EXPRESSION, PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A RECOMBINANT AMYLASE FROM THE YEAST Cryptococcus flavus

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The α-amylase (EC3.2.1.1) is one of the key enzymes of starch processing, and great importance in a wide field of applications: breweries, paper and textile industry, among others. However, the high costs to obtain this enzymes is an bottleneck to its effective use. Therefore, considering the industrial applicability of the α-amylases is necessary to produce enzymes with appropriate physico-chemical characteristics. In this work, the main goal was to produce a Cryptococcus flavus amylase in Escherichia coli and characterize it biochemically. Firstly, the ORF encoding to the amylase from C. flavus (AMY1) was synthesized with codons optimized for E. coli expression cells and cloned onto pET21a vector. In silico analysis have revealed a predicted molecular mass of 67 kDa, and isoelectric point of 4.46. The three dimensional structure prediction of recombinant amylase has been perfomed by homology using the Phyre2 Program. It was found that 98% of the recombinant amylase structure was modeled with accuracy. Subsequently cells of E. coli BL21 (DE3) pLys E and S pLys were transformed by heat shock method. The induction was carried out with Isopropyl β-D-1-thiogalactopyranoside (IPTG) 1mM and then the protein expression was evaluated by SDS PAGE analysis. No bands corresponding to a recombinant amylase was observe d. An alternative protein expression protocol was performed using a culture media containg 1% glucose e induction with 100mM lactose instead IPTG. Also, no protein bands correspond to AMY1 was visualized. These preliminary results suggests that the AMY1 can be toxic to E.coli cells. Others protocols are being tested. We thanks the Universidade Federal de São João Del Rei – Campus Centro Oeste, Divinópolis, MG for providing all the material, equipment and facilities to carry out the project.

Keywords: α-amylase, Cryptococcus flavus, recombinant expression.