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Characterization of Crude and Partially Purified Thermo Active and Thermo Stable Alkaline Protease Produced by *Bacillus cereus* FT1

Asha B [1, 2] and Palaniswamy M*[1]

[1]*Department of Microbiology, Karpagam University, Karpagam Academy of Higher Education, Coimbatore- 641021, Tamil Nadu, India*
[2]*SIAS Centre for Scientific Research, Vazhayoor, Malappuram- 673633, Kerala, India*

Abstract

The *Bacillus cereus* FT1 isolated from soil is found to be a producer of alkaline protease enzyme. The crude and partially purified enzymes are characterised by studying the effect of substrate concentration, pH, temperature, metal ions, detergents, surfactants and oxidising agents on enzyme action. The maximum enzyme activities of crude and partially purified enzyme are observed at substrate concentrations of 2 and 3% respectively. The maximum activity of crude enzyme is at pH 9.5 and partially purified enzyme is at pH 10. Maximum stability for crude and partially purified enzyme are at pH 9.5 when incubate for 1h with residual activities of 98 and 100% respectively. The optimum temperature for action is 60°C for crude enzyme and 65°C for partially purified enzyme. The crude enzyme retains more than 95% of residual activity at temperature ranges of 5°C to 55°C and partially purified enzyme retains more than 95% of residual activity at temperature ranges of 5°C to 60°C after 1h of pre incubation. The metal ions Ba^{2+} and Mn^{2+} enhance the activity of both. They maintain 90% and above of residual activity when incubate with surfactants and detergents for up to 1h and the detergent Sunlight even increases the activity to above 100% for both. Different concentrations of H_2O_2 also retain 84% and more of residual activity for both enzymes. The partially purified enzyme retains its optimum activity for almost eight weeks at room temperature and there after only an insignificant decrease is noted.

Key words: Thermo stable alkaline protease, characterisation, *Bacillus cereus*

INTRODUCTION

Proteases are the enzymes that breakdown proteins in to its component amino acid. They are studied intensively not only due to their importance in cellular metabolism, but also because they have gained importance in the industrial community [1, 2]. The demand for the proteolytic enzymes which are specific and stable towards a particular pH, temperature, surfactants, metal ions, and organic solvent are now rising and stimulating the searches for more new enzyme sources [3]. Proteases account for about 60% of total worldwide enzyme sale and find many applications in the detergents, food processing, pharmaceuticals, bioremediations, production of digestives and treatments of inflammation and virulent wounds [4, 5, 6, 7, 8].

Alkaline proteases are generally active and stable at pH ranges of 9-11 and find applications mainly in detergent industry as the pH at which the laundry detergents are active lie between 9 and 12 [3, 9, 10]. The proteases must exhibit important properties to be used as a suitable detergent additive, like optimum alkaline pH activity, effectiveness at low temperatures, stability at high temperatures, stability in presence of detergent ingredients and ability to degrade variety of proteins [11]. Based on all these properties, alkaline proteases are industrially applicable due to their high stability and high activity under harsh conditions [12, 13, 14, 15].

Among the bacterial strains reported to be producing alkaline proteases, *Bacillus* genus gained a notable importance as a significant source of neutral and alkaline proteases. They are found to be highly stable at extreme pH and temperature ranges [16, 17, 18, 19]. Alkaline proteases derived from *Bacillus* have been extensively used due to their significant proteolytic activity and stability, broad substrate specificity, low cost production and purification [9, 20].

The detergent industry now demands an alkaline protease enzyme which is specific and stable towards pH, temperature, organic solvents, metal ions and surfactants, stimulating the searches for new enzyme sources [3]. In the present study, an attempt is made to characterise the protease enzyme produced by soil isolated *Bacillus cereus* FT1 strain. The characterization and stability of the enzyme are aimed to study and determine a possible eco-friendly application of enzyme in the detergent industry.

MATERIALS AND METHODS

Microorganism

The microorganism that is used in this study is *Bacillus cereus* FT1, which was previously isolated and maintained in our laboratory, SIAS Centre for Scientific Research, Vazhayoor, Malappuram, Kerala. The isolate was identified based on the methods recommended in the Bergey's manual of determinative bacteriology and diagnostic microbiology depending upon it's morphological, cultural and biochemical characteristics [21] and further confirmed based on the 16S rRNA sequence analysis and BLAST identification.

Enzyme production

Protease enzyme production was carried out using the previously standardised media (Composition [%]: lactose 2%; casein 4%; KH_2PO_4 0.2%; K_2HPO_4 0.2%; $MgSO_4.7H_2O$ 0.1%; $MnSO_4$ 0.1%) with pH 9.5. Inoculated media was incubated at 35°C for 48 h at 200 rpm on a rotary shaker. Cell free supernatant was collected by centrifugation at 10,000 rpm in a cooling centrifuge for 20 min to obtain crude extract, which can be used as the enzyme source for characterization. The crude extract was also partially purified and characterised.

Assay of alkaline protease enzyme activity

The crude or partially purified enzyme (0.1ml) was mixed separately with 1 ml of casein solution (2% w/v in 0.05M Tris-HCl buffer having pH 9) and incubated for 10 min at 37°C in the water bath. The reaction was terminated by adding 3 ml of 20% ice cold TCA and the final volume was made 5ml by adding 0.9 ml of the distilled water. The mixture was then centrifuged at 5000 rpm for 15 min and the supernatant (0.5ml) was mixed with 2.5 ml of 0.5M Na_2CO_3 and kept for 20 min at room temperature. Finally, added appropriately diluted Folin's phenol reagent, kept for 10 min and absorbance was measured (660 nm). The amount of tyrosine liberated was calculated from a standard graph generated using standard tyrosine of 10-100 µg/ml and protease activity was calculated from tyrosine concentration. One unit of protease activity is equal to the amount of enzyme required to liberate 1 µg tyrosine per ml per minute under standard assay parameters. [22].