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The Role of Bacterial Protease Enzymes and In Silico Prediction on the Effectiveness of Medical Applications

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Abstract: Bacterial protease enzymes have a wide range of medical applications, particularly in the treatment of infected wounds and therapy for protein digestion disorders. Proteases produced by bacteria such as Bacillus pseudomycoides show great potential for medical therapy due to their ability to break down proteins in the human body. In silico prediction using bioinformatics software such as AutoDock and PyMOL allows researchers to analyze the three-dimensional structure and molecular interactions of proteases with target substrates before conducting laboratory experiments. This article examines the role of bacterial protease enzymes in medical applications and the benefits of using in silico prediction to study the therapeutic potential of proteases. With a combined approach of experimentation and in silico analysis, the process of developing protease-based therapies can be more efficient and faster. This study emphasizes the importance of bioinformatics approaches in accelerating the discovery of enzyme-based medical therapies for future clinical applications.

Keyword: Protease Enzyme, Protease Producing Bacteria, In Silico Prediction, Medical Applications, Bioinformatics

INTRODUCTION

Protease enzymes are a group of enzymes that have the ability to hydrolyze peptide bonds in proteins, making them very important in various biological processes and industrial and medical applications. Bacterial proteases are of major interest because of their stable, efficient, and easy-to-cultivate properties in various environmental conditions (Gupta et al., 2002). In the medical field, proteases play a role in wound debridement, antimicrobial therapy, and protein-based drug processing. Many studies have highlighted the potential of proteases as bioactive agents in the treatment of chronic diseases and infections (Rao et al., 1998). Therefore, further exploration of the protease character of bacterial isolates is crucial in the development of modern therapies.

Protease-producing bacterial isolates from extreme environments such as soil, waste, and sea show diversity in biochemical properties and enzymatic stability. For example, the genus Bacillus is widely reported as a major producer of proteases because of its ability to produce

enzymes in high amounts and is resistant to various pH and temperatures (Ananta et al., 2025). The advantages of protease from Bacillus are not only its stability but also its high activity against complex protein substrates. Various approaches have been carried out to identify the characteristics of protease, including purification, enzymatic activity testing, and kinetic studies. These studies underlie clinical applications such as enzymatic therapy in infectious wounds and other skin disorders.

Bioinformatics approaches are important tools in analyzing the structure and function of protease enzymes more efficiently and precisely. In silico prediction through molecular modeling and docking can identify substrate-enzyme interactions, active sites, and potential affinity for target compounds (Doytchinova & Flower, 2007). This is very helpful in the development of protein-based therapies and determining the effectiveness of enzymes in medical applications. In addition, computational methods accelerate the validation process without having to rely entirely on time-consuming and expensive laboratory experiments. The combination of bioinformatics analysis and characterization of bacterial proteases can open up new opportunities in the design of enzyme-based therapies.

As the need for biological-based therapies increases, including in the fields of regenerative pharmacy and chronic wound treatment, proteases are becoming one of the promising bioactive agents. In the treatment of infectious wounds, proteases work by breaking down necrotic tissue and accelerating the regeneration of new tissue (Jisha et al., 2013). This application is increasingly relevant in the context of increasing antibiotic resistance, where biological approaches are becoming an alternative solution. Proteases produced by microorganisms have high potential as adjuvant therapy in the treatment of wounds, especially those that are difficult to heal. Therefore, the exploration of proteases as active ingredients in drug formulations is an increasingly interesting topic in biomedical research.

In addition to wound treatment, proteases are also used in the pharmaceutical field for the manufacture of vaccines and the development of therapeutic proteins. Recombinant enzyme technology allows the production of proteases on a large scale, as well as the modification of their properties to suit clinical needs (Banik & Prakash, 2004). In the formulation process, proteases are used to digest target proteins so that they are more easily absorbed by the body or to activate drug precursors. The development of protease-based delivery technology is also being developed in smart drug delivery systems. This potential positions proteases as important candidates in the era of individual and precision therapy.

