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The Role of Bacillus pseudomycoides Isolates and Bioinformatics Modeling for Modern Pharmaceutical Applications: A Literature Review

Yorasakhi Ananta[1], Salsabila Dwi Fitri[2]
[1]Universitas Andalas, Padang, Sumatera Barat, Indonesia, yorasakhiananta27@gmail.com
[2]Universitas Jambi, Jambi, Indonesia, dwifitrisalsabila@gmail.com

Corresponding Author: yorasakhiananta27@gmail.com[1]

Abstract: This article presents a literature review on the role of Bacillus pseudomycoides isolates as protease enzyme producers and how bioinformatics modeling enhances their potential in modern pharmaceutical applications. B. pseudomycoides is known to produce proteases with superior characteristics such as temperature and pH stability, making them potential candidates for pharmaceutical formulations. Bioinformatics approaches such as protein structure modeling, molecular docking, and genomic analysis have accelerated the functional characterization of these enzymes. The combination of the utilization of microorganisms and computational approaches opens up new opportunities in drug development, wound therapy, and pharmaceutical biotechnology. This study recommends multidisciplinary integration in the utilization of microbial resources for future therapeutic needs.

Keyword: *Bacillus Pseudomycoides*, Protease Enzyme, Bioinformatics, Pharmaceutical Applications, Molecular Modeling.

INTRODUCTION

The development of microbiology has paved the way for the exploration of microorganisms as potential sources of biochemicals for medical and pharmaceutical applications. One of the microorganisms that has attracted attention is Bacillus pseudomycoides, which is known as an efficient producer of protease enzymes. Proteases play an important role in the development of pharmaceutical products, especially in the fields of wound healing and enzymatic formulation (Ahmad et al., 2022). With the increasing resistance of antibiotics and the need for biological-based therapies, protease enzymes from microbes have become an alternative solution. Therefore, it is important to review the potential of B. pseudomycoides from the perspective of modern pharmacy.

Protease enzymes produced by bacteria such as B. pseudomycoides have advantages such as high stability to various environmental conditions and low production costs. These enzymes are used in various medical applications, including in drug formulations, drug delivery systems, and chronic wound therapy (Hasan et al., 2023). The proteolytic activity of these enzymes can help clean necrotic tissue and accelerate tissue regeneration. In addition, microbial proteases

are easier to modify through genetic engineering techniques to increase their specificity or stability. This shows the strategic value of microorganism exploration in the development of pharmaceutical technology.

As technology advances, bioinformatics approaches become essential tools in mapping the biological potential of microorganisms. Techniques such as protein structure modeling, active site prediction, and molecular interaction simulation can accelerate the process of discovering and optimizing bioactive candidates. In this context, proteases from B. pseudomycoides can be analyzed more deeply for pharmaceutical applications using computational technology (Ali, 2023). Bioinformatics modeling also plays a role in identifying beneficial mutations and designing more stable enzyme variants. Therefore, the integration of microbiology and bioinformatics is a strategic combination in the development of pharmaceutical innovation.

Recent studies have shown that Bacillus pseudomycoides has the ability to produce proteases with high activity in various environmental conditions, including extreme environments such as high temperatures and alkaline pH. This ability provides significant advantages in pharmaceutical applications because enzyme stability greatly determines therapeutic effectiveness (Kurniawan et al., 2022). In product formulations such as wound healing ointments or drug carriers, enzyme stability determines the duration of biological activity in the body. Therefore, the selection of microorganisms that are able to maintain enzyme activity in physiological environments is the main key in the development of innovative pharmaceutical products.

The use of proteases in the pharmaceutical field has expanded, especially in wound therapy, destruction of necrotic tissue, and delivery of peptide-based drugs. Proteases from microbial isolates such as B. pseudomycoides have been shown to have cytolytic and anti-inflammatory effects that can accelerate tissue regeneration (Sari et al., 2023). Another advantage is the ability of this enzyme to work selectively on certain substrates, which minimizes side effects compared to conventional chemical compounds. The development of enzyme-based products also supports the trend of safer and more environmentally friendly biological therapies in the modern health industry.

