IMPROVEMENT OF CULTURE CONDITIONS AND PURIFICATION OF RECOMBINANT ?LPHA-AMYLASE OF THE YEAST *Cryptococcus flavus*

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Alpha-amylases are hydrolytic enzymes that catalyze the hydrolysis of internal a-1,4-glycosidic bonds in amylose and amylopectin. They are among the most important industrial enzymes with many applications in starch processing, brewing, alcohol production, textile and other. *C. flavus* is an amylolytic yeast secreting high levels of a-amylase, which is used as a complementary source to produce ethanol from stach. In this work we test three nitrogen sources for production of the recombinant *C. flavus* a-amylase (*Amy1*) expressed in *S. cerevisiae:* YNB (yeast nitrogen base plus amonium sulphate), urea and yeast extract. The yeast culture incubated with yeast extract for 72 hs at 27ºC on a shaker at 200 rpm, presented the highest growth (OD_{600} 7.22), better amylolytic activity (40 $U.mL^{-1}$) and highest amount of total secreted protein (37.85 $\mu g.mL^{-1}$). The culture was centrifuged at 2254*G* and the supernatant was dialyzed, lyophilized, and applied into a Q-Sepharose column equilibrated with 50 mM sodium acetate, pH 5.5 and 1 mM $CaCl_2$. The column was washed with three volumes of buffer and the proteins were eluted with a linear salt gradient from 0 to 0.5 M. The fractions with amylolytic activity were dialyzed and analyzed by SDS-PAGE to confirm the protein purity. The use of yeast extract improves the amylase production by recombinant *S. cerevisiae* strain which is the first step to optimize its biotechnology applications. The structural characterization of pure Amy1 is in progress.

Key words: a-amylase, *Cryptococcus flavus*, *Saccharomyces cerevisiae*, amylolytic activity.

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