Purification of extracellular α-amylase produced by *Bacillus licheniformis* ATCC 6346

Vengadaramana, A., Balakumar, S. and Vasanthy Arasaratnam

This study was aimed at the purification of α-amylase produced by *Bacillus licheniformis* ATCC 6346 in fermentation medium at 42°C and at 100rpm. The fermentation medium contained (g/l) soluble starch, 4.0; $(NH_4)_2SO_4$, 5.0; peptone, 6.0; $FeCl_3$, 0.01; $MgCl_2.6H_2O$, 0.01; $CaCl_2.2H_2O$, 0.01; KH_2PO_4, 4.0 and K_2HPO_4, 7.5. The spent medium contained 37.5 Uml^{-1} α-amylase activity and 1.77 $mgml^{-1}$ protein. To purify the enzyme it was subjected to fractional precipitation by adding solid $(NH_4)_2SO_4$ from 10 to 70% saturation. Highest enzyme activity was precipitated at 50% saturation with $(NH_4)_2SO_4$. Hence for the purification studies the enzyme was precipitated with 50% saturation of $(NH_4)_2SO_4$. The precipitate was dissolved in distilled water and dialysed against distilled water. The residue contained α-amylase activity of 125Uml^{-1} and 1.907 $mgml^{-1}$ protein. The recovery of α-amylase by $(NH_4)_2SO_4$ precipitation was 66.6% showing specific activity of 65.54Umg^{-1} protein. The dialyzed enzyme was loaded on ion-exchange (DEAE-Sephorose) column (1 x 5 cm) and equlibrated with 0.01M Tris buffer (pH 8.0). The enzyme was eluted with the same buffer containing 0-0.8 M NaCl. Fractions 37-47 showed enzyme activity and poole of 11 fractions (purified) containd 38.33Uml^{-1} enzyme activity and 0.2205$mgml^{-1}$ protein. The recovery of α-amylase by ion-exchange was 55.2% showing specific activity of 174.14Umg^{-1} protein . The enzyme sample purified by ion-exchange chromatography was seperated by Sodium dodecyl sulfate-polyacrylamide gelelectrophoresis. The electrophorogram showed a single band for the purified enzyme, with molecular weight of the purified enzyme was 55.54 kDa.

Department of Biochemistry, Faculty of medicine, University of Jaffna, Sri Lanka