Prospects of ultrafiltration in supporting the Development of *Matabacillus* sp. CS-2 antinecrosis protease as a wound debridement agent - A bibliography study

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Abstract. Debridement is a critical step in wound healing, which involves removing necrotic or infected tissue from wounds Specific enzymes, including fibrinolytic protease from *Metabacillus* sp. CS-2 has shown anti-necrosis activity and potential as a wound debridement agent. However, efforts to enhance the enzyme's activity to improve its performance as a debridement agent remain unexplored. This review highlights the potential of ultrafiltration to boost bacterial protease activity based on data from Google Scholar and PubMed. Using the dimension.ai database with keywords “ultrafiltration”, “bacterial protease”, and “protease activity”, a literature review over the last ten years was conducted, supported by network visualization via VOSviewer. The findings suggest that ultrafiltration is an important part of a study that should be conducted to support the development of *Metabacillus* sp. CS-2 protease as a debridement agent.

1 Introduction

Debridement is an important cleaning step in treating chronic wounds, a health condition that often leads to amputation and death. Debridement plays an important role in managing necrosis, by removing dead tissue and improving blood circulation around the wound [1]. Debridement aims to create a healthy environment needed for wound healing by removing material that could be a source of infection or inhibit new tissue growth [2].

Chronic wounds where excess fibrin accumulates in the wound area can lead to tissue death or necrosis [3]. The application of protease enzymes for the treatment of chronic wounds, which trigger infections that lead to sepsis, amputation, and death in many countries

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is currently in demand [4]. Proteases offer a promising role in the debridement process to promote wound healing [5,6]. Proteases have a natural ability to destroy protein peptide bonds. Fibrinolytic proteases in particular can destroy fibrin clots, which form in response to injury or infection [7].

Indonesia is known as a center of marine diversity, including algae and their symbiont microorganisms [8]. Bacteria-produced fibrinolytic proteases can aid in the debridement process by destroying fibrin clots that can encapsulate and protect necrotic and infected tissue [9]. Fibrinolytic protease is an enzyme that has an important role in the process of breaking down fibrin, a protein involved in the formation of blood clots [10]. To improve the effectiveness and purity of these fibrinolytic proteases, specialized techniques such as ultrafiltration become relevant. Studies related to the purification of enzymes using ultrafiltration methods are very important to determine enzymes with a high level of purity without damaging the structure and activity of these enzymes [11].

Ultrafiltration as a molecular size-based separation method, can help improve the purity and concentration of these enzymes [12]. Separating molecules based on membrane pore size, ultrafiltration can remove inhibitory substances or contaminants that may affect fibrinolytic protease activity [13]. Ultrafiltration helps to improve the clarity and purity of the *Metabacillus* sp. CS-2 enzyme.

This study aims to explore and highlight the potential of ultrafiltration as a method to enhance the activity of bacterial protease, specifically from *Metabacillus* sp. CS-2, to improve its effectiveness as a wound debridement agent. The study seeks to address the gap in research regarding the enhancement of this enzyme's activity by reviewing relevant literature from the past ten years, using databases like Google Scholar, PubMed, and dimension.ai, and utilizing network visualization through VOSviewer to support its findings.

2 Materials & Method

This bibliography analysis aims to show the research trend and gap related to the "Ultrafiltration of protease enzymes". Steps to meet the study objective was initiated by collecting information mainly from database http://app.dimensions.ai, which then stored as *.ris type of files. This type of file is recognized by various map visualization software. The search for bibliography analysis was conducted using the keyword "Ultrafiltration of protease enzymes" published in the research range from 2015 to 2024 by searching titles and abstracts. A table review was constructed based on Google Scholar and PubMed databases. The steps are as follows:

2.1 Journal Eligibility Criteria

All references used in bibliography analysis were obtained using computerized search tools from dimension.ai database. Journal exclusion criteria in this study were journals that were related to the ultrafiltration of protease enzymes published in 2015-2024. For table review, the main database used were Google Scholar and PubMed database. For the table, the determination of journal eligibility was based on the inclusion criteria set as follows: (i) Protease; (ii) Ultrafiltration; (iii) *Metabacillus* sp.; (iv) reported in Indonesian or English; (v) search for review journals were also published in 2015-2024.

2.2 Journal Selections

Journal selection was based on Pigott and Polanin's (2020) guidelines to identify journals that met the inclusion criteria listed in this journal publication. Careful identification and data

analysis resulted in titles and abstracts that could be used to identify inappropriate sources that needed to be excluded. The resulting journal articles were also reviewed and evaluated to see if they met the inclusion criteria.

The bibliographic analysis was conducted by using PubMed and Google Scholar databases published from 2015-2024 that discussed "purification by ultrafiltration method of enzymes from *Metabacillus* sp. CS-2 bacteria". The article search used Medical Subject Title Headings (MeSH) with several combinations including "protease", "protease enzyme", "protease bacteria", "ultrafiltration", "*Metabacillus* sp.", and "protease activity". This journal review aims to determine the protease enzyme activity of *Metabacillus* sp. CS-2 bacteria.

2.3 Research Bias Control

The risk of bias or quality assessment in this journal review includes the following: (i) the rigor of the information provided regarding the bacterial protease *Metabacillus* sp. CS-2; and (ii) selective reporting of results. The overall acceptable risk of bias was considered minimal when all requirements were met.