Most recent studies emphasize the importance of molecular analysis in understanding the mechanism of action of bacterial proteases. Identification of coding genes, prediction of three-dimensional structures, and modeling of interactions with substrates are crucial approaches in improving the effectiveness and specificity of enzymes (Kumar & Takagi, 1999). This approach also makes it easier for researchers to engineer proteases with certain properties, such as high stability to extreme temperatures or pH. With this technology, the efficiency of protease use in pharmaceutical products can be significantly increased. Therefore, the integration of bioinformatics and biotechnology is a major pillar in current enzymatic research.

One of the bioinformatics methods used in protease analysis is molecular docking, which allows simulation of protease interactions with target molecules such as membrane proteins or pathogenic compounds. This technology is useful in predicting enzyme effectiveness against specific pathogens or bioactive compounds (Morris et al., 2009). The use of this method has been combined with molecular dynamics simulations and pharmacophore analysis to improve prediction accuracy. The results are used to design proteases that are more selective and have minimal side effects. This strategy is very important in medical applications that require high safety and efficacy.

In the context of sustainability and efficiency, many researchers are now turning their attention to local microorganisms and environmental isolates as new sources of proteases. This not only increases the availability of resources but also allows the adaptation of enzymes to

local conditions of the human body or application environment. For example, isolates from mangrove waters or agricultural soils have shown high protease activity and good safety profiles (Ananta et al., 2025). These studies also used metagenomic approaches to explore the hidden potential of uncultivated microbiota. Therefore, exploring local microbial sources remains a strategic step in protease research.

Although the potential of bacterial proteases is quite promising, there are challenges in terms of stability, immunogenicity, and therapeutic efficacy when used in biological systems. Therefore, a systematic approach is needed that combines bioproduction optimization with in silico simulations to estimate the efficacy and safety of their applications. Enzyme stability enhancement can be achieved through targeted mutations or structural modifications based on bioinformatics prediction results. This strategy has been shown to improve enzyme efficiency in various health and pharmaceutical products. Integration of experimental and computational approaches is key to realizing the full potential of proteases in medicine.

Against this background, this article aims to review the current literature on the characteristics of bacterial protease enzymes, as well as the use of in silico approaches in predicting their effectiveness for medical applications. A systematic review is conducted of various studies related to production, characterization, optimization, and bioinformatics simulations. It is hoped that this article can contribute to the development of enzyme-based strategies in modern pharmaceutical and therapeutics. In addition, this article highlights the potential synergy between laboratory technology and computational modeling as the future of biotherapy. The main focus is given to innovative medical applications with high translational value.

METHOD

This article was compiled using a systematic literature review approach using sources from scientific databases such as PubMed, ScienceDirect, Scopus, and Google Scholar. Inclusion criteria included publications discussing bacterial protease enzymes, medical applications, and in silico predictions such as molecular docking and protein structure modeling, with a period of 2000–2025. The search strategy used a combination of relevant keywords and Boolean methods.

Each selected article was evaluated based on research methods, topic relevance, and its contribution to medical applications. The collected data were analyzed thematically to identify enzyme characterization, production optimization, and utilization of in silico techniques. The results are presented in the form of a narrative synthesis to highlight the integrative role of biotechnology and bioinformatics in improving the effectiveness of bacterial protease use in the health sector.

RESULTS AND DISCUSSION

Protease enzymes produced by bacteria show diversity in their activity and stability depending on the species and isolation environment. For example, bacteria from the genus Bacillus, such as Bacillus subtilis and Bacillus licheniformis, have been widely reported to produce proteases with high activity over a wide range of temperatures and pH (Joo et al., 2003). This diversity is important in determining the potential for medical applications because enzyme stability under physiological conditions is a major prerequisite for its use in human biological systems.

Previous studies have shown that protease enzymes from Bacillus pseudomycoides have good proteolytic capacity, especially in hydrolyzing complex proteins (Hasan et al., 2020). This property opens up opportunities for the application of this enzyme in wound therapy, necrotic tissue cleaning, or as part of an enzyme-based antimicrobial treatment strategy. Optimization of production conditions such as pH, temperature, and substrate type has significantly increased the yield of protease production from these isolates.

