**M.Phil. in Biochemistry**

# Isolation of a thermostable alkaline protease producing bacterial strain and kinetic studies on the enzyme

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## Abstract

This study focuses on the isolation and identification of alkaline protease producing bacteria and the enzyme production by the selected bacteria. For this purpose bacterial strains were isolated from dog (61Nos), beef (17Nos) and fish (14Nos) decaying soil. Single colonies of the isolated bacterial strains were purified by repeated streaking and cultivating in nutrient-agar medium at 40$^{o}$C for 24h. Among the 92 bacterial strains, selected 36 strains produced alkaline protease activity, above 4 UmL$^{-1.}$ Among the 36 alkaline protease producers, 4 strains which gave alkaline protease activity in the range from 35 to 54 UmL$^{-1}$ ($DDS_2$, $DDS_{21,}$ $DDS_{33}$ and $DDS_{47}$) were selected. Based on the morphological and biochemical tests isolates $DDS_2$, $DDS_{21,}$ $DDS_{33}$ and $DDS_{47}$ were identified as *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus laterosporus* and *Bacillus cereus* respectively. To select the best alkaline proteases produced by B. *thuringiensis*, B. *subtilis*, B. *laterosporus* and B.*cereus* were characterized and they showed zero order kinetics up to 10, 15, 10 and 15min respectively. Among the selected isolates B. *subtilis* and B. *cereus* produced alkaline protease with optimum pH of 10.5 for the activity, while the protease produced by B. *thuringiensis* and B. *laterosporus* showed optimum pH of 9.5. Thus B. *subtilis* and B. *cereus* were selected and B. *subtilis* produced highest alkaline protease and its protease showed highest activity at 72$^{o}$C and pH 10.5 and good thermostability (Half life-48 min) without additives. The optimized culture conditions for *B. subtilis* were 37$^{0}$C, the fermentation medium to flask volume ratio 1 : 20, inoculum size 17% (v/v) of 18h old inoculum from 36h old slant culture and agitation speed of 200rpm and fermentation time was 92h. The optimization studies increased the protease production by 2.1 fold while the time taken to produce highest protease activity was reduced by 28h. Optimization of fermentation medium was studied. Calcium free medium was found to be best for protease production. $MgSO_4.7H_2O$ of 0.35gL$^{-1}$ gave highest growth at 24 hours and protease activity at 92h and 15gL$^{-1}$NaCl and 0.1gL$^{-1}$ $ZnCl_2$ were most suitable for protease production. Optimization of peptone as 8gL$^{-1}$ and yeast extract as 8gL$^{-1}$ improved the protease production by 1.08 and 1.12 folds respectively. When the peptone and Yeast extract were replaced with different nitrogen sources such as $(NH_4)_2SO_4$, soyabean, casein, beef extract, tryptone, milk powder and malt milk powder, tryptone 25gL$^{-1}$ was more effective in improving alkaline protease production from *Bacillus subtilis* [887 (±6.9)UmL$^{-1}$]. Among the tested nitrogen sources, tryptone was selected as the best nitrogen source for highest alkaline protease production. Therefore 1.9 fold increase in protease activity was achieved after optimizing the concentration of the best nitrogen source. Among the carbon sources used (sucrose malt extract and starch) glucose was more effective for alkaline protease production [987.3(±6.9) UmL$^{-1}$]. By the optimization of culture conditions and culture medium the protease production was improved by 12.6 fold. Based on the properties of the protease produced by *B. subtilis,* the enzyme can be used in industries regarding alkaline proteases.