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Research Article

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Isolation and screening of protease producing bacteria from marine waste

S. Sneha, Merina Paul Das* and L. Jeyanthi Rebecca

Department of Industrial Biotechnology, Bharath University, Chennai, India

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ABSTRACT

The aim of present study was to isolate protease producing bacteria from soil samples collected from marine waste. The isolation was done by serial dilution and plating methods. All the isolates were screened for proteolytic activity on skim milk agar plate. A total of 7 from 18 bacteria were screened based on the zone of proteolysis. The maximum proteolytic activity was evaluated by primary and secondary screening methods. The significant isolate was identified using standard identification parameters and selected for further studies.

Keywords: Protease; Screening; Proteolysis; Marine waste.

INTRODUCTION

Protease is one of the most important enzymes known and is of great significance having approximately 60 % of the total industrial enzyme market. Proteases are also known as peptidyl –peptide hydrolases which catalyse the hydrolysis of peptide bond from protein molecule [1]. This proteolytic enzyme constitute one of the most important group of industrial enzymes and in recent years, the use of alkaline proteases in a variety of industrial processes involving detergents, food, leather and silk has increased remarkably [2-5]. This vast diversity of proteases, in contrast to the specificity of their action has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications [6]. To meet the upward trend in demand, studies on cost effective production of industrially important enzymes have become the need of today. Proteases of commercial importance are produced from microbial, animal and plant sources [7], but proteases from microbial sources are preferred to the enzymes from plant and animal sources since they posses almost all characteristics desired for their biotechnological applications [8] and because of their fast growth rate, easy to manipulate for getting highly stable enzymes through genetic engineering and requires shorter time for production and purification steps [6, 9]. Microbial proteases account for approximately 40% of the total worldwide enzyme sales. Microorganisms elaborate a large array of proteases, which are intracellular and/or extracellular. Intracellular proteases are important for various cellular and metabolic processes, such as sporulation and differentiation, protein turnover, maturation of enzymes and hormones and maintenance of the cellular protein pool. Extracellular proteases are important for the hydrolysis of proteins in cell-free environments and enable the cell to absorb and utilize hydrolytic products [10]. In this line, our present study was carried out to isolate, and screening of potent protease producing bacteria from waste material.

EXPERIMENTAL SECTION

Collection of samples

Soil samples were collected from marine waste of different places of Chennai, India. Soils were taken from 2-3 cm depth and kept in sterile plastic bag. To prepare soil suspension, about 1 g of each soil sample was incubated with 5 ml of sterile distilled water for 10min.

Isolation of protease producing bacteria

Protease producer bacteria were isolated by serial dilution and spread plating techniques. An aliquot of 0.1 ml of the serially diluted sample (from 10^{-5} to 10^{-7} dilutions) was transferred to skim milk agar plates using spread plate

method. The plates were maintained at a temperature of 37 ºC for 48 h. The colonies obtained through spread plate method were sub-cultured in nutrient agar slants, in duplicates, incubated at 37 ºC for 24 h and then stored at 4 ºC.

Primary and secondary screening of protease producing bacteria

Primary screening was performed by spot inoculating all the protease producing bacterial isolates on 1 % skim milk agar (SMA) plate using toothpick heads of 2 mm diameter and incubated at room temperature. The zone of clearance due to proteolysis was recorded up to 3 d. The bacterial isolates producing clear zones over 10 mm alone were selected and subjected to secondary screening. Secondary screening was performed with the culture filtrates of the 7 selected bacterial isolates using well diffusion method. All the 7 isolates were grown in Nutrient broth (NB). One ml of each test bacterial inoculums with 0.5 OD was inoculated to 100 ml of medium and incubated at 150 rpm in a rotary shaker at room temperature. After two days of incubation, the cultures were harvested, centrifuged at 10000 rpm for 15 min at 4°C and the supernatant was collected. Skim milk (1%) agar plates were prepared and wells were made using 9 mm sterile cork borer. Culture filtrate of each isolate was placed at 100 µl in each well and incubated at 37°C for 48 h. Then flooded with 10% tannic acid solution and incubated for 24h at room temperature. After the incubation period, isolates capable of producing protease were screened on the bases of clearance zone: colony size (cz/cs) ratio on SMA plates. So the desired colonies were picked and streaked for culturing pure colonies.

