IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, 2022

ISOLATION, IDENTIFICATION AND PRODUCTION OF PROTEASE ENZYME PRODUCING BACTERIA FROM BUTCHER SHOP MATERIAL

Dr. Jenitha K.

Assistant Professor, Department of Microbiology,
Scott Christian College (Autonomous), Nagercoil – 629 003.
(Affiliated to Manonmaniam Sundaranar University, Abishekapatti,Tirunelveli – 627 012
Tamil Nadu, India.

Abstract

In the present study protease enzyme producing bacteria was isolated from blood swab samples collected from butcher shop material. The isolates were identified by morphological and biochemical characteristization. The bacterial isolates identified were *E.coli* and *Salmonella sp*. These isolates were screened for the production of protease enzyme and then transferred to the production media for enzyme protease production. These enzymes can be extracted further by downstream processing and applied in the industries such as leather, detergent, pharmaceuticals and textiles. The enzymes that are of microbial origin are really cost effective than artificial enzymes that are man-made.

Key words:- Protease, *E.coli*, *Salmonella sp*., Downstream processing, screening, Butcher shop.

INTRODUCTION

Enzymes occur in all living organisms and catalyze bio chemical reactions necessary to support life (Olympska-Beer*et al.*, 2006). A wide array of enzymes are extracted from plant sources; they have many advantages including cost production and stability of products (Hood, 2002). An ample range of sources are used for commercial enzyme production from a broad spectrum of plant species. Non- microbial sources provide a larger proportion of these, at the present time. Microbes are preferred to plants and animals as sources of enzymes because (Hasan *et al.*, 2006).

- They are generally cheaper to produce.
- Their enzyme contents are more predictable and controllable,
- Regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media.

Microbial proteases

Proteases have been successfully produced by researches from different microbial sources. These enzymes have gained interest not only due to their vital role in metabolic activities but also due

ISSN PRINT 2319 1775 Online 2320 7876
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to their immense utilization in industries (Rao *et al.*, 1998; Sandhya *et al.*, 2005; Younes and Rinaudo, 2015). Proteases produced by microbial sources are classified into groups based on their acidic or basic properties. They are also classified based on the presence of functional groups and the position of peptide bond (Panda *et al.*, 2013).

Alkaline proteases: The genus *Bacillus* is vital for commercially important alkaline protease which is active at alkaline pH ranging between 9 and 11. These alkaline protease producers are distributed in water, soil, and highly alkaline conditions.

Acidic Proteases: Acid proteases are stable and active between pH 3.8 and 5.6 and are frequently used in soysauce, protein hydrolysate, and digestive aids and in the production of seasoning material. Acid proteases are also exploited for use in clearing beer and fruit juice, improving texture of flour paste, and tenderizing the fibril musle (Zhang *et al.*, 2010).

Neutral Proteases: Neutral proteases are defined as, such as they are active at a neutral or weakly acidic or weakly alkaline pH. Mostly neutral proteases belong to the genus *Bacillus* and with a relatively low thermo tolerance ranging from pH 5 to 8. They generate less bitterness is hydrolysis of food proteins.

Sources of proteases: Among microbes, *Bacillus* sp. are extensively studied for protease production in a large scale, and they are exploited in various industries like leather, detergent, pharmaceuticals, and textile.

AIM

The aim of the study is to isolate and identify the bacteria that produce protease enzyme from butchershop material.

MATERIALS AND METHODS

Collection of Sample: The blood samples were collected using sterile swaps from butcher shop near Krishnankovil, Nagercoil, Kanyakumari District, TamilNadu. The enzyme producing bacteria was isolated from these samples (Plate 1A, Plate 1B).

Plate 1A. Sample collection site

Plate 1B. Collected swab sample

PLATE 1

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, 2022

Processing of Sample: The collected blood swab samples were directly inoculated into sterile nutrient agar plate by spread plate method. The colonies on the plates were observed after 24-48 hours at 37^0C. The isolated bacterial colonies were selected and subcultured.

