Purification and characterization of a heat-stable alkaline protease from Bacillus stearothermophilus F1

ABSTRACT

A thermophilic Bacillus stearothermophilus F1 that produced an extremely thermostable alkaline protease was isolated from decomposed oil palm branches. The isolated protease was purified to homogeneity by heat treatment, ultrafiltration and gel filtration chromatography with a 128-fold increase in specific activity and 75% recovery. The protease, which is a serine-type enzyme, has a relative molecular mass of 33 500 by sodium dodecyl sulphate-polyacrylamide gel electrophoresis but only 20 000 by gel-filtration chromatography. The enzyme was optimally active at pH 9.0 and was stable for 24 h at 70° C and in the pH range from 8.0 to 10.0. It was capable of hydrolysing many soluble and insoluble protein substrates but no esterase activity was detected. The enzyme activity was markedly inhibited by Co^{2+} and Hg^{2+}, whereas Mg^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+} and Sr^{2+} had little or no inhibitory effect. However, Mn^{2+} strongly activated the protease activity. The protease exhibited a high degree of thermostability [$t_{1/2}$ (85° C) = 4 h, (90° C) = 25 min]. The stability at higher temperatures (85° C and above) was shown to be dependent on the presence of Ca^{2+}.

Keyword: Bacillus stearothermophilus F1; Thermostability; Protease; Enzyme purification