

E2-70: Purification and characterization of intracellular alpha galactosidase from an *Aspergillus* species

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Alpha galactosidase is used in industry in soy milk processing and beet sugar production to remove raffinose sugars, paper and pulp industry to hydrolyze hemicellulose and in production of gelling agents. In industrial applications, intracellular alpha galactosidase producing fungi are used as the enzyme source. In this study we have isolated an *Aspergillus* species producing intracellular alpha galactosidase activity of 3645 mU/g after 96 h of cultivation.

Alpha galactosidase was purified to homogeneity as determined by polyacrylamide gel electrophoresis. Recovery was 8% and folds purification was 51, Michaelis constant was 3.3×10^{-4} M and maximum velocity was 2.26×10^{-2} $\mu\text{mol}/\text{min}/\text{g}$. Studies on effect on pH showed that the enzyme has pH optima of 3.5 and 5.0. Enzyme was stable between pH 3.5-7.0. Optimum temperature for enzyme activity was observed at 50°C and enzyme was stable between 20-60°C. Studies on raffinose hydrolysis showed that free mycelium (3g) stirred in a 1% raffinose solution (100ml) hydrolyzed 12% of the sugar in 4 h. Fungal mycelium (3g) immobilized on 5% polyacrylamide gel and illuted with 1% raffinose solution (100ml) at a flow rate of 60ml/h, hydrolyzed 51% of the sugar solution in 4 h.

Aspergillus species isolated has high intracellular alpha galactosidase activity of 3645mU/g. Enzyme is heat stable upto 60°C and has a low Michaelis constant and high maximum velocity. Immobilized in polyacrylamide gel it shows high raffinose hydrolyzing activity. These results indicate that the *Aspergillus* species isolated could be used industrially to hydrolyze raffinose sugars.