rebioin journal
OF BIOSCIENCES

Extraction of enzymes from Protease-Producing Bacteria.

admin@reboin.com www.rebioin.com

Extraction of enzymes from Protease-Producing Bacteria.

*Tanuja Bisht[1]

[1] Department of Microbiology,

Sai Institute of Paramedical & Allied Sciences,

Dehradun

*Corresponding Author's Email- tanubisht228@gmail.com

Abstract

Protease enzymes are widely used in industries such as food processing, pharmaceuticals, detergent industry and biotechnology. This study focused on extracting protease enzymes from protease-producing bacteria. This study looks at how enzymes from bacteria, *Staphylococcus aureus* and *Klebsiella*, can help make detergents better at removing stains. Bacterial strains were isolated from environmental samples and screened for protease production using specific media. The most efficient strain was selected for enzyme extraction. The protease enzyme was then obtained through centrifugation and filtration of the bacterial culture. The extracted enzyme was analysed for its activity and stability under different conditions. The results showed significant protease activity, indicating its potential for industrial applications. By adding natural enzymes from microbes into cleaning products, the industry can create more eco-friendly and effective cleaning solutions.

Keywords- Protease-Producing Bacteria, Detergent Industry, Stain removal, Microbes, Staphylococcus aureus, Klebsiella pneumonia

1. INTRODUCTION

Microbes, often referred to as microorganisms, are tiny life forms that can be found in nearly every corner of our planet. They display an astonishing variety and encompass a wide array of organisms, including bacteria, archaea, fungi, protists and viruses. [1]

Within ecosystems, these microorganisms play vital roles such as aiding in nutrient cycling, assisting in decomposition processes and forming symbiotic relationships.

Microbes are found everywhere in nature and play a crucial role in the overall health of holobionts. They are considered an abundant source of highly diverse and versatile enzymes. The increasing demand for industrial enzymes, driven by the necessity for sustainable solution to environmental challenges, has amplified the need for these microbial enzymes. They are employed in various sectors, including food and feed processing, polymer production, pharmaceutical manufacturing, detergent and paper production, textile and biofuel refinement. [2]

Detergent manufacturers are increasingly embracing the use of enzymes that are compatible with their products, marking a growing trend in the detergent industry. These enzymes include cellulases, lipases, proteases, and amylases, which are commonly employed in detergent formulations. [3]

Most of the Detergent industry used microbial enzyme. Proteases are the most commonly used enzymes in industry. Important bacteria that produce these enzymes include *Pseudomonas* species, *Bacillus* species, *Staphylococcus*, and *Klebsiella pneumoniae*. [4]

2. MATERIALS AND METHODS

2.1 Collection of Sample and serial dilution- 1 gram of Soil sample is collected from the sewage area. It was then weighed aseptically into 100 mi of sterile distilled water. The samples were then serially diluted in 5 different test tubes. [5]

2.2 Isolation of Bacteria- The bacteria were isolated and cultured on NAM (Nutrient Agar Medium) plates using the streaking methods. The cultures were then incubated at 37 °C for 24 Hours. [6]

2.3 Characterization of bacteria

2.3 a) Gram staining- Gram staining of the bacterial isolates revealed the presence of both Gram-positive and Gram-negative bacteria. The Gram-positive bacteria appeared as purple and the Gram-negative bacteria appeared as pink-coloured. [7-10]

2.3 b) Microscopic Examination- The stainless stained samples were observed under a microscope to characterize the bacteria based on their morphology [11]. The Gram-positive bacteria appeared as purple, spherical cocci arranged in clusters, characteristic of Staphylococcus aureus the Gram-negative bacteria were pink-stained, rod-shaped cells with a mucoid appearance, consistent with Klebsiella spp. These findings confirm the distinct morphological and staining characteristics of the two bacterial species (Fig.1 and Fig.2).

