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Production and Characterisation of an Alkaline Protease from *Bacillus amyloliquefaciens* isolated from the Soil

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Abstract: *Bacillus amyloliquefaciens* is a gram positive bacilli which was isolated from the soil sample. The organism was charactertized as *B amyloliquefaciens* by standard biochemical tests. The organism was inoculated in medium from which alkaline protease enzyme was produced. The partial purification was done by using ammonium sulfate precipitation and subjected to dialysis which was used for further study. The optimization of the enzyme was done by various parameters such as effect of pure soluble sugars, various nitrogen sources, and effect of pH and temperature on protease production. The enzyme activity was observed at the temperature above 40°C and pH range of about 9. These enzymes can be used in the field of molecular biology and increasingly studied due to its importance and subsequent application in industry and biotechnology.
Keywords: Alkaline Protease, *Bacillus amyloliquefaciens,* Soil.

Introduction

Enzymes are organic substances produced by living cells which catalyse physiologically significant reaction. They have extra ordinary catalytic power, for greater than that of synthetic catalyst. They have high degree of specificity and they accelerate specific biochemical reaction. Proteases are among the most studied protein explaining the bases of enzyme function, proteolytic activity has been involved in the processing and regulation of enzymes, hormones in the blood coagulating processes, fertilization and embryo development. Microbial proteases are divided into acidic, neutral and alkaline proteases on the basis of pH range in which their activity is optimum. Alkaline proteases are the most important group of enzymes which are secreted by both neutrophilic and alkanophilic bacilli represent a major source of commercially produced proteolytic enzymes[1]. Variety of micro organisms such as bacteria, fungi are known to produce these enzymes. Alkaline proteases produced by *Bacillus* spp are most important group of enzymes and find major application in industries. In present study protease enzyme was isolated from *Bacillus amyliquefaciens* from soil source and optimization of the enzyme was studied using various carbon and nitrogen source.

Materials and Methods

Sample Collection: A soil sample was collected in a sterile plastic bag from the agricultural land in Thiruninravur and processed in the Microbiology Laboratory of Jaya College of Arts and Science.

Isolation of Proteolytic Bacterial Species: 1 gm of soil sample was dissolved in 100 ml of saline solution which were serially diluted. Then the samples were plated on casein yeast extract agar by pour plate method from which the organisms were isolated and placed on skimmed milk agar and purified by streaking on Nutrient agar slant and maintained as a pure culture by storing at 4º C.

Identification of Bacterial Strain: The Bacterial strain was identified by doing Gram staining, motility, various biochemical reaction as per Standard procedure.

Production of Proteolytic enzyme: Conical flask containing 250 ml of Nutrient broth was inoculated with a 12 hours old culture and kept on a shaker for 48 hours at 37ºC. After incubation the culture was centrifuged at 10000 xg for 15 mins and cell free supernatant fluid was used as a source of crude enzyme[2,3].

Partial purification of Enzyme: Cell free supernatant fluid was precipitated using solid ammonium sulphate to 60% saturation. The precipitate was collected by centrifugation at 15000 xg for 15 mins and dissolved in 0.2 M Na_2CO_3 and further subjected to dialysis.

Optimization of Protease enzyme:

(a) Effect of pure soluble sugars on protease production : 30 ml of basal media containing different carbon and nitrogen sources was inoculated with 0.5 ml of the enzyme solution keeping the rest of the media the same and incubated at 37º C for 24 hours.

(b) Effect of organic nitrogen sources on production of protease: Peptone was replaced with 1% soluble compounds as Nitrogen source keeping the rest of the media and the activity of enzyme in fermented broth was measured after 24 hours.

(c) Effect of temperature on protease production: Different temperatures such as 10ºC, 20ºC, 30ºC, 40ºC, 50ºC, 60ºC, 70ºC and 80ºC were selected for maximum production of enzyme. The production medium was inoculated with 0.5% of culture of suspension was incubated at selected range of temperatures for 24 hours.

(d) Effect of pH on the protease production: To optimize the pH for obtaining higher activity, pH of the selected media was adjusted in the range of 5-12. Culture suspension of 0.5% amount was inoculated on to 30ml of the media. The production of enzyme was read at 660nm[4,5,6].

Results

Isolation of Proteolytic Bacteria: the organism producing protease enzyme was identified by the presence of zone of clearance around the colonies on casein yeast extract agar.

Identification: The isolated bacterial strain was identified morphologically and gram staining of bacterial isolates results was gram positive bacilli. The physiological and biochemical test were carried out on the isolated strain by the methods described in Bergys manual of determinative bacteriology and Cowan and steels manual for identification of bacteria.

Production and Partial Purification of Protease Enzyme: The isolated organism was cultivated at 37ºC in the production media in the shaker. The pH was maintained at (7-9). The enzyme was produced and purified by ammonium sulphate precipitation and dialysis method.

