Some Preliminary Studies on the Production and Properties of -amylase from Bacillus licheniformis ATCC 6346

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Thermostable α-amylase is produced by a mesophilic organism, *Bacillus licheniformis*. Single colony of *B. licheniformis* ATCC 6346 from nutrient-agar slants (grown at 37°C for 24h) was transferred to activation medium and incubated at 42°C in a rotary shaker (100rpm) for 12h and used as inoculum. The nutrient-agar medium contained (gl^{-1}) nutrient agar, 25.0 and soluble starch, 3.0 and the activation medium contained (gl^{-1}) nutrient broth, 25.0 and soluble starch, 3.0. The fermentation medium was inoculated with inoculum (20%, v/v) and incubated at 42°C and 100rpm. The Fermentation medium contained (gl^{-1}) soluble starch, 2.0; $(NH_4)_2SO_4$, 2.0; peptone, 2.0; NaCl, 1.0; $FeCl_3$, 0.005; $MgCl_2$ $6H_2O$,0.005; $CaCl_2$. $2H_2O$, 0.005; KH_2PO_4 , 1.0 and K_2HPO_4, 2.5. The strain *B. licheniformis* ATCC 6346 reached log phase at 12h. Highest Optical density (OD_{600nm}) 1.905 was at 12h and the highest α-amylase activity (20.58Uml^{-1}) was obtained at 33h. Amounts of soluble starch and $(NH_4)_2SO_4$ in the fermentation medium were optimized to improve the enzyme production. The soluble starch concentration in the medium was varied in the range of 2-10gl^{-1} while all other contents of the fermentation medium was kept the same. The highest α-amylase activity (28.7 Uml^{-1}) was produced in the fermentation medium containing 4gl^{-1} soluble starch. Then to the fermentation medium containing 4gl^{-1} soluble starch, different concentrations of $(NH_4)_2SO_4$ (2-9gl^{-1}) was added and the highest α-amylase activity (39.60Uml^{-1}) was obtained in the medium containing 5gl^{-1} $(NH_4)_2SO_4$. Kinetic properties of the α-amylase produced by *B. licheniformis* were studied. Enzyme activity with time was determined and the reaction time was fixed as 10 min. The optimum pH was determined as pH 7.0 for enzyme activity. The activity was measured at different temperatures ranging from 40 to 95°C and the optimum temperature for the enzyme activity was 85°C when soluble starch was used as the substrate. Michaelis constant of α-amylase for soluble starch was 0.47gl^{-1} at pH 7.0 and 85°C. The enzyme pre incubated at 85°C and at pH 7.0 lost 25% of its original activity in 5min while that pre incubated at 75°C retained 75% of its initial activity at 90min. When the enzyme was incubated at 85°C and pH 9.0 retained 73% of its initial activity at 30min; and at pH 8.0 retained 63% of its initial activity at 10min. At pH 7.0 and 8.0 and at 85°C the enzyme lost the total activity at 60min and 50min respectively. Further studies are in progress to increase the enzyme production and to purify the enzyme.