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Protease Enzyme Producing Microorganisms and Their Application in Disinfection of Infected Wounds

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Abstract: Wound infection is a serious clinical challenge, mainly due to increasing antibiotic resistance and biofilm formation by pathogenic microorganisms. Protease enzymes produced by various microorganisms, especially bacteria, have shown potential in decomposing necrotic tissues, destroying biofilms, and enhancing the efficacy of other antimicrobial agents. The literature shows that microorganisms such as Bacillus subtilis, Pseudomonas aeruginosa, and Streptomyces spp. produce proteases with significant pharmacological activities. The application of these enzymes in topical formulations, bioactive wound dressings, and combination therapies has become the focus of research in the fields of pharmaceuticals and medical biotechnology. This article aims to review the characteristics of protease-producing microorganisms, the mechanism of action of enzymes in the wound disinfection process, and the challenges and prospects of their future applications. This review is expected to be a scientific basis for the development of biotechnology-based wound therapy.

Keyword: Protease Enzymes, Microorganisms, Wound Disinfection, Biofilm, Antimicrobial Therapy

INTRODUCTION

Infected wounds are a serious health problem and can significantly slow the healing process. Pathogenic microorganisms, especially bacteria, often colonize wounds and form biofilms that are resistant to conventional treatments. This condition not only inhibits tissue regeneration but also increases the risk of systemic complications. The uncontrolled use of systemic antibiotics further exacerbates global antimicrobial resistance. Therefore, an alternative approach that is more effective and environmentally friendly is needed (Sharma et al., 2020).

One promising approach in the treatment of infected wounds is the use of protease enzymes produced by microorganisms. These enzymes can degrade necrotic tissue, break down biofilms, and increase the penetration of other antimicrobial agents into the wound area. Microorganisms such as Bacillus subtilis, Pseudomonas aeruginosa, and Streptomyces spp. are known to have a high capacity to produce active proteases. The use of proteases from biological sources not only increases the effectiveness of therapy but also reduces the risk of side effects compared to chemical approaches (Alnahdi, 2012).

In the context of pharmaceutical and health biotechnology, exploration of enzyme-producing microorganisms has grown rapidly in the last two decades. Microbial proteases have been used in topical gel formulations, bioactive wound dressings, and as components in combination therapies with antibiotics. Recent studies have even shown the ability of proteases to target structural components of biofilms such as proteins and polysaccharides, thereby increasing the chances of complete wound healing (Kang et al., 2021). This potential makes proteases a prime candidate in the development of biotechnology-based wound therapies.

Protease-producing microorganisms can be obtained from extreme environments such as soil, sea, and even industrial waste, which show high resistance and efficiency in enzyme production. Fermentation techniques and growth medium optimization have succeeded in increasing the production of microbial proteases on an industrial scale. In addition, genetic engineering technology has also been applied to improve the stability and substrate specificity of the enzymes produced. This supports the development of proteases as future therapies in the treatment of complex infectious wounds (Banerjee & Ray, 2023).

Although the prospects for their use are promising, there are several challenges that still need to be overcome in the clinical application of microbial proteases. The stability of enzymes under physiological wound conditions, the potential for immunological reactions, and their effectiveness against various types of pathogenic microorganisms are still active research topics. Therefore, a thorough understanding of the characteristics of proteases from various microorganisms is essential to optimize their use. The existing research so far provides a strong foundation, but further validation through clinical trials and in vivo studies is needed (Ravindran et al., 2020).

With the increasing interest in non-antibiotic and bio-based therapies, the focus on microbial proteases as topical disinfectant agents is becoming increasingly relevant. In vitro and in vivo studies have shown that these enzymes are able to accelerate wound cleansing and reduce the population of resistant microbes. Moreover, the success of proteases in overcoming biofilms makes them superior to traditional therapies that often fail in cases of chronic wounds. Therefore, this approach is in line with the global trend in finding more specific and efficient therapeutic solutions (Ali, 2023).

In addition, the integration of proteases in modern wound dressings opens up great opportunities for the development of innovative medical products. Enzyme-based wound dressings offer gradual release and more targeted activity, and can be tailored to the specific conditions of the patient's wound. The use of hydrogel or nanotechnology-based delivery systems is also being developed to maximize enzyme stability and activity in the wound environment. This shows a strong collaboration between microbiology, pharmacy, and material engineering in developing new therapies (Zhang et al., 2022).

Based on this background, this article aims to systematically review the role of protease-producing microorganisms and their applications in disinfection of infected wounds. The main focus is to identify potential microbial types, the mechanism of action of proteases in inhibiting biofilms, and the clinical implications and challenges of their application. This review is based on current literature covering laboratory studies, preclinical studies, and technological approaches in enzyme production and formulation. It is hoped that this review can provide a comprehensive overview as a basis for the development of protease-based wound therapies in the future.

METHOD

This article uses a systematic literature study method by reviewing various scientific publications from databases such as PubMed, Scopus, and Google Scholar. Inclusion criteria include articles discussing microorganisms producing protease enzymes and their applications in disinfection of infected wounds, published between 2013 and 2024. Data are classified based on the type of microorganism, enzyme type, and effectiveness in the context of wound healing.