Maintenance of isolated colonies BSB1, BSB2: The isolated colonies obtained in the plates are picked up and cultured separately on their respective growth media at specified growth condition for individual pure cultures.

Characterization and Identification of Protease Enzyme Producing Bacteria

The various types of morphological and biochemical tests were carried out for the identification of bacterial isolates according to Bergey's Manual of Determinative bacteriology.

Morphological Characterization: The isolated colonies were tested for their size, shape, occurance, etc. By Gram staining technique and the motility as determined by Hanging drop technique.

Biochemical Characterization namely IMVic, Catalase, TSI, Nitrate reduction test were performed.

Screening of protease enzyme producing bacteria:

The isolated colonies were streaked on the skimmed milk agar plate. The plates were incubated at 37^0C for 24 hrs. After incubation period the skimmed milk agar plates were observed for zone of clearance around the colonies.

Protease enzyme production:

The protease enzyme producing bacteria were inoculated into 100ml of peptone broth and incubated at 37°C for 72 hours .After proper incubation, the media was observed for production of protease enzyme.

RESULTS

Isolation and Identification of protease enzyme producing bacteria

The identification of protease enzyme producing bacteria was done by Gram's staining, motility and biochemical characterization (Table 1, Table 2, Table 3, Table 4, Plate 1A, Plate 1B).

ISSN PRINT 2319 1775 Online 2320 7876
Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, 2022

Table 1. Gram's staining of isolated protease enzyme producing bacteria

Isolates	Gram's Staining
BSB1	Gram Negative rods
BSB2	Gram Negative rods

Key : BSB1 Blood swab bacteria 1, Blood swab bacteria 2

- Gram's staining technique was performed for the isolated culture BSB1,it showed the presence of Gram negative rods .
- Gram's staining technique was performed for the isolated culture BSB2 ,it showed the presence of Gram negative rods .

Table 2. Hanging drop technique performed for isolated protease enzyme producing Bacteria

Isolates	Results
BSB1	Motile
BSB2	Motile

Key : BSB1 Blood swab bacteria 1, Blood swab bacteria 2

- Hanging drop technique was performed for the isolated culture BSBI ,it showed motility along the border line of the droplet culture.
- Hanging drop technique was performed for the isolated culture BSB2 ,it showed motility along the border line of the droplet culture.

Table 3. Biochemical characterization of protease enzyme producing bacteria BSB1

Biochemical Test	Results
Inodle test	Negative
Methyl Red test	Positive
Vogesproskauer Test	Negative
Citrate Utilization Test	Negative
Triple sugar Iron Agar Test	Alkaline slant/ Acid butt
Nitrate Reduction Test	Positive

- The isolated culture BSB1 showed positive result for the biochemical tests namely MethylRed test, Triple sugar iron agar test, Nitrate reduction test.
- In MethylRed test ,red colour formation was observed on addition of methylred indicator solution.

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- In Triple sugar iron agar test showed the presence of Alkaline slant /Acid butt which indicates H_2S production .
- All the above observations confirms that the isolated culture BSB1 is *Escherichia coli.*

Table 4. Biochemical characterization of protease enzyme producing bacteria BSB2

Biochemical Test	Results
Indole test	Positive
Methyl Red	Positive
Vogesproskauer Test	Negative
Citrate Utilization Test	Negative
Nitrate reduction	Positive

- The isolated culture BSB2 showed positive result for the biochemical tests namely Indole test ,MethylRed test, nitrate reduction test.
- In Indole test ,Cherry red ring was showed on addition of Kovac's reagent .
- In MethylRed test, red colour formation was observed on addition of methylred indicator solution.
- In Nitrate reduction test red colour formation was observed on addition of Sulphanilic acid and Alpha napthalamine solution.
- All the above observations confirms that the isolated culture BSB2 is *Salmonella* sp.