The effectiveness of proteases in medical applications is also influenced by their structural characteristics. Bioinformatics studies have shown that in silico prediction of the three-dimensional structure of a protease can provide important information regarding the location of the active site, substrate affinity, and possible interactions with other molecules (Rawlings et al., 2018). This is very useful in designing recombinant protease molecules or directing targeted mutagenesis to improve enzyme performance.

Molecular modeling of bacterial proteases is also used to analyze thermal stability and resistance to protease inhibitors. For example, a study by Ahmad et al. (2021) utilized molecular dynamics simulations to assess the stability of protease structures in physiological environments and the results were used to guide the design of more durable and target-specific enzymes. This is crucial for enzyme applications in the human body, such as protein-based therapies.

One of the major advantages of the in silico approach is its ability to virtually screen potential inhibitors or substrates, which can then be tested experimentally. A study by Sharma et al. (2022) showed that molecular docking modeling can predict the binding affinity between proteases and various bioactive compounds, providing early insights before in vitro or in vivo experiments are performed. In medical applications, proteases have great potential for use in chronic wound therapy and infections due to their ability to decompose dead tissue and pathogenic biofilms. Protease enzymes from Bacillus spp. have been tested in vitro for this ability, and have shown promising results in inhibiting the growth of bacterial biofilms such as Pseudomonas aeruginosa (Nazari et al., 2020). This approach is very important considering the increasing antibiotic resistance.

The use of proteases in the pharmaceutical field also includes their use as enhancers in drug delivery systems. These enzymes can modify protein matrices or break down biological barriers to facilitate drug absorption in the body. Several studies have developed nanoparticle-based delivery systems with proteases as part of their active components to increase drug release efficiency (Patel et al., 2021).

Optimization of protease enzyme production from bacteria is carried out through a modified fermentation approach. For example, Ananta, Alamsjah, and Agustien (2025) optimized protease production from PUA-14 isolates originating from mangrove waters by manipulating pH, temperature, incubation time, and substrate concentration. The results showed a significant increase in enzymatic activity, indicating the importance of environmental control in the production of high-quality enzymes for medical applications.

In silico studies of proteases from marine isolates showed significant amino acid sequence variations in the catalytic site compared to terrestrial proteases. This variation allows the development of enzymes that are more resistant to extreme environments or specific to certain substrates. Such characteristics are highly desirable in medical applications that require high precision and functional stability under human body conditions (Chen et al., 2021).

Phylogenetic and domain structure analysis of bacterial proteases also provide important insights into understanding the evolution and biological functions of these enzymes. The use of databases such as MEROPS and InterPro has helped in the classification of proteases based on their families and functional domains. With this approach, a new protease from the genus Bacillus that has not been widely explored but has high potential for pharmaceutical applications can be identified (Rawlings et al., 2018).

One of the main challenges in the clinical application of bacterial proteases is immunogenicity. Therefore, in silico prediction of immunogenic epitopes becomes important. Several studies have used platforms such as IEDB to predict the potential antigenicity and allergenicity of a particular protease, thus allowing protein modification before therapeutic use (Kumar et al., 2022).

Protease enzymes are also used in vaccine production, both as adjuvants and in the antigen purification process. In some cases, proteases are used to cleave antigens in a form that is more

easily recognized by the immune system, increasing the effectiveness of vaccines (Singh & Sinha, 2020). This expands the scope of bacterial protease applications beyond their traditional catalytic role.

The use of homology modeling and molecular docking techniques is essential in understanding the interaction of proteases with substrates or inhibitors. A study by Alavi et al. (2022) showed that this method can predict the effects of mutations on enzyme activity and assist in the design of proteases with improved substrate affinity. This is important for clinical applications where efficiency and specificity are highly required. In cancer therapy, proteases play a role in degrading the extracellular matrix and increasing drug penetration into tumor tissues. Several studies are exploring the use of proteases from marine microorganisms in oncology therapy due to their more selective nature towards tumor tissues (García-Carreño et al., 2020). This opens up great opportunities for protease enzymes as additional therapeutic agents.

