E2-47 Purification and characterization of raw starch hydrolysing alpha amylase from *Aspergillus* sp. RSH-I

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Raw starch hydrolysing alpha amylase from 36h culture supernatant of *Aspergillus* sp. RSH-I was purified to homogeneity by 50% ammonium sulfate fractionation and DEAE A-25 anion exchange column chromatography.

The total activity of the culture supernatant after ammonium sulfate fractionation was reduced to 9202mU from 27778mU for soluble starch and to 6160mU from 29760mU for raw starch. Total protein content was reduced to 88mg from 600mg. The specific activity for soluble and raw starch was increased to 105mU/mg from 46mU/mg and 70mU/mg from 50mU/mg respectively. Purification and recovery fold for soluble starch was 2.3 and 33% respectively while for raw starch it was 1.4 and 20.7% respectively.

Four protein peaks were observed during DEAE gel chromatography. These were designated as SOI, RSI, RSII and RSIII in the order of elution from the column. SOI and RSI showed only soluble starch hydrolysing activity. The total activity of RSII for soluble and raw starch was 679.4mU and 2948mU respectively. The specific activity of the same fraction for soluble and raw starch was 1014mU/mg and 4400mU/mg respectively. The increase in purification folds were 21.6 and 88.7. Recovery was 2.5 and 10 for soluble and raw starch respectively.

RSIII fraction showed 139.7mU and 1970mU of soluble and raw starch hydrolysing activity and the specific activities of 209mU/mg and 2940mU/mg for soluble and raw starch respectively. Recovery as a percentage of total activity of extract was 0.5 and 6.62 and the purification folds were 4.5 and 58 for soluble and raw starch respectively.

Polyacrrylamide gel electrophoresis showed a single protein band for RSII fraction indicating that it is purified to homogeneity. This purified enzyme showed highest raw starch hydrolysing alpha-amylase activity at 40°C. The enzyme has its activity in the pH range 3.5-8.0 giving maximum activity at pH 8.0.