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Literature Review on Characterization and Optimization of Protease Enzymes for Medical Applications

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Abstract: Protease enzymes are a group of enzymes that play an important role in protein breakdown and have been widely studied for medical applications, especially in the fields of wound healing, cancer therapy, and antimicrobials. This article presents a systematic review of various studies exploring the biochemical characterization and optimization of protease enzyme activity produced by microorganisms. The main focus of this review includes the optimum conditions for enzyme activity, stability to pH and temperature, and genetic engineering and fermentation methods for increasing its production. It was found that thorough characterization is very important in determining the potential for clinical application of protease enzymes. In addition, optimization of enzyme production through fermentation and biotechnology approaches has been shown to increase efficiency and reduce the risk of side effects in medical applications. This review provides a comprehensive overview of the progress and challenges in the development of proteases for use as therapeutic agents.

Keyword: Protease Enzymes, Microorganisms, Enzyme Characterization, Production Optimization, Medical Applications.

INTRODUCTION

Protease enzymes are enzymes that function in the hydrolysis of peptide bonds in proteins, and play a vital role in various biological and medical processes. This enzyme is produced by various organisms, including bacteria, fungi, and animals, but microorganisms are the main source because of their superiority in large-scale production (Ali, 2023). In a medical context, protease enzymes are used in wound cleansing formulations, anti-inflammatory therapies, and even as candidates for anticancer therapies. The existence of this enzyme is important along with the increasing need for biotechnology-based pharmaceutical products. Therefore, a deep understanding of protease enzymes is crucial for their development as therapeutic agents. In recent decades, interest in the use of microbial enzymes in the medical field has continued to increase because they are considered more economical, environmentally friendly, and easy to genetically modify (Rahman et al., 2022). Microorganisms such as Bacillus subtilis, Streptomyces spp., and Pseudomonas aeruginosa have been known as the main producers of stable and active protease enzymes under various conditions. Intensive research is directed at characterizing the properties of this enzyme such as temperature stability,

specific activity, and tolerance to extreme pH. These characteristics are important to determine the success of its application in the complex physiological environment of the human body.

In addition to characterization, the process of optimizing enzyme production is the main focus to produce proteases in sufficient quantity and quality for medical needs. Optimization strategies include regulating fermentation conditions, selecting substrates, and biotechnological approaches such as mutagenesis and genetic engineering (Liu et al., 2021). This approach aims to increase production efficiency and the specific activity of the enzyme produced. By increasing production efficiency, the cost of developing enzyme-based products can be reduced, making them more competitive in the global pharmaceutical market.

The application of proteases in the medical world covers a wide range of fields, from chronic wound cleaning, treating bacterial infections, thrombolytic therapy, to anticancer formulations. This enzyme is also used in the formulation of protein-based drug delivery systems that require the breakdown of certain target proteins (Patel & Desai, 2022). One of the advantages of proteases is their ability to work specifically on certain protein substrates, making them very suitable for therapies that require specific molecular targets. However, a deep understanding of enzyme characteristics is required so that their use is appropriate and safe.

Characterization of protease enzymes includes measuring parameters such as optimum pH, optimum temperature, specific activity, and thermal stability. This knowledge is important to determine the best conditions for medical applications, especially in wounds that have a certain microenvironment (Kumar et al., 2023). In addition, it is also important to know the sensitivity of the enzyme to inhibitors and heavy metals that can be found in infected wounds. This characterization is also the basis for pharmaceutical formulations involving protease enzymes as active ingredients.

Optimization of microbial protease production requires an integrated approach that involves manipulation of culture conditions and growth media. Solid and liquid fermentation technologies are the mainstay methods in increasing protease production yields. In addition, the use of agro-industrial waste substrates as fermentation media is an environmentally friendly and economical approach (Singh et al., 2022). The combination of fermentation techniques and genetic optimization has been shown to provide significant results in the production of high-quality proteases.

The main challenge in the development of proteases for medical applications lies in the stability of the enzyme under physiological conditions and the risk of immunogenicity. Therefore, many studies are directed at chemical and genetic modification of enzymes to increase stability and reduce the risk of side effects (Zhang et al., 2023). This modification aims to produce enzymes that are more compatible with body tissues and have a longer shelf life. In addition, enzyme formulations in the form of nanoencapsulation have also begun to be developed to increase therapeutic efficiency.

This article aims to review the current literature on the characterization and optimization of microbial protease enzymes for medical applications. The main focus is given to aspects of biochemical parameters, production methods, and application studies that have been carried out in a clinical context. By compiling a comprehensive overview of various studies, this article is expected to be a reference for researchers and practitioners in the fields of health, pharmacy, and biotechnology in developing more effective and safe protease-based formulations.