The analysis was carried out qualitatively to identify trends, benefits, and challenges of microbial protease applications in the health sector. This approach aims to provide a comprehensive overview of current research developments relevant to the topic. All literature reviewed has gone through a peer-review process to ensure the validity of the information.

RESULTS AND DISCUSSION

Microorganisms Producing Protease Enzymes

Bacillus subtilis is known as one of the main producers of protease enzymes that are stable and active at various pH and temperature conditions. Protease from B. subtilis has been widely used in medical and industrial applications because of its ability to degrade necrotic tissue without damaging healthy tissue. A study by Jisha et al. (2013) showed that protease from this strain is effective in cleaning chronic wounds. Another advantage is the ease of cultivating this microorganism in cheap media. In addition, B. subtilis is safe and has long been used in pharmaceutical products. Therefore, this strain is widely used as a model in proteolytic enzyme research (Jisha et al., 2013).

Pseudomonas aeruginosa also produces proteases such as elastase and alkaline protease which are effective in decomposing biofilms. However, due to its pathogenicity, its application is limited only to enzyme extraction and not the use of live microbes. Nevertheless, proteases from P. aeruginosa show very high activity against protein components in biofilms, making them active ingredients in wound disinfectant formulas. Research by Kessler et al. (1993) stated that this microbial elastase is able to increase the effectiveness of antibiotics by opening the biofilm matrix. The development of enzyme delivery systems from these microbes remains a focus of research. The use of nanocapsules or coformulation with antibiotics has been tried to reduce their toxicity (Kessler et al., 1993).

Streptomyces spp. produce neutral and acidic proteases that are widely studied because they have non-pathogenic properties and high enzyme production capabilities. Proteases from this genus are thermostable and can be used in various topical formulations. A study by Younes et al. (2018) showed that proteases from Streptomyces are effective in reducing bacterial loads and accelerating wound epithelialization in animal models. In addition, its ability to degrade fibrin helps clean wounds faster. These characteristics make it an attractive choice for modern pharmaceutical development. With a relatively easy fermentation process, these microorganisms are very promising commercially (Younes et al., 2018).

In addition to bacteria, several fungi such as Aspergillus niger and Penicillium are also able to produce active proteases with medical potential. Proteases from these fungi tend to be more active at acidic pH and can be used in wounds with local acidic conditions. The proteolytic activity of these fungi has been developed for the formulation of creams and wound irrigation solutions. A study by Kumar et al. (2020) showed that proteases from Aspergillus accelerated the healing time of diabetic wounds in mice. However, safety and allergy aspects are still considerations in the use of these fungi. Therefore, toxicological evaluation is essential before being widely applied (Kumar et al., 2020).

Extreme environments such as hot springs, high salinity, and industrial waste are also habitats for extremophile microbes that produce unique proteases. Proteases from these microorganisms are usually more stable to changes in temperature and pH, and more resistant to natural inhibitors. Examples of microbes such as Halobacterium and Thermus aquaticus have been explored as stable sources of proteases in chronic, difficult-to-treat wounds. Research by Gupta et al. (2013) showed that enzymes from extremophiles have high activity in wound environments with mixed infections. The industrial potential of these microbes has not been fully utilized in the pharmaceutical sector. However, exploration of them can provide new alternatives in the treatment of infectious wounds (Gupta et al., 2013).

Proteases and Their Effect on Wound Healing

Bacterial proteases not only help clean wounds from necrotic tissue, but also play an important role in the wound healing process. These proteases help in wound debridement, which is the process of removing dead cells that prevent new tissue regeneration. Research by Sharmin et al. (2015) shows that proteases produced by Bacillus subtilis play a role in accelerating wound healing through the mechanism of increasing angiogenesis. This accelerates the formation of new blood vessels needed to provide oxygen and nutrients to the wound area. In addition, these proteases also help regulate collagen levels needed for optimal healing (Sharmin et al., 2015).

Microbial protease activity also has a positive impact on the management of wound infections. Proteases can break down proteins in bacterial biofilms that protect pathogens from antibiotic treatment. A study by Hernández et al. (2017) confirmed that the use of microbial proteases can increase the effectiveness of antibiotics by reducing biofilm protection, which is often a major barrier to healing infected wounds. Proteases such as elastase from Pseudomonas aeruginosa have the potential to open biofilms covering the wound surface and make it easier for antibiotics to destroy pathogens (Hernández et al., 2017).

One of the latest applications of microbial proteases is in the development of gels or ointments that can be used to treat chronic wounds. A study by Zhang et al. (2019) showed that a gel formulation containing protease from Streptomyces has the advantage of reducing inflammation and accelerating wound healing. This gel not only contains proteolytic enzymes, but also anti-inflammatory compounds that support tissue regeneration. The formulation has been tested on wounds in animal models and showed better results compared to conventional treatments (Zhang et al., 2019).

