maximum amylase activity observed after 96 hours of cultivation, having hydrolyzed around 96% of the substrate in the reaction medium. Subsequent efforts should be made to optimize the fermentation medium, verifying the effect of mineral cofactors, such as calcium, or organic cofactors, such as amino acids, on the secretion of amylase by this isolate. In addition, its genomic DNA was extracted and stored for later identification at species level.

Keywords: Endophytic fungi. *Manihot esculenta*. *Penicillium sp.* Amylase.

1 INTRODUCTION

Amylases are a large family of enzymes that cleave the α-glycosidic bonds of starch, releasing various products, including dextrin and progressively smaller polymers made up of glucose units[1]. These are divided into groups, classified as α-amylase, β-amylase and amyloglucosidase, and can be obtained from different sources, including plants, animals and microorganisms, with filamentous fungi and bacteria being the main producers[2]. In addition, they are of great importance to biotechnology, with applications in industry sectors as food, detergent, textile, paper and cellulose, sugar and energy / biofuels.

Face to the growing applicability of enzymes in various industrial sectors, is necessary a sampling effort to screening for new microorganisms that make possible to discover species that produce amylases, as well as to characterize their amylolytic potential. A fungus previously isolated from the tuberous roots of *Manihot esculenta* showed a positive result in the qualitative plate amylase test (enzyme index > 2.0). It was identified as a member of the genus *Penicillium* based on the micro-morphology of the hyphae and fruiting body (figure 1). The aim of this study was to evaluate the secretion of α-amylase by the fungus *Penicillium sp.* in a submerged fermentation process.

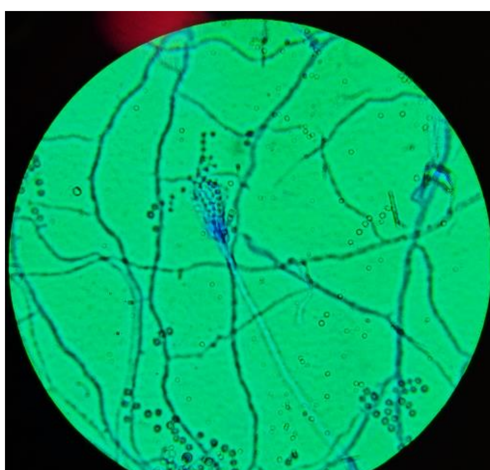

Figure 1 – Micro-morfologia das hifas de *Penicillium sp.* isolado de *Manihot esculenta.*

2 MATERIAL & METHODS

Penicillium sp., preserved in sterilized distilled water [3], was reactivated in Petri dishes containing YM culture medium (yeast extract 5 g/L, malt extract 5 g/L, dextrose 10 g/L and agar 20 g/L). To carry out the quantitative test of α-amylase secretion in submerged fermentation, the pre-inoculum was prepared in 100 mL Erlenmeyer flasks containing 50 mL of liquid culture medium, composed

of tryptone (10 g/L), glucose (15 g/L) and starch (3 g/L), inoculated with 1.84 x 10^7 spores counted in a Neubauer chamber. The flasks were incubated for 72 hours at 28 °C and 120 rpm. After this time, all the contents were transferred to a 500 mL Erlenmeyer flask containing 150 mL of fermentation medium composed of glucose (3 g/L), starch (15 g/L) and tryptone (10 g/L).

Each 24 hours, 2 mL aliquots were collected and the α-amylase activity was checked using the FUWA method[4] with modifications. The aliquots were centrifuged (12000 rpm, 10min) to sediment the biomass, and the supernatant was used as an enzyme broth. The enzymatic assay was carried out by mixing 8 µL of 50 mM sodium acetate buffer (pH 6,0), 20 µL of 1% soluble starch, and 12 µL of enzyme broth, incubated at 40 °C for 10 minutes. The reaction was stopped with 40 µL of 1 M acetic acid, 40 µL of FUWA was added and the total volume of 2 mL was completed with distilled water. The absorbance was checked at 660 nm and the concentration of residual starch was calculated according to equation 1 (R^2= 0.9954). From the concentration of residual starch, the enzymatic activity was calculated based on the percentage of hydrolyzed substrate, as previously described [4]. The submerged fermentation trials were performed in triplicate.