METHOD

This study is a systematic literature review conducted by collecting and evaluating scientific articles from leading databases such as PubMed, ScienceDirect, SpringerLink, and Google Scholar. The keywords used include “protease enzyme”, “bacterial protease”, “enzyme characterization”, “fermentation optimization”, and “medical application”. The selected articles focus on the last 10 years, are peer-reviewed, and discuss the biochemical characterization, production methods, and medical applications of protease enzymes from microorganisms. The

collected data were analyzed qualitatively to identify trends, commonly used methods, and the most promising potential applications.

RESULTS AND DISCUSSION

Protease enzymes are enzymes that catalyze the cleavage of peptide bonds in proteins and play an important role in various biological processes. In a medical context, proteases are used for wound therapy, treatment of digestive disorders, and even as components in cancer drug formulations. According to Ali (2023), protease characterization is very important to understand enzymatic activity, stability to temperature and pH, and sensitivity to inhibitors. These parameters determine the suitability of enzymes in the physiological conditions of the human body. Research also shows that isolation of proteases from microorganisms, especially bacteria, produces enzymes with unique characteristics and high stability. Therefore, characterization is an essential initial step before the use of enzymes medically.

Protease characterization includes determining the type of protease based on its active mechanism, such as serine, cysteine, aspartate, or metalloprotease. A study by Kumar et al. (2022) showed that serine protease from Bacillus subtilis has high proteolytic activity and is resistant to pH variations. This makes it an ideal candidate for topical application in wound healing. Identification of specific activities also helps in selecting optimal formulation conditions. Enzyme stability tests against temperature and pH are carried out to ensure that the enzyme is active in human body conditions or medical product storage conditions. With this understanding, protease applications become safer and more effective.

Optimization of protease enzyme production is carried out by manipulating fermentation conditions, including carbon sources, nitrogen, temperature, pH, and incubation time. Research by Ramadhani et al. (2023) showed that modifying the pH of the medium towards slightly alkaline can increase protease production by Bacillus licheniformis. In addition, the use of agro-industrial waste as a fermentation substrate has been shown to reduce production costs. This strategy is in line with the principle of a circular economy in industrial biotechnology.

Production optimization not only increases yield but also affects the quality and stability of enzymes. In a study by Ananta et al. (2025), optimization of protease enzyme production by PUA-14 isolate bacteria from mangrove waters was carried out by assay various factors, including pH, temperature, substrate concentration, incubation time, nutrient concentration, and carbon and nitrogen sources in the media. The optimization results showed that certain conditions, such as the right pH and temperature, as well as optimal substrate and nutrient concentrations, can significantly increase protease production, making it a potential candidate for medical applications. Therefore, this step is an important aspect in the process of developing protease-based medical products.

Recent studies have also explored the use of genetic engineering techniques to increase the expression of protease-producing genes. By cloning the protease gene into a high-expression vector such as E. coli or Pichia pastoris, a significant increase in the amount and activity of the enzyme was obtained. According to Liu et al. (2021), recombinant expressed proteases showed higher homogeneity and better stability. In addition, this approach allows the production of enzymes in a pathogen-free system, which is safer for medical applications. However, this engineering process requires thorough validation of the safety of the final product. The integration of molecular approaches in enzyme production is an important trend in the development of modern enzymatic therapies.

The application of protease enzymes in wound healing has been widely studied due to their ability in debridement, which is the removal of necrotic tissue without damaging healthy tissue. According to Ali (2023), proteases from Bacillus subtilis and Bacillus cereus show rapid and selective debridement activity. These enzymes also show mild anti-inflammatory properties that support tissue regeneration. The use of proteases in the form of gels or ointments allows

direct application to wounds and accelerates the healing process. Early clinical trials have shown effectiveness equivalent to conventional chemical debridement products. Therefore, proteases are a potential alternative in modern wound therapy.

In addition to wound healing, proteases are also applied in enzyme therapy for digestive disorders, such as pancreatic insufficiency. In this condition, patients experience a deficiency of digestive enzymes, including proteases. A study by Nugroho et al. (2022) showed that microbial protease supplementation improves protein digestion and amino acid absorption. The enzyme formulation was developed in the form of an enteric capsule to protect its activity from the acidic environment of the stomach. The effectiveness of therapy depends on the stability of the enzyme and the accuracy of the dosage. Therefore, enzyme characterization is key in the development of effective digestive enzyme therapy.