To optimize the utilization of protease from B. pseudomycoides, a thorough characterization of its biochemical and molecular properties is needed. This includes analysis of enzyme activity, thermal stability, optimum pH, and the effect of metal ions on enzyme activity. With this data, scientists can adjust the most ideal formulation conditions for the use of the enzyme in pharmaceutical applications. In addition, molecular characterization such as sequencing of the protease-encoding gene provides important information for genetic engineering and increased enzyme production (Rahman et al., 2024).

In recent years, bioinformatics approaches have played an important role in accelerating the characterization of protease enzymes. Technologies such as homology modeling, secondary and tertiary structure prediction, and molecular dynamics simulations are used to understand the structure and function of enzymes more efficiently. This allows for the identification of active sites, prediction of structural stability, and the design of mutations that can improve the efficiency or resistance of enzymes in pharmaceutical applications (Ananta et al., 2025). The combination of laboratory experiments and computer simulations makes the enzyme development process faster and more cost-effective.

In addition, the in silico approach also allows the identification and validation of pharmacological targets of microbial proteases against human proteins or tissues. The molecular docking process provides insight into the interaction between enzymes and target molecules, which is important in the development of enzyme-based drugs. This further strengthens the relevance of B. pseudomycoides proteases in modern therapies, such as the degradation of toxic proteins or the treatment of chronic infections through enzymatic means

(Ali, 2023). This technology also allows rapid screening of potential inhibitor compounds for applications in regulating enzyme activity.

This literature review is very important because currently there are limited explorations and specific utilization of *Bacillus pseudomycoides* in the context of modern pharmaceutical applications, although its potential is very promising. Various studies have focused more on other Bacillus species such as *B. subtilis* and *B. licheniformis*, whereas *B. pseudomycoides* shows unique characteristics in the production of protease enzymes. Therefore, this article aims to present a comprehensive summary of existing research results, as well as to strengthen the urgency of utilizing this isolate in biotechnology-based pharmaceutical practices.

The main objective of this review is to systematically examine the role of Bacillus pseudomycoides isolates as protease producers and how bioinformatics modeling can support the characterization and optimization of these enzymes for pharmaceutical applications. By reviewing various recent scientific publications, this article is expected to provide a comprehensive overview of the synergy between microbiology and bioinformatics in the development of enzyme-based therapeutic solutions. The integration of these approaches is believed to open up innovative opportunities in the modern pharmaceutical industry, especially in the development of biologically based therapies.

METHOD

This study is a systematic literature study conducted by searching scientific articles from trusted databases such as PubMed, ScienceDirect, SpringerLink, and Google Scholar. The keywords used in the search include: "*Bacillus pseudomycoides*", "protease enzyme", "bioinformatics", "pharmaceutical application", and "enzyme characterization". The selected literature focuses on research in the last 10 years and is published in national and international accredited journals. Relevant articles are classified based on their focus on bacterial isolates, production and characterization of protease enzymes, and the use of bioinformatics techniques. All articles are reviewed in depth to assess the quality of the methods, results, and contributions to the development of pharmaceutical applications.

RESULTS AND DISCUSSION

Bacillus pseudomycoides isolates are commonly found in extreme environments such as alkaline soil and industrial wastewater, which show high resistance to environmental conditions. Research by Hasan et al. (2022) showed that this isolate was able to produce significant protease when incubated in solid nitrogen-rich media. Molecular identification through the 16S rRNA gene confirmed its closeness to the Bacillus cereus group, but with certain phenotypic differences. Successful isolation requires optimization of temperature, pH, and selective media composition to support optimal growth.

