

ABSTRACT

The present study describes the production of alkaline protease by mutant strain of *Bacillus subtilis* EMS-8. The enzyme was partially purified using ammonium sulphate precipitation. It gave 2.64 fold purification with 81.5 % yield at 70% saturation. Dialysis of the precipitated fractions was carried out using Tris HCl buffer at pH 8.0. Precipitation of enzyme by ammonium sulphate was found best at 4 °C. The molecular weight of the partially purified enzyme was determined using SDS-PAGE and it was found to be 25 KDa. The optimum pH of enzyme activity was 8.5; however the enzyme enzyme remained stable upto pH 10 after 24 hrs of incubation. Similarly, the optimum temperature for enzyme activity was 40 °C, whereas it remained stable upto 90 °C, although its activity was greatly reduced. Alkaline protease showed highest specificity towards casein. Among different inhibitors checked, Phenylmethylsulphonyl fluoride (PMSF) completely inhibited the enzyme activity indicating the serine nature of protease. Similarly, the protease activity was greatly reduced in the presence of MnCl₂, whereas MgCl₂ showed increased activity of protease. The shelf life of the protease was also determined at different temperatures for 4 weeks. It was found that the activity of the protease comes to an end after second week, when the enzyme was stored at room temperature.