Proteases also play a role in accelerating recovery from burns. Burns are often accompanied by more extensive tissue damage and inflammatory processes that inhibit healing. Research by Oliveira et al. (2018) showed that proteases produced by Aspergillus niger can accelerate the removal of dead tissue in burns and support faster skin regeneration. The results of this study support the use of proteases in the treatment of burns that are more efficient and reduce the treatment time required (Oliveira et al., 2018).

Another advantage of using microbial proteases is their ability to repair damage caused by mixed infections. Wound infections involving several types of bacteria can slow the healing process and trigger chronic inflammation. Microbial proteases have the ability to target various pathogens in wounds and accelerate wound debridement. Research by Munkhtsog et al. (2020) showed that a combination of proteases from Bacillus subtilis and Pseudomonas aeruginosa can repair wounds infected by various pathogens and facilitate faster healing (Munkhtsog et al., 2020).

In addition to clinical benefits, the use of microbial proteases can also improve sustainability in wound treatment. The use of proteases from natural microbial sources reduces the need for antibiotics and synthetic chemicals that often have side effects and long-term negative impacts. Research by Patel et al. (2021) showed that proteases from Streptomyces used in wound healing are not only effective in debridement but also more environmentally friendly compared to chemical alternatives. This opens up great opportunities in the development of more natural and sustainable wound therapies (Patel et al., 2021).

Microbial proteases also show potential in wound management in diabetic patients, who often experience difficulty in wound healing due to impaired circulation and chronic infections. Research by Gupta et al. (2018) showed that proteases from Bacillus cereus were able to reduce bacterial colonies in diabetic wounds and support faster healing. In diabetic patients, the use of microbial proteases not only accelerated wound healing but also reduced the risk of recurrent infections, which are often a problem in diabetic wounds (Gupta et al., 2018).

Despite its benefits, the use of microbial proteases in wound healing also faces challenges. One of the main challenges is the potential for allergic reactions in some individuals who are sensitive to foreign proteins. Research by Zhang et al. (2021) identified that some patients experience allergic reactions to certain proteases, especially with long-term use. Therefore, it is important to perform a skin compatibility test before using proteases in medical applications. Nevertheless, the potential of proteases in wound therapy remains high, with further development in delivery and formulation technology (Zhang et al., 2021).

Another challenge is controlling the stability of protease enzymes in therapeutic formulations. Proteases tend to be easily degraded by temperature and unstable environmental conditions, which can reduce their effectiveness in wound therapy. Research by Chen et al. (2020) suggests the use of nanoparticles or more advanced delivery systems to improve the stability of microbial proteases in topical formulations. With better delivery technology, the effectiveness of proteases in wound healing can be more optimal and sustainable (Chen et al., 2020).

In addition, it is important to pay attention to the regulation and licensing of microbial protease-based products in clinical medicine. The development of pharmaceutical products using protease-producing microbes requires further research on the safety standards and regulations that apply in various countries. Research by Matos et al. (2019) shows that although microbial proteases have great potential, regulatory approval and the licensing process in the medical market require considerable time and cost. Therefore, collaboration between the pharmaceutical industry and regulatory agencies is needed to accelerate the adoption of this technology (Matos et al., 2019).

The use of microbial proteases in wound therapy also requires further research on the optimal dose and frequency of application. Research by Kumar et al. (2020) showed that proteases used in excessive amounts can cause skin irritation and increase the risk of secondary infections. Therefore, further studies are needed to determine the right dose and formulation to achieve maximum results without side effects (Kumar et al., 2020).

Even so, the integration of microbial proteases in wound treatment can accelerate the development of more innovative and affordable wound therapies. Proteases have great potential for use in various types of wounds, including burns, diabetic wounds, and other chronic wounds that are difficult to treat conventionally. Research by Roberts et al. (2018) shows that the use of proteases in combination with new technologies such as nanomedicine can further increase the effectiveness of wound therapy. This opens up new opportunities in the medical field based on biotechnology and microbiology (Roberts et al., 2018).

In the context of infected wound therapy, microbial proteases can act as a more environmentally friendly and cost-effective solution compared to conventional antibiotics. Reducing the use of antibiotics in wound care is essential to reduce the increasing antibiotic resistance. A study by Williams et al. (2020) showed that microbial proteases can replace most of the role of antibiotics in infected wound therapy without increasing the risk of resistance. This paves the way for the development of more sustainable and effective therapies in the management of infected wounds (Williams et al., 2020).

CONCLUSION

Proteases produced by microorganisms show great potential in the treatment of infected wounds, with applications ranging from wound debridement to bacterial biofilm reduction. Various protease-producing bacteria, such as Bacillus subtilis, Pseudomonas aeruginosa, and Streptomyces, have been shown to be effective in accelerating wound healing by increasing angiogenesis, reducing inflammation, and resolving bacterial infections. In addition, microbial proteases play a role in the repair of burns, diabetic wounds, and chronic wounds that are often difficult to treat with conventional methods. However, challenges related to enzyme stability, allergic reactions, and regulatory use remain to be addressed. Nevertheless, the use of microbial proteases has the potential to reduce the dependence on antibiotics and offer a more environmentally friendly solution in the treatment of infected wounds. Further research on optimal dosage, formulation, and development of delivery technologies will accelerate the implementation of microbial proteases in clinical practice.

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