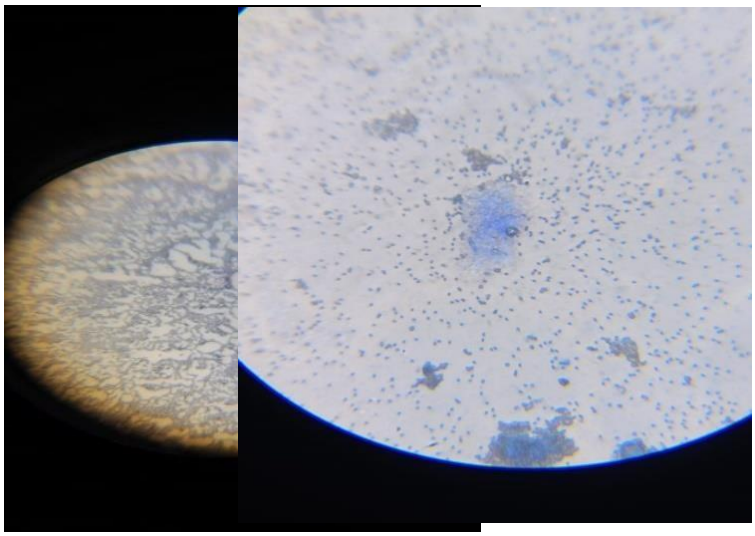

Fig.1 Showing staining is Staphylococcus aureus

Fig.2 Showing staining is Klebsiella pneumonia

2.4 Biochemical Testing- Standard biochemical tests were performed on the isolated bacteria to identify their metabolic and enzymatic properties. [12-17]

Table no. 1 Biochemical test describe the characterization of isolated bacteria.

S.no.	Biochemical Test	S. aureus Results	Klebsiella Results
1.	Catalase Test	Negative	Negative
2.	Sugar Test: • Dextrose • Sucrose • Maltose • D-Mannitol	 Positive Positive Positive Positive	 Positive Positive Negative Positive

3.	Citrate Test	Negative	Negative
4.	Nitrate Test	Positive	Negative
5.	Urease Test	Negative	Negative
6.	H_2S	Positive	Positive
7.	MR Test	Positive	Positive
8.	MR-VP Test	Negative	Negative
9.	Indole Test	Positive	Positive
10.	Amylase	Positive	Positive
11.	Protease	Positive	Positive

3 Enzyme Extraction- The extraction of crude enzymes is performed for the study of the use of S. aureus and Klebsiella Bacteria in the Detergent Industry. This extraction contains a mixture of enzymes released from bacterial cells. While the extraction method is relatively simple and less time consuming compared to more advanced purification methods, it may yield a mixture of various enzymes and other cellular components. As a result, the crude enzyme preparation may not be as pure as highly purified enzymes obtained through specific purification techniques. [18-20]

The crude enzyme extract can be used for various research applications, such as enzyme characterization, enzyme activity assays, and its various use in different industries.

Evaluation of Stain Removal Abilities and Assessment of Detergent Industry Applications.

3.1 a) Stain Removal Test- To test how effective the proteases from *Staphylococcus aureus* and *Klebsiella pneumoniae* are at removing stains, we compared their performance using three different washing solutions. [21-24]

We check their compatibility test to remove stain from Detergent, S. aureus and Klebsiella.

Requirements: In the stain removal test we used Ink, 3 piece of Fabric , 3 Jar , Detergent , Klebsiella enzyme extract , Staphylococcus enzyme extract ,Water.

Procedure:

- Take Clean fabric and cut it three pieces.
- Apply a single drop of ink to each of the fabric samples.
- Allow the fabric samples to dry completely.
- Take three jars. In one jar, prepare a mixture of water and detergent. In the second jar, mix water with *Staphylococcus aureus* culture. In the third jar, combine water with *Klebsiella pneumoniae* culture.
- Immerse the inked fabric samples in the respective solutions (detergent-water, *Staphylococcus aureus*-water, and *Klebsiella pneumoniae*-water) for 5 minutes to facilitate thorough exposure.
- Subsequently, rinse the samples with clean water and allow them to air dry completely.

Before

Fig. 3

After

Fig. 4

1. In compatibility Test first one is Detergent test clean stain after 2-3 wash but after the 2-3 wash that is not clean properly. It leaves small spots.
2. Second test is Staphylococcus aureus and Detergent clean stain after 2 washes.
3. Third test is Klebsiella and Detergent clean stain in 1st wash.

4. Discussion and conclusion

The extraction of protease enzymes from bacteria is essential for their application in the detergent industry, where they serve as key additives for improving stain removal and fabric care. This method proved effective in concentrating active protease enzymes while maintaining their stability and efficiency, which are crucial for detergent formulations.

The choice of bacterial strain significantly influenced enzyme yield, with some strains demonstrating superior protease production under optimized conditions. Additionally, environmental factors such as pH and temperature played a critical role in enzyme activity. The extracted protease exhibited optimal activity in alkaline conditions and demonstrated stability across a broad temperature range, aligning with the requirements for detergent applications. These properties ensure that the enzyme remains active in washing environments, even under varying conditions.

Future research should focus on optimizing the purification process and exploring genetic and metabolic engineering approaches to enhance enzyme yield. Genetic modifications could improve bacterial strains' protease production capacity, making them more efficient and cost-effective for large-scale detergent manufacturing. Additionally, studies on enzyme immobilization techniques may further enhance protease stability and reusability, making them more suitable for repeated wash cycles.