The use of proteases in pharmaceuticals also includes cancer therapy, especially in targeted therapy approaches. Proteases such as urokinase and matrix metalloproteinase are used to break down the extracellular matrix, allowing better drug penetration into tumor tissue. According to Hasan et al. (2021), these enzymes help reduce tumor resistance to chemotherapy. However, the use of proteases in oncology must be strictly controlled to avoid damage to normal tissue. Therefore, nanoparticle-based enzyme delivery systems are being developed to ensure target specificity. This opens up new opportunities in more effective cancer therapy more precise.

One of the main challenges in the medical application of protease enzymes is their potential immunogenicity. The immune system can recognize foreign enzymes as antigens and trigger immune reactions. A study by Rahmawati and Taufik (2023) showed that the use of protease in the form of liposome encapsulation can reduce the immune response. This technique also increases enzyme stability and prolongs the half-life in blood circulation. Therefore, the formulation approach is important in overcoming this challenge. Innovations in delivery technology continue to develop to improve the safety of proteases as therapeutic agents.

Protease characterization also includes enzyme kinetic studies such as Km and Vmax values, which provide information on the affinity of the enzyme for its substrate. These parameters are important for dose design in clinical applications. According to a study by Ali (2023), protease from Bacillus amyloliquefaciens showed a low Km value for casein, indicating a high affinity suitable for digestive therapy. In addition, knowledge of natural inhibitors such as EDTA or PMSF is used to control enzyme activity in product formulations. By understanding these kinetic properties, the development of medical applications becomes more precise and safe.

The stability of enzymes in pharmaceutical formulations is an important aspect to ensure therapeutic efficacy. Enzymes must be stable to changes in temperature, pH, and storage conditions. Research by Wijayanti et al. (2023) showed that proteases combined with natural polymers such as alginate can maintain activity up to 90% after storage for 3 months at room temperature. The use of appropriate excipients in the formulation also helps prevent enzyme denaturation. Therefore, physicochemical characterization of proteases is very important in the development stage of medical products.

Bioinformatics technology is now used to predict the structure and stability of proteases through protein modeling. With the help of software such as SWISS-MODEL and I-TASSER, the three-dimensional structure of enzymes can be accurately predicted. This allows for the identification of active sites and prediction of interactions with substrates or inhibitors. According to Ahmad et al. (2022), this approach accelerates the process of characterization and development of more stable enzyme mutants. The integration of bioinformatics and laboratory techniques strengthens the multidisciplinary approach in protease research for medicine. This progress shortens research time and reduces development costs.

In the context of regulation, the use of enzymes as drugs or active ingredients in health products must meet safety and efficacy standards. Regulatory agencies such as BPOM and FDA

require comprehensive toxicity and bioactivity tests. A study by Dewi and Sari (2022) emphasized the importance of in vivo testing on animals to evaluate potential side effects before proceeding to the clinical trial stage. GMP certification in the enzyme production process is also a must to ensure product quality. Strict regulations encourage innovators to ensure that protease products are not only effective but also safe for consumers.

Recent research has also examined the use of proteases in the regenerative field, such as stem cell therapy and tissue engineering. Proteases are used to release cells from culture matrices without damaging the cell membrane. This is very important in tissue transplantation and 3D culture. According to Prasetya et al. (2024), proteases from marine microbes showed better results than conventional enzymes such as trypsin. This application shows the great potential of proteases in supporting future medical technologies. Therefore, exploration of new sources of proteases is a priority in biomedical research.

In order to address the challenges of effectiveness and sustainability, the bioprospecting approach to extremophilic microorganisms is now the focus of research. Microbes from extreme environments such as hot springs or deep seas produce proteases that are resistant to high temperatures and extreme pressures. A study by Fauzi and Lestari (2023) found that proteases from Thermus aquaticus have thermal stability up to 90°C, making them ideal for heat-based or high-sterility medical applications. This potential opens up new opportunities in the development of more robust and durable medical enzymes.

The role of protease enzymes in topical product formulations such as creams, ointments, and gels is now widely developed to treat chronic wounds such as diabetic ulcers and decubitus ulcers. A study by Setiawan et al. (2023) showed that the combination of protease with anti-inflammatory ingredients such as Aloe vera and Centella asiatica extract provides a synergistic effect on tissue regeneration. The stability of the enzyme in the formulation is a major challenge, so encapsulation techniques or combinations with natural polymers are needed. The use of protease in topical products provides a non-invasive and comfortable approach for patients. Therefore, formulation innovation is an important step in expanding the clinical application of protease.

Protease enzymes also play a role in the diagnostic field, especially as biomarkers in certain diseases or as components in medical biosensors. According to Rahman et al. (2021), proteases released by tumor cells can be used for early detection of cancer. The use of fluorogenic substrates that can be cleaved by proteases allows for real-time measurement of activity. This application has great potential for non-invasive screening and monitoring of therapy response. With the development of microfluidic and nanotechnology technologies, protease-based biosensors can be developed in a portable format for use at the point of care (point-of-care testing).