$$\boldsymbol{Y = 0,758 * X - 0,9954} \quad (1)$$

3 RESULTS & DISCUSSION

The first sample collected at the start of submerged fermentation showed amylolytic activity, having hydrolyzed 66.7% of the substrate supplied in 10 minutes. This result indicates that the initial pre-inoculum conditions were efficient in inducing the secretion of amylases by *Penicillium sp* in the first 32 hours in contact with starch. Maximum amylase activity was detected at 96 hours of cultivation, when 95.9% of the starch had been degraded. Amylolytic activity became undetectable at 120 hours of cultivation. The complete results are shown in figure 2.

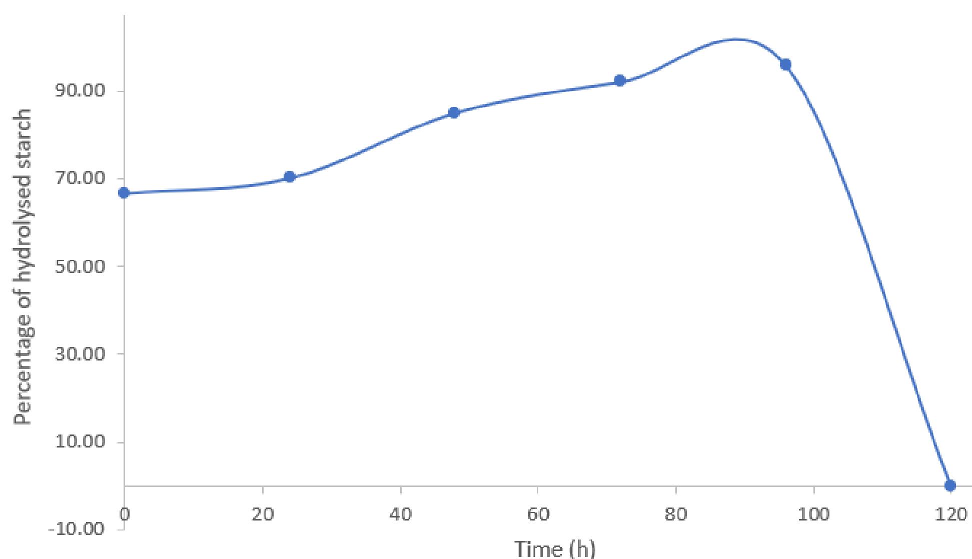

Figure 2 Percentage of hydrolyzed starch along submerged fermentation.

Similar results were obtained by Selvam et al. [5] when they obtained peak activity at 96 hours by cultivating *Bacillus sp.* on cassava waste as a substrate for amylase production. Several factors can influence amylase activity, such as the pH or temperature of the assay. In this assay, tryptone was provided as the sole source of nitrogen, which may explain the depletion of enzyme activity after 120 hours of cultivation, as observed in previous assays. [5,6]

Other relevant factors are the type of flask used and the way in which enzyme production was conducted. Production in agitated flasks is a limiting factor for enzyme production due to the difficulty in maintaining physicochemical properties in the production medium, such as temperature, pH, secretion of metabolites and other metabolism by-products. In addition, the simple continuous conduction mode favors low enzyme secretion due to the depletion of nutrient concentrations and the accumulation of potential inhibitors, which may explain the drop-in enzyme activity after 96 hours of production.

4 CONCLUSION

Manihot esculenta is a potential habitat for natural producers of α-amylases. The isolate of *Penicillium sp.* was sufficiently able to produce amylases after 72h of pre-inoculum, being capable of hydrolysate about 96% of the available substrate along the enzymatic assay.

Subsequent efforts will be employed to improve the amylase activity (U/mL) by this isolate, assessing the effects of mineral supply, as calcium and nitrogen sources, and organic factors, as amino acids and carbon sources. Furthermore, the genomic DNA was extracted and stored for posterior species taxonomic identification.

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