This study highlights the potential of bacterial proteases in the detergent industry and their significance in improving cleaning efficiency. The findings underscore the importance of optimizing extraction and purification techniques to maximize enzyme performance in detergent formulations. With continued research and technological advancements, bacterial proteases can be further developed to create more sustainable and effective detergent products.

Acknowledgment

I would like to express my sincere gratitude to Dr. Manjit Kaur, Senior Scientist at Rapture Biotech, Dehradun, for their invaluable guidance, encouragement, and constructive feedback throughout t this study. Their insights were instrumental in shaping the direction of this research.

I am grateful to my colleagues and peers, for their insightful discussions and technical assistance.

REFERENCES

1. Fasiku SA, Ogunsola OF, et al. Isolation of bacteria with potential of producing extracellular enzyme (amylase, cellulase, and protease) from soil sample. J Adv Microbiol. 2020;20(3):21-26.
2. Thapa S, et al. Biochemical characteristics of microbial enzyme and their significance from industrial perspectives. Mol Biotechnol. 2019;61:579-601.
3. MacWilliams MP. Citrate test protocol. Am Soc Microbiol. 2016.
4. Saha RN, Wissink EM, Bailey ER, Zhao M, Fargo DC, Hwang JY, Daigle KR, Fenn JD, Adelman K, Dudek SM. Rapid activity-induced transcription of Arc and other IEGs relies on poised RNA polymerase II. Nat Neurosci. 2011;14(7):848-856. doi:10.1038/nn.2839.
5. Akita H, Matsushika A, Kimura Z. Enterobacter oligotrophica sp. nov., a novel oligotroph isolated from leaf soil. Microbiol Open. 2019;8:e843.
6. Tripathi N, Sapra A. Gram staining. StatPearls Publishing; Treasure Island, FL; 2020.

7. Facklam R, Elliott JA. Identification, classification and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. ASM J. 1995;8(4):1-10.
8. Facklam R, Elliott JA. Identification, classification and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. ASM J. 1995;8(4):1-10.
9. Bartholomew JW, Finkelstein H. Relationship of cell wall staining to gram differentiation. J Bacteriol. 1958;75:77-84.
10. McClelland R. Gram's stain: The key to microbiology. MLO Med Lab Obs. 2001;33:20-22, 25.
11. Barenfanger J, Drake CA. Interpretation of Gram stains for the non-microbiologist. Lab Med. 2001;32:7.
12. Holt RD, Lowton JH. The ecological consequences of shared natural enemies. Annu Rev Ecol Syst. 1994;25:495-520.
13. MacWilliams MP. Citrate test protocol. Am Soc Microbiol. 2016.
14. Biochemical tests for identification of medical bacteria. J Clin Pathol. 1981;34(5):572.
15. Hemraj V, et al. A review on commonly used biochemical test for bacteria. Innovare J Life Sci. 2013;1(1):1-10.
16. Chapman, et al. Food research. 1937;2:349-367.
17. Dutta JR, Dutta PK, Banerjee R. Isolation and characterization of a newly isolated Bacillus strain for alkaline protease production and optimization of its enzyme production. Biotechnol Bioeng. 2004;88(3):324-336.
18. Maurer KH. Detergent proteases. Curr Opin Biotechnol. 2004;15(4):330-334.
19. Karray A, Ellouz-Chaabouni S, Fakhfakh N. Proteases from extremophiles and their potential industrial applications. Extremophiles. 2018;22(2):157-169.
20. Cavello I, Hours R, Cavalitto S. Biotechnological applications of proteases in the textile industry. Adv Enzyme Res. 2013;1(2):41-49.
21. Zhao L, Wang Y. Recent developments in enzyme-based stain removal strategies. Biotechnol Adv. 2017;35(3):243-254.
22. Singla R, Singh S. Bioengineering of proteases for detergent applications: A review. Bioprocess Biosyst Eng. 2018;41(6):737-749.
23. Saad R, Hassan H. Comparative analysis of microbial proteases in detergent industry applications. Int J Biochem Mol Biol. 2016;7(4):67-79.
24. Rai S, Saxena R. Enzyme-based detergents and their role in sustainable cleaning applications. Sustainable Chem J. 2015;2(3):129-145.

rebioin journal
OF BIOSCIENCES

Unlocking Enzymes: Harnessing Power from Protease-Producing Bacteria

Plot no 977, GMS Road, near Balliwala Flyover, opposite Cubic Plaza,
Dehradun, Uttarakhand 248001

admin@reboin.com

www.rebioin.com