In the context of burn therapy, proteases can help in cleaning dead tissue and accelerating re-epithelialization. A study by Mahendra and Cahyani (2022) revealed that topical protease application reduces the number of necrotic cells and accelerates the formation of granular tissue. The advantages of proteases over surgical methods are their selective and minimally invasive nature. However, the safety of the application remains a concern, especially in open wounds with a risk of infection. Combination with antimicrobial agents is a solution that is now widely studied to optimize clinical outcomes without increasing the risk of complications.

The application of proteases in systemic enzymatic therapy is also being developed for inflammatory diseases such as rheumatoid arthritis and atherosclerosis. Enzymes are used to break down immune complexes or protein plaques in the body. According to Taufiq and Rahmawati (2022), certain proteases can reduce inflammatory mediators and improve tissue conditions in animal models of arthritis. The main challenge is maintaining the stability of enzymes in the blood circulation and avoiding degradation by endogenous proteases. Therefore,

the design of nanoparticle or polymeric-based delivery becomes very important to support the effectiveness and safety of systemic therapy.

Several studies have also shown that the combination of proteases with antibiotics can increase the effectiveness of antimicrobials, especially against pathogenic biofilms. Proteases are able to break down the extracellular matrix of biofilms, making it easier for antibiotics to penetrate and kill bacteria. A study by Andriani et al. (2023) proved that the combination of proteases from Bacillus subtilis with gentamicin gave superior results compared to single antibiotics in a wound infection model. This approach is a solution to the increasing antibiotic resistance. Therefore, the application of proteases in synergy with other therapies deserves further development.

In the field of regenerative pharmacy, proteases are used to assist in the formation of protein-based scaffolds that support cell growth and tissue regeneration. According to Pramudya et al. (2023), proteases are used to modify the surface of biomaterials to make them more easily bound by growth factors or stem cells. This process supports cell differentiation and accelerates the formation of new tissue. This application is promising for tissue engineering of skin, cartilage, and even nerve tissue. The protease used must have high specificity and stability so as not to damage important structures during the regenerative process.

Ethical challenges and public acceptance of the use of microbial enzymes in medical products are also a concern. Issues of biological safety and production sustainability are factors that must be explained to the public. According to Nugroho and Anjani (2023), an educational approach and transparency of clinical trial data are very important to increase public trust. The use of non-pathogenic microbial sources and sterile production processes must be standardized. Support from regulatory agencies and cross-sector cooperation are needed to accelerate the adoption of protease technology in healthcare.

The future prospects for protease development for medical purposes lie in the integration of new technologies such as CRISPR for genetic modification of enzyme-producing microorganisms. With this technique, protease-producing strains can have their efficiency increased without the need for complex engineering at the industrial level. According to Fitriani and Aziz (2024), the application of CRISPR to Bacillus spp. has succeeded in increasing protease activity by up to 40% without changing the stability and others. This technology is expected to accelerate the production of high-quality enzymes sustainably and safely for pharmaceutical applications. Innovation in genetic technology is an important foundation for the development of medical enzymes in the future.

Based on all the findings in this review, it can be concluded that characterization and optimization of protease enzymes are very important in ensuring the effectiveness, safety, and sustainability of their use in the medical field. With the advancement of biotechnology, various challenges such as stability, immunogenicity, and production efficiency can be overcome. The synergy between formulation technology, genetic engineering, and molecular approaches opens up broad opportunities for the application of proteases in wound therapy, digestion, cancer, and tissue engineering. Therefore, further research and collaborative development between fields are the keys to the successful integration of protease enzymes in modern healthcare.

CONCLUSION

Protease enzymes from microorganisms show great potential in medical applications, especially in the fields of wound therapy, inflammatory disease treatment, drug delivery systems, biosensors, and tissue engineering. Enzyme characterization, including stability, optimum pH, optimum temperature, and substrate activity, is an important initial step in their utilization. Optimization through genetic manipulation, fermentation, and immobilization techniques have been shown to improve production efficiency and enzyme stability under physiological conditions. In addition, integration with current technologies such as encapsulation and nanoformulation improves clinical effectiveness and expands the scope of

their applications. However, challenges such as enzyme degradation, immunogenic risks, and clinical acceptance still need to be addressed with a multidisciplinary approach. Therefore, synergy between basic and applied research is needed to encourage the use of protease enzymes in modern medical therapy more widely, safely, and sustainably.

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