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# Purification and comparison properties of crude enzyme with purified α-amylase from *Bacillus licheniformis* ATCC 6346

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**ABSTRACT**

*The α-amylase from Bacillus licheniformis ATCC 6346 was purified by ion-exchange chromatography (DEAE-Sepharose). The spent medium contained 37.5 $UmL^{-1}$ α-amylase activity and 1.77 $mgL^{-1}$ protein. Highest specific activity (65.54$Umg^{-1}$) was obtained at 50% $(NH_4)_2SO_4$ saturation and 66.6% recovered. The precipitated and dialyzed enzyme was purified using DEAE-Sephorose at pH 8.0, and eluted with the 0.01M Tris buffer containing 0-0.8 M NaCl. The recovery of α-amylase by ion-exchange chromatography was 7.5%, with 8.2 fold purification, showing the specific activity of 173.8$Umg^{-1}$ protein. The purified α-amylase was tested for purity by SDS-PAGE. The purified enzyme showed a single band with an apparent molecular weight of 55.54 kDa. Crude α-amylases showed zero order kinetics for 10min while purified α-amylase showed zero order kinetics for 8min. The optimum temperature for the activities of crude and purified enzymes was 85°C. The optimum pH was 7.0 for the crude and purified at 85°C. When the crude enzyme was pre-incubated at 85°C and at pH 7.0, it lost 40% of its initial activity at 10min while the purified enzyme lost 75% of its initial activity at 10min. Crude and purified enzymes showed 119, 77.7 & 20.3 and 107, 60, & 20% of relative activities respectively with amylose, amylopectin, and maltose when compared to soluble starch at 85°C and pH 7.0. Both crude and purified enzymes showed no activity with cellulose, sucrose and pullulan. Therefore substrate specificity indicated, that both purified and crude α-amylases were able to hydrolyse mainly starch, amylose and amylopectin.*

**Key words:** Purification, α-Amylase, *Bacillus licheniformis*, Enzyme stability, DEAE-Sepharose.

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