

Kinetic Studies of the Crude Alkaline Proteases From Bacterial Strains Isolated From Dog Decaying Soil

Anpalagan, V.C., Balakumar, S. and Vasanthi Arasaratnam

Department of Biochemistry, Faculty of Medicine, University of Jaffna

Proteases are very active under alkaline and thermo stable conditions and have great potential in the industrial application. The objective of this study is to determine the kinetic properties of alkaline proteases from locally isolated bacterial strains. Two bacterial strains were isolated from dog decaying soil. Single colony of the selected bacterial strain was obtained by cultivating the organism in nutrient-agar medium at 40°C for 24h. The nutrient agar medium contained (gL⁻¹) nutrient broth, 10.0; peptone, 10.0; sodium chloride, 5; and bacteriological agar, 17.5. The bacterial cells grown on the slants were transferred into the activation medium, incubated in shaker water bath at 40°C and 120 rpm for 18h. Both activation and fermentation media were same and contained (gL⁻¹) glucose, 10.0; peptone, 5.0; yeast extract, 5; K₂HPO₄, 10.0; MgSO₄·7H₂O, 0.2; and Na₂CO₃, 10. Two bacterial strains named DS₁ and DS₂ were cultivated for 130h and the spent media were used as alkaline protease enzymes sources. Both the bacterial strains produced the proteases at pH 9.5 and at 40°C and were used for the kinetic studies. Crude alkaline proteases showed zero order kinetics for 10 minutes. When the activities of crude proteases from strains DS₁ and DS₂ were measured at different temperatures ranging from 30 to 90°C and at pH 9.5, the optimum temperatures for the activities of the crude enzymes obtained from DS₁ and DS₂ were 75 and 50 °C respectively. Both enzymes obtained from strains DS₂ and DS₁ showed the optimum pH at 8.5 and 50, 75°C respectively.