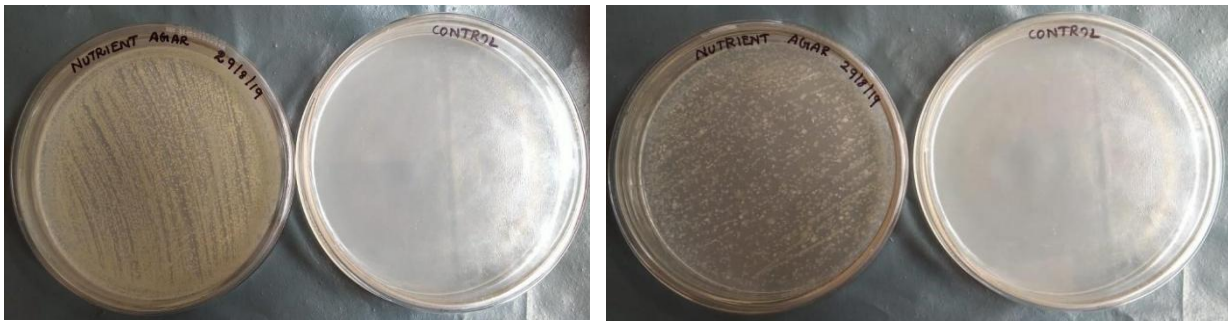

Plate 1A **Plate 1B**

PLATE 1. ISOLATION OF PROTEASE ENZYME PRODUCING BACTERIA FROM BUTCHER SHOP MATERIAL

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 9, 2022

Screening of protease enzyme producing bacteria

After incubation the skim milk agar plates showed clear zones around the line of streaks (Plate 2A, Plate 2B).

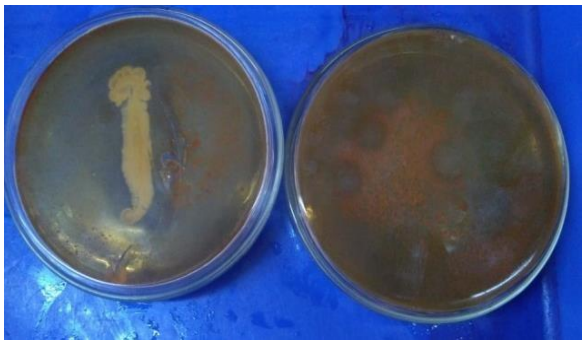

Plate 2A. Proteolytic activity of BSB1

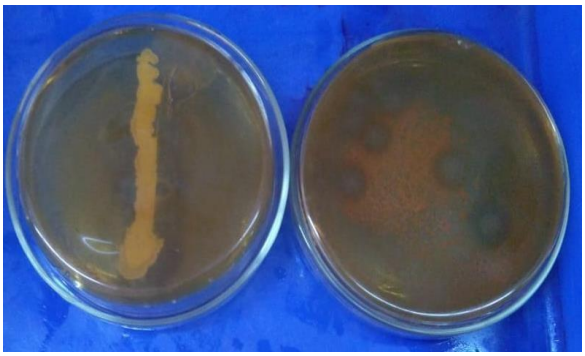

Plate 2B. Proteolytic activity of BSB2

PLATE 2. SCREENING OF PROTEASE ENZYME PRODUCING BACTERIA

Protease Enzyme production

The enzyme of produced by enzyme producing bacteria by using peptone broth media. After incubation turbidity showed the production of protease enzyme (Plate 3A, Plate 3B).

Plate 3A. Protease enzyme producing broth of BSB1

Plate 3B. Protease enzyme producing broth of BSB2

PLATE 3 PROTEASE ENZYME PRODUCTION

DISCUSSION

In the bacterial isolates were found to be *E.coli* and *Salmonella* sp. Protease enzyme produced by *Salmonella*sp was screened using skim milk agar medium. In situ protease production from the potential isolates were identified by the clearing of opaque milk proteins in the area surrounding colonies growing on the surface (Nehra *et al*., 2004).

CONCLUSION

The current study is a biological approach for producing proteases for application on Industries which will be economical and replace the noxious chemicals of the day with teh environmental friendly products for the betterment of life on the planet.

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