Thesis Title	Studies on the Amylase Enzyme from Thermoactinomyces sp. that was Isolated from a Soil Sample
Name	Pilarat Maneerat
Concentration	Biology
Department	Biology
Academic Year	1997

ABSTRACT

Thermoactinomyces sp., a Thermophilic filamentous bacteria, was identified as *Thermoactinomyces vulgaris*. When it was cultivated on STYA-10 pH 7-9, it produced white aerial mycelia upon which abundant single spores on short sporophores were found and the mycelium was stained gram-positive.The organism was able to grow at 35 -65^{0}C pH 7-9,but not grow at room temperature 70^{0}C pH 5 and 11.Tt optimal temperature for growth was 55 - 60^{0}C. STYB-30 pH 8 incubated at 55^{0}C was the best condition for amylase production. Maximum amylase activity (130 D.U./ml) was obtained in 48 hours.Growth and amylase production were determined at 55^{0}C in a fermentor containing STYB-30 pH 8, and maximum dried weight 3 g/l and amylase activity 136 D.U./ml were achieved in 12 hours and 48 hours,respectively.When Glucose was added to the fermentor at the 8th hour,maximum activity 102 D.U./ml was found in 60 hours.This suggested that glucose catabolite repression regulated the enzyme synthesis.Crude enzyme with 52,47,28 or 26% yields were obtained after dialyzing against Tris-HCl pH 8 containing 0,1,5 or 10 mM Ca^{++} ,respectively. Crude enzyme had optimal pH range from 5.5-8.5. Calcium ion (2 mM) in the substrate did not affect the activity of the crude enzyme at various temperature tested and optimal temperature 65^{0}C was determined from this assay.However,the increase in calcium ion concentration caused the increase in heat stability of the enzyme. The crude enzyme was stable at 60^{0}C.It had half life greater than 15 minutes under this condition.The enzyme denatured rapidly at 68^{0}C or above.Some metal ions(0.5 mM Na^{+} ,Cu^{++} ,Ca^{++} and Fe^{+++}) neither activated nor

inhibited the activity of the crude enzyme.Metal ions,such as 0.5 mM of Mg^{++} ,Mn^{++} ,and Ag^{+},and 5 mM of Ca^{++} ,and Na^{+} ,slightly inhibited the activity and 81-88% of the activity of the control was obtained.Zinc ion (0.5 mM),Na^{+}(50-150 mM),Ca^{++} (10 mM) and EDTA (0.5 mM) inhibited the activity moderately since 57-74% of the activity of the control was determined.Among the metal ions tested,the mercuric ion (0.5 mM) was the most potent inhibitor because only 16% of the activity remained.When calcium ion existed in crude enzyme,binding of the enzyme to the Q-Sepharose Fast Flow was reduced and high percent recovery was obtained.This result was similar to the results obtained from the binding experiments performed in eppendorfs.Therefore,Ca^{++} not only caused less binding to the Q-matrix but also enhanced stability of this enzyme.