In addition, bioinformatics modeling of proteases is also useful in predicting enzyme stability in various pharmaceutical preparations such as ointments, capsules, and controlled release systems. This stability is important to maintain enzyme activity until it reaches its therapeutic target. In silico stability studies save time and costs before physical stability tests are carried out in the laboratory (Ahmad et al., 2021). 16. Innovations in the development of recombinant proteases enable the production of enzymes with high efficiency and better purity. Using gene cloning and heterologous expression techniques, protease-encoding genes from bacteria can be expressed in systems such as E. coli, Pichia pastoris, or Bacillus subtilis, facilitating industrial-scale production processes (Hasan et al., 2020). This strategy also allows the development of enzyme variants with specific characteristics for certain medical therapies.

Proteases also have applications in the field of diagnostics, especially as biomarkers or in enzymatic-based detection systems. For example, proteases from Staphylococcus aureus have been used in the rapid detection of bacterial infections through specific substrates that produce optical signals when digested (Chen et al., 2021). The integration of bioinformatics approaches allows the selection of substrates that best match the characteristics of the target protease.

The utilization of proteases from bacteria in extreme environments, such as halophiles and thermophiles, is also a growing topic. Enzymes from these bacteria tend to be more stable at high temperatures and extreme pH, making them ideal for medical applications that demand high enzyme resistance (Ahmad et al., 2021). Bioinformatics plays an important role in identifying structural motifs that support this stability.

Another challenge is controlling the activity of protease enzymes so as not to damage healthy tissues. Therefore, protease engineering to increase substrate selectivity is very important. Molecular docking studies have been used to predict the affinity of proteases for various types of substrate peptides, thus facilitating the design of safer therapies (Kumar et al., 2022).

Proteases from the genus Bacillus, including Bacillus pseudomycoides, show great potential in medical applications due to their high adaptability and secretion capabilities. By combining experimental and bioinformatics approaches, recent studies have been able to identify active domains, predict tertiary structures, and optimize modification points (Ananta et al., 2025).

Several in silico approaches are also used to evaluate the potential interactions of proteases with target proteins in the human body. The goal is to assess side effects or potential interference with important biological pathways. Molecular dynamics simulations can depict the dynamics of enzyme-substrate structures in detail, providing a deeper understanding of the stability of these interactions (Rawlings et al., 2018).

The combination of omics and bioinformatics data has helped in the comprehensive analysis of protease activity and its relationship with metabolic pathways. This approach is very useful in identifying the effects of proteases on the immune system or wound healing processes.

The integration of transcriptomic and proteomic analysis also facilitates the identification of new therapeutic targets (Singh & Sinha, 2020).

Research related to the clinical application of bacterial proteases is growing, with a focus on safety, efficacy, and stability. Future studies are expected to lead to the development of proteases that are not only efficient but also spatially and temporally controllable in the patient's body. The integration of bioinformatics and protein synthesis techniques is key in directing the application of this enzyme towards precision therapy (Nazari et al., 2020).

Bioinformatics also supports the reporting and documentation of protease research results. Through gene mapping, structural modeling, and protein interaction prediction, researchers can compile new functional databases for therapeutic enzymes. This not only enriches scientific repositories but also accelerates protein-based drug discovery (Alavi et al., 2022).

Overall, the synergy between bacterial protease exploration and bioinformatics modeling offers great potential in the development of modern medical therapies. Not only in infection and wound therapy, but also in drug delivery systems, diagnosis, and treatment of complex diseases. For this reason, multidisciplinary development involving microbiology, pharmacy, and bioinformatics is a must in the future.

CONCLUSION

Protease enzymes produced by various types of bacteria show great potential in medical applications, both as therapeutic agents, wound cleaning agents, and biomolecules for drug delivery systems. Characteristics such as high activity, stability to various environmental conditions, and genetic engineering capabilities make proteases a prime candidate in the development of enzyme-based therapies. In silico approaches through bioinformatics techniques such as molecular modeling, molecular docking, and genomic analysis play an important role in understanding the structure and function of proteases in more depth. The combination of exploration of protease-producing microorganisms and bioinformatics approaches can accelerate innovation in safe and effective precision medical therapies. With the increasing need for smarter and more efficient therapies, bacterial protease research and in silico prediction have become strategic areas in the fields of modern health and pharmacy.

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