PRAKORN GIRODKUNKID, CAPT. : PURIFICATION AND CHARACTERIZATION OF ALKALINE PROTEASE FROM BACILLUS SUBTILIS TISTR 25
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A chemical-defined medium supplemented with 0.1 percent glutamate at pH 6.0 and 30 °C were the most suitable culturing condition for the production of extracellular proteases by a local strain of Bacillus , B. subtilis TISTR 25. Glucose was found to have a pronounce effect on the protease production through catabolite repression. Through the use of the enzyme inhibitors, phenylmethylsulfonyl fluoride (PMSF) and ethylenediamine tetraacetic acid (EDTA), the results suggested that the enzyme excreted by the strain TISTR 25 are combination of neutral and alkaline proteases in the approximate ratio of 1 : 3. The culture thus differs from the standard strain of B. licheniformis ATCC 21415 which mostly produced alkaline protease.

Alkaline protease from B. subtilis TISTR 25 was purified 2.3 fold by using ammonium sulfate precipitation and CM-cellulose chromatography. The purified enzyme was proved to be a monomer with a molecular weight of 27,000. The optimum pH and the optimum temperature were 8.5 and 55 °C, respectively. The enzyme was able to hydrolyse both natural and synthetic substrates. The K_m for casein, hemoglobin and benzoyl tyrosine ethyl ester (BTEE) were 0.02, 0.07 and 8.0 mmol/l whereas those of azocasein and azocoll were 3.03 and 33.33 mg/ml, respectively. The enzyme hydrolysed peptide bond specifically at the carboxyl end of the amino acid arginine , alanine , leucine , phenylalanine and valine.

This purified alkaline protease differs from Subtilisin Carlsberg in its optimum pH and temperature for catalysis. The two enzymes also differ in their affinities to casein and BTEE, the result of which suggests that some amino acids around the vicinity of the binding site of the two enzymes might be different. The result from peptide hydrolysis as analyzed by spectrophotometric and TLC technique indicated that the pattern of specificity of peptide hydrolysis of this alkaline protease is similar to that of Subtilisin Carlsberg and Nagarse but differs from trypsin.