The protease enzyme produced by B. pseudomycoides belongs to the serine-protease group and has high enzymatic activity in the neutral to alkaline pH range. A study by Ahmad et al. (2021) stated that optimum proteolytic activity was achieved at a temperature of 50–55°C and pH 9.0. These characteristics indicate that the protease from this bacterium has high potential for use in the pharmaceutical field, especially as a wound debridement agent and enzymatic therapy.

The characterization of the protease enzyme was carried out by SDS-PAGE analysis to determine the molecular weight and stability of the enzyme. In addition, the Michaelis-Menten kinetic test was used to determine the Km and Vmax values of the enzyme. According to research by Sari et al. (2023), the protease enzyme from the B. pseudomycoides isolate showed a low Km value, indicating a high affinity for its substrate. This is an indicator that this enzyme works efficiently at low substrate concentrations, which is important in pharmaceutical applications.

Some of the proteases produced have antibacterial effects against skin pathogens such as Staphylococcus aureus and Pseudomonas aeruginosa. This activity is associated with the ability of the protease to hydrolyze the membrane proteins of pathogens. A study by Dewi & Pratama (2022) showed that culture supernatants containing proteases significantly inhibited the growth of gram-positive and negative bacterial colonies. This opens up opportunities for the use of proteases in topical antimicrobial therapy.

In silico techniques are used to predict the 3D structure of proteases using software such as SWISS-MODEL and I-TASSER. This modeling aims to evaluate the active site and predict affinity for substrates and inhibitors. The results of the analysis by Yusuf et al. (2023) showed that the protease from B. pseudomycoides has an active site similar to commercial therapeutic proteases, which strengthens its potential application as a drug candidate.

Molecular docking is used to determine the interaction of proteases with target proteins or biological substrates. The docking results from the AutoDock Vina application show stable hydrogen bonds and electrostatic interactions between the protease and the target protein. A study by Hasanah et al. (2023) showed that the protease is able to bind strongly to inflammatory proteins, opening up the possibility of its use in chronic wound therapy or systemic inflammation.

Molecular dynamics simulation for 100 ns showed the stability of the protease structure in a physiological environment. This is important to validate that the enzyme is not easily denatured when applied topically or systemically. The simulation also showed low fluctuations in the active domain, indicating high stability in enzyme activity (Ahmad et al., 2021).

Protease from B. pseudomycoides has potential as an active agent in pharmaceutical products such as wound ointments, antiseptic gels, or necrotic tissue cleansers. Its effectiveness as a debridement agent showed comparable results to papain and bromelain in comparative tests. In addition, its low level of irritation makes it an ideal candidate for dermatological applications (Hasan et al., 2022).

Biocompatibility tests on fibroblast cell cultures showed that the protease enzyme did not cause cell death at therapeutic concentrations. This indicates its safety for clinical use, although further in vivo testing is still needed. Research by Ahmad et al. (2021) also stated that no significant hemolytic activity was found in in vitro tests against human red blood cells.

The stability of protease enzymes is very important in their application as active pharmaceutical ingredients. Research shows that enzymes from Bacillus pseudomycoides remain active after heating at 60°C for 30 minutes, and are resistant to mild organic solvents such as ethanol and methanol (Hasan et al., 2022). This stability allows its use in various formulations, including alcohol-based products or mild heating.

Some metal ions such as Ca^{2+} and Mg^{2+} are known to increase the stability and activity of protease enzymes, while heavy ions such as Hg^{2+} are inhibitory. A study by Sari et al. (2023) showed that the addition of 5 mM $CaCl_2$ increased protease activity by up to 20%, indicating the importance of the role of co-factor ions in enzymatic formulations.

In silico analysis of the amino acid sequence of protease yielded a pI of around 8.5, which means the enzyme is more active in basic conditions. This profile is important for pharmaceutical applications, especially for chronic wound products that are usually basic (Yusuf et al., 2023). Knowledge of pI is also useful in enzyme purification and formulation.

Protease enzymes need to be formulated in a compatible carrier system to remain stable and effective. Literature studies have shown that hydrogel and liposome-based carriers can maintain enzyme activity and allow controlled release (Dewi & Pratama, 2022). This is very important for long-term topical pharmaceutical applications.

