

ISOLATION AND CHARACTERIZATION OF PROTEASE PRODUCING BACTERIAL FROM SOIL AND ESTIMATION OF PROTEASE BY SPECTROPHOTOMETER

ABSTRACT

About 25 isolates were recovered from different soil samples collected from different field nearer to Ravulapalem village, East Godavari district, Andhra Pradesh, India. On the basis of colony size, texture, and microscopic characteristics, the isolates were categorized into 10 types. Among these, five isolates showed good proteolysis activity, three isolates showed moderate activity, 2 isolates showed poor activity, and eight isolates exhibited very poor activity. Structural, staining and biochemical activity results have revealed that four of five active enzyme producing bacteria are *Bacillus* spp, and one is *Staphylococcus* spp. One Bacteria shown high Enzyme producing activity than the other. Estimation of enzyme producing activity of bacteria isolate was studied by Spectrophotometry. Crude enzyme extract was studied for protein degrade activity and producing amino acid amount was measured by using folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity was expressed in microgram of tyrosine released by 1 ml of enzyme in 30 minutes at 30°C on tyrosine equivalent.

KEY WORDS: Protease enzyme, Bacteria, *Bacillus*, Spectrophotometer.

INTRODUCTION

The present investigation was designed to evaluate the presence of Protease bacteria in soil. A protease (also termed peptidase or proteinase) is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein (1-8). Today, proteases account for approximately 40% of the total enzyme sales in various industrial market sectors (Gupta et al., 2002). The most important applications of protease are used in laundry detergents, leather processing, brewing, food and pharmaceutical industries (Abdel-Naby et al., 1997).

Microbial enzyme production on an industrial scale was initiated by Takamine, in 1890, and started the production of Takadiastase enzyme preparation which was mainly an α -amylase preparation, but it contained a substantial amount of protease (Aunstrup, 1980). Since the initiation of microbial enzyme production, many protease preparations took part in the market. Although, variant microorganisms were used to produce protease, the genus *Bacillus* have being so far the most important group of enzymes produced commercially (Ferrero et al., 1996).

This work was undertaken to obtain a new obligate microorganism which had a well known protease production. Fifteen bacterium strains were isolated and characterized from soil samples that were collected at different regions of Ravulapalem, East Godavari (Dt), A.P. One, of which had the highest photolytic activity, was selected and estimated the proteolytic activity by spectrophotometer.

MATERIALS AND METHOD

Equipment: Spectrophotometer, Centrifuge, Hot Air oven, Autoclave, Incubator, Laminar air flow, Petri plates, pH Meter.

Media used: NAM medium, Skim milk agar media, CMC agar medium, Starch agar medium

Reagents: Protease, Potassium Phosphate, Dibasic, Trihydrate, Casein, Trichloroacetic Acid, Folin & Ciocalteu's Phenol Reagent, Sodium Carbonate, Anhydrous Sodium Acetate, Trihydrate, Calcium Acetate, L-Tyrosine, Congo red.

Isolation and identification

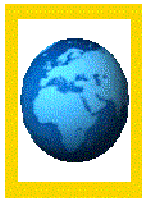
Soil samples were collected from different regions of Ravulapalem, West Godavari (dt), Andhra Pradesh, India and 25 bacterium strains were isolated. Strains were assayed for proteolytic activity and a bacterium strain which had the most Proteolytic activity was selected. The selected strain was identified using biochemical and morphological tests. Bergey's manual of systematic bacteriology was used as reference (Sneath et al., 1986). Gram stain, endospore forming, motility test, catalase test, glucose fermentation and nitrate reduction characteristics were detected. The results showed that the bacterium was a *Bacillus* strain.

Protease assay

Proteolytic activity was carried out according to Casein-Pholine method (Boethling, 1975). Culture media was centrifuged at 7200 rpm for 10 min and supernatant was used as enzyme source. However, 1% casein (in 0.1 M phosphate buffer and pH 7.0) was used as substrate. 1 ml each of enzyme and substrate was incubated at 50°C for 60 min. The reaction was terminated by adding 3 ml of Trichloroacetic acid (TCA). One unit of protease activity was defined as the increase of 0.1 unit optical density at 1 h incubation period. Then it was centrifuged at 5000 rpm for 15 min. From this, 0.5 ml of supernatant was taken, to this 2.5 ml of 0.5M sodium carbonate was added, mixed well and incubated for 20 min. Then it was added with 0.5 ml of folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity was expressed in microgram of tyrosine released by 1 ml of enzyme in 30 minutes at 30°C on tyrosine equivalent.

Cellulose activity:





Amylase activity:



S.NO	ISOLATES	PROTEASE	CELLULASE	GRAMSTAIN	AMYLASE	CATALASE	SPOREFORMATION	ORGANISM
1	S1A	+Ve	+Ve	+Ve	-ve	+ve	-ve	Strpeptococcus
2	S1B	+Ve	+Ve	-ve	+Ve	+ve	-ve	Bacillus
3	S1C	+Ve	+Ve	+Ve	+Ve	+ve	+ve	Cocci
4	S1D	+Ve	+Ve	+Ve	+Ve	+ve	-ve	Bacillus
5	S1E	+Ve	+Ve	-ve	+Ve	+ve	-ve	Streptobacillus
6	S2A	+Ve	+Ve	-ve	+Ve	+ve	-ve	Bacillus
7	S2C	+Ve	+Ve	+Ve	+Ve	+ve	-ve	Streptococcus
8	S2D	-ve	+Ve	+Ve	+Ve	+ve	-ve	Cocci
9	D2E	+Ve	+Ve	-ve	+Ve	+ve	-ve	Cocci
10	S2F	-ve	+Ve	+Ve	+Ve	+ve	+ve	Bacillus
11	S2G	-ve	+Ve	+Ve	+Ve	+ve	-ve	Bacillus

Protease activity



RESULTS

About 25 isolates were recovered from different soil samples collected from different field. On the basis of colony size, texture, and microscopic characteristics, the isolates were categorized into 10 types. Isolated bacterial strains were screened for protease producing ability on skim milk agar. The zone formation around the bacterial colony indicated the protease positive strain which may be due to hydrolysis of casein. Hence the strains were identified as a protease producer and it was taken for further experimental studies and biochemical test. The pictures of zone forming isolates are given below. Among them, five isolates showed good proteolysis activity, three isolates showed moderate activity, 2 isolates showed poor activity, and eight isolates exhibited very poor activity. Structural, staining and biochemical activity results have revealed that four of five active enzyme producing bacteria are Bacillus sps, and one is staphylococcus sps.

One *Bacillus* strain showed high Enzyme producing activity than the other. Estimation of enzyme producing activity of bacteria isolate was studied by Spectrophotometer. Crude enzyme extract was studied for protein degrade activity and producing amino acid amount was measured by using folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity was expressed in microgram of tyrosine released by 1 ml of enzyme in 30 minutes at 300C on tyrosine equivalent. Results of Proteolytic activity tests were given Table 2.

CONCLUSION

Various bacterial isolates from soil were studied for protease producing activity. Proteolytic activity was measured for high enzyme producing strain. Structural, staining and biochemical activity results have revealed it is *Bacillus* sps, The novel *Bacillus* strain has then identify by 16S rDNA phylogenetic analysis.

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