Identification of potent strain

Microscopic observation

The bacterial isolates were gram stained and observed under a high power magnifying lens in light microscope. Endospore staining, capsule staining and motility test were performed to observe the morphology and motility of the cells [11].

Biochemical characterization

The bacterial isolates were characterized biochemically by indole test, methyl red test, voges proskauer test, Simmons citrate test, catalase test, oxidase test, urease test, nitrate reduction test, starch hydrolysis test, acid formation from sugars [12].

RESULTS AND DISCUSSION

Isolation and screening of protease producing bacteria

Total nine marine waste samples were collected and processed by serial dilution and spread plate method for the isolation of marine bacteria. A total 18 different bacterial strains were isolated on the basis of zone of proteolysis. All isolates were primarily screened for protease production on skim milk agar plate method. Among 18 isolates, 7 isolates were showing significant inhibitor zone (Table 1). Among these, strain P-5 exhibited maximum zone of proteolysis. This strain was identified and used for further studies.

Table 1. Determination of zone of proteolysis by isolated strains

Isolates	Zone of inhibition (mm)
P-1	14
P-2	21
P-3	15
P-4	12
P-5	29
P-6	11
P-7	11

Identification of the isolates

The maximum protease producing strain was selected depending on the highest clear zone. It was found that the bacterial strain P-5 showed largest zone of clearance of 29 mm and this strain was selected for further use. The isolate showed positive results for catalase, oxidase, voges-proskauer, starch hydrolysis, and negative results for gram staining, methyl red, urease, and acid formation from sucrose. Based on the morphological studies and biochemical test, the selected strain was tentatively identified as *Alcaligenes* sp. P-5 which has the highest protease activity among the all isolates. This identified potent strain was used for future research.

Table. 2 Morphological, and biochemical characteristics of the isolated strain P-5

Characters	Results
[1] Morphology	Rod shaped, gram -ve, aerobic
[2] Motility	+ve
[3] Catalase	+ve
[4] Oxidase	+ve
[5] Citrate utilization	+ve
[6] Voges- Proskauer	+ve
Hydrolysis of starch	+ve
[7] Hydrolysis of casein	+ve
[8] Methyl Red	-ve
[9] Acid formation from glucose	+ve
[10] Hydrolysis of urea	-ve
[11] Acid formation from sucrose	-ve
[12] Formation of indole	-ve
[13] Nitrate reduction	+ve

CONCLUSION

Protease occurs naturally in all organisms and is an essential constituent for all the existing live forms. Microorganisms such as bacteria and fungi and yeast are the main source of protease enzyme. Here *Alcaligenes* sp. P-5 was isolated from marine waste and can be used for protease production as protease has an enormous applications in different industries. The pilot scale production of protease, fermentation methodology to be adopted for maximum production and assay of enzyme kinetics at various steps is still to be studied.

REFERENCES

[1] Pradeep Palsaniya; Rinki Mishra; Neha Beejawat; Sonia Sethi; B. Lal Gupta. *J. Microbiol. Biotech. Res*., **2012**, 2 (6), 858-865.
[2] AA Kembhavi; A Kulkarni; A Pant. *Appl. Biochem. Biotechnol*., **1993**, 38, 83-92.
[3] A Gessesse; BA *Gashe. Biotechnol. Lett*., **1997**,19, 479-481.
[4] CG Kumar; H Takagi. *Biotechnol. Adv*., **1999**, 17, 561-594.
[5] PL Manachini; MG Fortina. *Biotechnol. Lett*., **1998**, 20, 565-568.
[6] MB Rao; TM Aparna; GS Mohini; DV Vasanti. *Microbiol. Mol. Biol.Rev.*, **1998**, 62, 597-635.
[7]PR Patel. Enzyme isolation and purification. In: Biotechnology: Applications and Research. Technomic Publishing Co. Inc., USA. **1985**, 534-564.
[8] R Gupta; QK Beg; P Lorenz. Appl. *Microbiol. Biotechnol*., **2002**, 59, 15-20.
[9] MJ North MJ. *Microbiological Rev*., **1982**, 46, 308-340.
[10]HM Kalisz. *Adv. Biochem. Eng. Biotechnol*., **1988**, 36, 1–65.
[11]K Sharma. Manual of Microbiology: Tools & Techniques, Ane Books, Delhi, India, 1, 141-171.
[12]HAP Sneath; GJ Halt. Bergey's Manual of Systematic Bacteriology. Vol 2 Baltimore, MD: Williams and Wilkins. **1986**.