ABSTRACT

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF EXTRACELLULAR α-AMYLASE FROM *Aspergillus fumigatus* HBF125

Öznur KOÇ

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Supervisor: Assist. Prof. Dr. Kubilay METİN
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In our study, screening of industrial enzymes and temperature limits of the 91 fungi was done. *Aspergillus fumigatus* HBF125 strain was chosen as the best one showing amylase activity from thermotolerant and thermophilic fungi. Culture conditions was optimized for this fungus. The best enzyme production from *A. fumigatus* HBF125 strain was determined in medium including: 7 days sporulation medium, 5% inoculum ratio, temperature 35 °C, starting pH 5.0 and 1.5% bran as carbon source. Extracellular amylase produced by *A. fumigatus* HBF125 in optimal growth medium was purified 54.4 fold with a recovery of 4.7% using starch affinity chromatography. Molecular weight of enzyme was found to be about 160 kDa by SDS-PAGE method. Those two subunits of molecule weight of the enzyme was found to be 86.2 and 73.8 kDa. The temperature and pH for maximum activity of the enzyme were 60 ^{o}C and 5.5, respectively. In a wide range of pH and temperature of the enzyme was found to be stable. It was found that the Km and Vmax of amylase were 1.45 mg/mL and Vmax 909 U/mL. In addition, the amylase exhibited broad substrate specificity. The enzyme activity was markedly inhibited in the presence of NBS suggesting that tryptophan residues play an important role in the catalytic process. The enzyme activity was not affected by detergents as Tween 20, but it was found inhibited by 1.4-dioxan and n-propanol. The enzyme by Hg^{2+} was strongly inhibited while Mn^{2+}, Co^{2+}, Ca^{2+} and Ba^{2+} were activated. The salt tolerance of the enzyme was found to be very good. The amylase was found to be metalloenzyme.

Keywords: Thermotolerant fungi, enzyme production, purification and characterization.