

Properties of Alkaline Protease C45 Produced by Alkaliphilic *Bacillus* Sp. Isolated from Chitu, Ethiopian Soda Lake

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A total of 240 alkaliphilic bacteria were isolated from samples collected from alkaline soda lakes of Ethiopia and were screened for the production of alkaline proteases. Of these, 30% were protease positive indicating the abundance of protease producing microorganisms in these habitats. The *Bacillus* sp. designated as C45 that grow in solid state medium was selected based on the property of the enzyme for further study. The protease produced by the *Bacillus* sp. was active in the pH range of 6.5-11.5, with optimum activity at pH 8-9; and stable at alkaline pH. The optimum temperature for activity was 40°C and 50°C in absence and presence of 5 mM of Ca²⁺, respectively. The enzyme displayed appreciable activity and stability at low temperature. These properties suggest that protease C45 could find potential application for dehairing and detergent at moderate temperature. When protease C45 was added to raw hide it enabled dehairing, suggesting the potential usefulness of the enzyme in the leather industry.

Proteases are essential constituents of all forms of life on earth which are classified based on chemical nature of the active site, the reaction they catalyze, and their structure and composition. The major classes are again classified in to sub classes based on pH, catalytic site on polypeptide, occurrence, and so on. Based on the catalytic site on the substrate, proteases are mainly classified in to endoproteases that prefer to act at the inner region of the polypeptide chain and exoproteases preferentially act at the end of the polypeptide chain. Based on their optimal pH proteases are also classified as: acid proteases which are active in the pH ranges of 2-6 and are mainly of fungal in origin, alkaline proteases which are optimally active in the alkaline range (pH 8-13), though they maintain some activity in the neutral pH range as well and are obtained mainly from neutralophilic and alkaliphilic microorganisms such as *Bacillus* and *Streptomyces* species, and neutral proteases which are active at neutral, weakly alkaline or weakly acidic pH and are mainly of plant in origin. Neutralophilic and alkaliphilic microbial alkaline proteases that are isolated from alkaline soda lakes possess a considerable industrial potential due to their biochemical diversity and stability at extreme pH environments, respectively. These application areas include: as processing aid like dehairing in leather tanning industries, as detergent additive to remove dirt and stains that are difficult to remove, in protein hydrolysis for production of high quality protein hydrolysates, in silver recovery from waste X-ray films, in pharmaceuticals production, and in chemical synthesis. Ethiopia is endowed with a range of alkaline habitats in Rift valley region. However, only few reports have been found on isolation of alkaline protease producers from these soda lakes. Therefore, the aim of this study was to isolate proteolytic alkaliphilic bacteria from Ethiopian soda lakes and characterize its enzyme.

Conclusions and Recommendations:

Based on the results of this work, the following conclusions can be drawn:

- The alkaline protease produced by this *Bacillus* sp. was active and stable at high alkaline and salt concentration.
- The protease stability and high enzyme activity at moderate (30°C-50°C) and at room temperatures, respectively, was an attractive feature to develop enzyme based industrial processes at room temperatures.
- Hide dehairing experiment at laboratory also confirmed the usefulness of this enzyme for application in leather industries.

Therefore, the following activities for future research recommended:

1. Searching for more potent alkaline protease producers from Ethiopian soda lakes.
2. Testing the alkaline protease from C45 for other suggested potential industrial applications.
3. Test of the enzyme dehairing capability at the tannery experimental level.