In addition to direct antimicrobial activity, proteases can also increase the effectiveness of antibiotics by disrupting pathogenic biofilms, which are generally resistant to treatment. The results of the enzyme combination test with gentamicin showed a synergistic effect against P.

aeruginosa (Ahmad et al., 2021), indicating the potential use of protease as a combination therapy.

Protease from B. pseudomycoides shows high activity against collagen and fibrin, which are the main components of wound tissue. This makes this enzyme very suitable as a selective debridement agent that does not damage healthy tissue. The study by Ananta et al. (2025) supports this by showing that the enzyme from the PUA-14 isolate effectively dissolves necrotic tissue without excessive side effects.

Protease that works selectively is able to clean dead tissue and stimulate its granulation process, thereby accelerating wound healing. Literature studies state that topical application of proteolytic enzymes increases the expression of growth factors and migration of fibroblast cells (Hasanah et al., 2023).

The application of foreign enzymes can cause immune reactions, so it is necessary to evaluate the immunogenic potential. Several early studies have shown that proteases from B. pseudomycoides have relatively low peptide sequences in human T lymphocyte activation, indicating low immunogenicity potential (Yusuf et al., 2023).

Bioinformatics technology allows the design of site-specific mutations to improve enzyme activity and stability. With an in silico approach, critical residues in the catalytic domain can be modified to produce superior variants, as shown in simulations by Hasan et al. (2022). Bioinformatics is also used to model metabolic pathways and large-scale protease production through metabolic engineering. Literature data shows that protease expression in recombinant Bacillus subtilis can increase production yields without changing its enzymatic activity (Sari et al., 2023), paving the way for technology-based industrial production.

Despite its many advantages, protease formulation for pharmaceutical applications faces challenges related to long-term stability and interactions with other formulation ingredients. Therefore, encapsulation strategies and stabilizer use are important in product development (Dewi & Pratama, 2022).

The use of enzymes in pharmaceutical products must meet the safety and effectiveness standards set by regulatory bodies such as BPOM and FDA. Evaluation of toxicity, efficacy, as well as stability and compatibility tests of the formulation are the main requirements for product approval (Hasanah et al., 2023). The role of Bacillus pseudomycoides and its protease enzymes in the pharmaceutical world is predicted to grow rapidly, especially in wound therapy, antimicrobial formulations, and therapeutics. enzyme-based pi. The combination of laboratory and bioinformatics approaches opens up new opportunities to develop more precise, effective, and safe pharmaceutical products (Ahmad et al., 2021; Yusuf et al., 2023).

The study results show that proteases from B. pseudomycoides have high potential to be developed into clinical products, either as topical drugs, wound dressings, or components of combination therapies. The main challenges remain in the scale-up process, clinical trials, and regulatory approval, but the prospects are promising (Ananta et al., 2025).

The integration of bioinformatics in enzymatic research has accelerated the process of screening, mutation design, and molecular analysis. This helps researchers understand the structure-function of enzymes in depth without having to go through long and expensive laboratory stages, thereby increasing the efficiency of developing modern biotechnology-based pharmaceutical products (Sari et al., 2023).

CONCLUSION

This literature study confirms that Bacillus pseudomycoides is a potential source of promising protease enzymes for various modern pharmaceutical applications. The characteristics of the protease from this isolate, such as stability at extreme temperatures and pH, and high substrate specificity, make it a leading candidate in the medical field, especially in wound therapy and enzyme-based drug formulation. Bioinformatics approaches, especially through in silico modeling, have made a major contribution to understanding the structure and

function of proteases molecularly. The combination of isolate bioprospection and bioinformatics modeling opens up great opportunities in the development of more efficient, specific, and safe enzyme-based pharmaceutical products. Further research is still needed for experimental validation and development of protein engineering to improve the efficacy and stability of protease enzymes from B. pseudomycoides.

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