Optimization of Protease Enzyme

a) **Effect of carbon source on growth of *Bacillus amyloliquefaciens*** – The effect of growth profile of *Bacillus amyloliquefaciens* with different carbon source of 1% concentration. It has been observed that Protease production was highest in raffinose and shown the maximum growth with OD above 3.0 at 24 hours (Table 1).

b) **Effect of nitrogen source on growth of *Bacillus amyloliquefaciens*** - using 1% nitrogen source for growth of protease it has been found that casein exhibited the maximum growth within 24 hours and the Tryptone exhibited less growth (Table 2).

c) **Effect of pH on growth of *Bacillus amyloliquefaciens*** - Growth of isolated bacterial species at different pH range of 5 – 12 has been provided. Maximum growth was in the alkaline pH and optimum pH was found to be in 9.0 (Table 3).

d) **Effect of temperature on growth of *Bacillus amyloliquefaciens*** – The optimum growth was found to be maximum at 40ºC (Table 4)[7].

Table I showing effect of pure soluble sugars on protease activity

SUGARS (1%W/V)	TEST ISOLATE
Sucrose	0.12
Galactose	0.22
Fructose	0.31
Salicin	0.25
Xylose	0.1
Mannose	0.06

Table II showing effect of organic nitrogen source on production of protease activity

NITROGEN SOURCE (1%W/V)	TEST
Peptone	0.20
Meat Extract	0.30
Caesin	0.40
Gelatin	0.25
Yeast Extract	0.14

Table III showing effect of pH on protease activity

pH	TEST
5	0.12
6	0.25
7	0.28
8	0.32
9	0.26
10	0.18
11	0.09
12	0.04

Table IV showing effect of temperature on protease activity

TEMPERATURE ºC	TEST
10 ºC	0.02
20 ºC	0.04
30 ºC	0.08
40 ºC	0.12
50 ºC	0.11
60 ºC	0.06
70 ºC	0.03
80 ºC	0.01

Discussion

Proteases are the hydrolases enzymes that hydrolyse the peptide bond. The term peptidase is used as synonymous with peptide hydrolyase for an enzyme. Proteases are among the most valuable commercial enzyme due to their wide application in the industrial process. Plants,animals and microbial sources are employed for protease production. Microbes serve as a preferred source because of their rapid growth. Proteases from *Bacillus amyloliquefaciens* is most widely used enzyme in the detergent industry owing to its

thermal stability. *Bacillus amyloliquefaciens* strains are non-pathogenic and non-toxigenic. The soil samples were collected and organism was isolated by serial dilution. It is then plated on casein yeast extract agar, the proteolytic activity was significant. On the basis of grams reaction and biochemical tests, the organism was identified as *Bacillus amyloliquefaciens*. Extracellular protease production in micro organism is greatly influenced by pH, temperature, carbon and nitrogen sources. Protease yield from isolated *Bacillus amyloliquefaciens* was high in media containing casein as soul nitrogen source. The results of the protease enzyme were found to be similar[8]. The addition of carbon source in the form of either monosaccharide or polysaccharide may influence the production of enzymes. The isolated *Bacillus* spp gave higher yield of protease in the presence of fructose. The results of Protease enzyme from the *Bacillus* spp were found to be similar[4]. Temperature and pH have profound effect on activity of protease. The activity of Protease enzyme was detected over wide range of assay pH and temperature. The activity of enzyme was optimal when assay was carried out at pH 9.0 and temperature 40ºC[9]. The production of Protease enzyme was profound and the enzyme activity was found to be 27.7µg/L additional work is necessary to enable full characterization of the protease.

References

1. Ramanathan T, Manivannan S and Nagavally D., Alkaline protease production and optimization in estuary isolate of *Aspergillus spp* Asian Journal of Microbiol. Biotech. Env. Sci. 2005, 7,495-500.
2. Cheng S W., Production and Characterization of protease of a feather degrading *Bacillus licheniformis* PWD-1. Biosci, Biotechnol, Biochem 1995, 59, 2239-2243.
3. Hiroshi onish, Tatsuromori, Setsuo Takeuchi, Keiko, Tani, Takekazu Kobayashi and Masahiro Kamekura ., Halophilic Nuclease of a moderately Halophilic Bacillus spp Production, Purification and characterization. Applied and Environmental Microbiology, 1983, 45, 24-30.
4. Dinesh Kumar, Harish Gajju and Tek Chand Bhalla., Production of Thermostable Protease by *Bacillus spp*. Asian Journal of Microbiol. Biotechnology Env. Sci. 2002, 4,533-540.
5. Ellaiah P, AdiNarayana, Rajyalakshmi P, and Srinivasulu.B., Optimisation of Process parameters for alkaline Protease Production under Solid State Fermentation by Alkalophilic *Bacillus spp*. Asian Journal of Microbiol. Biotech. Env. SC. 2003, 5, 49-54.
6. Mehta A, Ferosh S, Singh A, Anjana Sharma and Mehta P., Isolation and Characterization of extracellular protease producing Bacteria. Indian Journal of Microbiology, 2004, 44, 277-280.
7. Adesh Kumar, Archana Sachdev, Balasubramanyan S.D., Saxena A.K. and Lata., Optimisation of conditions for production of Neutral and Alkaline Protease from Species of *Bacillus* and *Pseudomonas*. Indian Journal of Microbiology, 2002, 42, 233-236.
8. Meenu Medan, Santosh Dhillon and Randir Singh., Production of alkaline protease by a UV – mutant of *Bacillus polymyxa*. Indian Journal of Microbiology, 2000, 40, 25-28.
9. Jitender Sharma, Avtar Singh, Rajesh Kumar., Partial purification of an alkaline protease from a new strain of *Aspergillus oryzae* awt 20 and its enhanced stabilization entrapped ca-alginated beads 2006.

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