2.4 Bibliometric Data and Network Visualization

VOSviewer software was used to visualize the bibliometric network or scientific publication data needed. VOSviewer can import bibliographic data from various sources like Web of Science, Scopus, PubMed, and Dimensions.ai. This data typically includes information on publications, citations, authors, keywords, and affiliations. It pre-processes the data to create a network of items, such as authors, journals, or keywords. These items can be linked based on co-authorship, co-citation, or co-occurrence relationships. VOSviewer was selected because it can map the relationships between keywords or terms that frequently appear together in publications, illustrating the main topics and emerging trends in a field [14-15].

3 Results and Discussion

Using the database of http://app.dimensions.ai/ resulted in 73,611 publications of scientific articles or journals published in the 2015 to 2024 data range. **Fig. 1** shows the number of journals published on "protease enzyme purification" each year. **Fig. 1** shows that between 2015 and 2024, there was an increase in the number of studies conducted on the topic of protease enzyme purification. The peak of such research is in 2022.

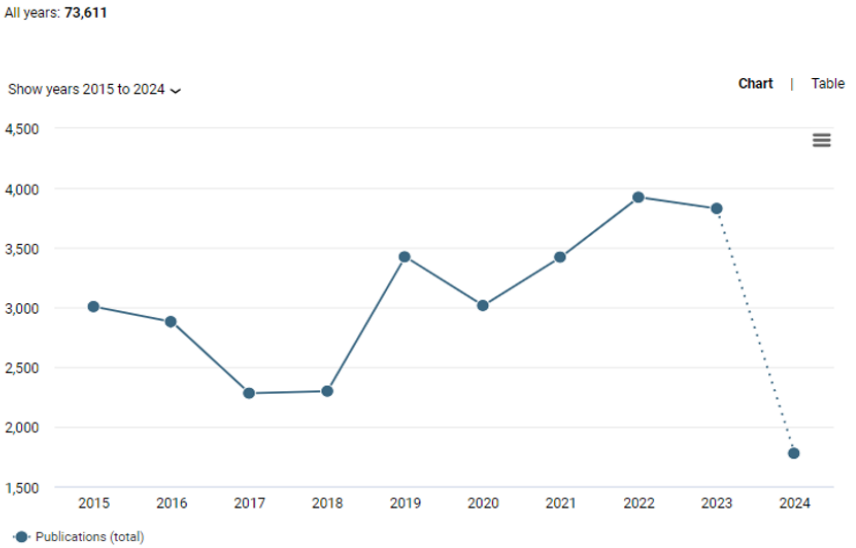

Fig. 1. Total of publications on "Purification enzyme protease" from 2015 to 2024 (source: http://app.dimensions.ai/)

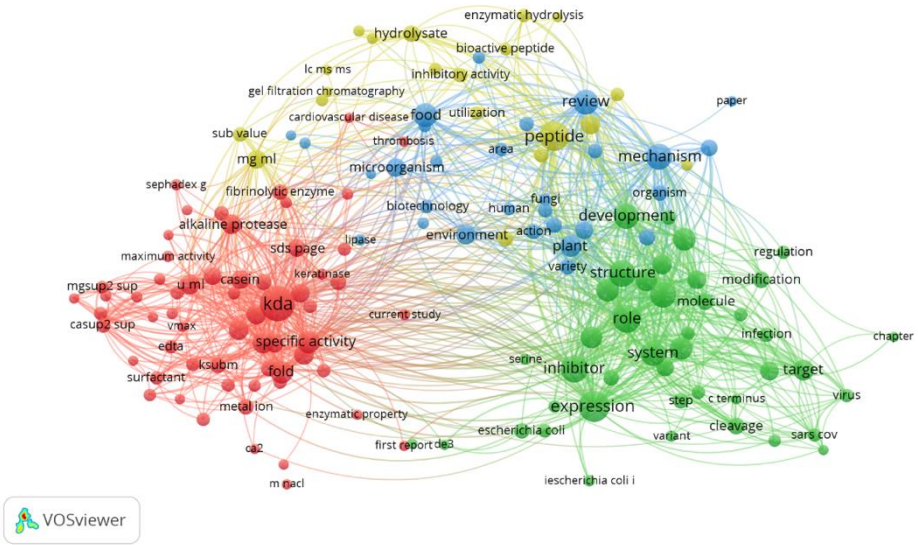

Fig. 2. Network Visualization of "Purification enzyme protease" (Source: VOSviewer and http://app.dimensions.ai/)

To display the overall data, the VOSviewer software was used to create a network visualization map. VOSviewer offers density visualizations, showing areas with high concentrations of items (e.g., popular topics or prolific authors) with more intense colors, providing a quick overview of hotspots in the research field. The visualizations and networks generated by VOSviewer can be used to interpret the structure, development, and trends within a research domain [14]. These insights are valuable for researchers, policymakers, and funding bodies to understand the landscape of scientific research. **Fig. 2** displays the network visualization of 152 terms.

Fig. 3 displays the density visualization of 152 terms. **Fig. 2** and **Fig. 3** show the network and index of research on protease enzyme purification, but they do not mention ultrafiltration as a protease enzyme purification method. Therefore, ultrafiltration as an attempt to improve the activity of bacterial protease enzymes has not been reported. VOSviewer is a powerful tool for bibliometric analysis, enabling researchers to map and explore the structure of academic research through visualizations of bibliographic data. By analyzing co-authorship, co-citation, and co-occurrence networks, VOSviewer helps to identify key trends, influential works, and collaboration patterns within a research field [15-16].

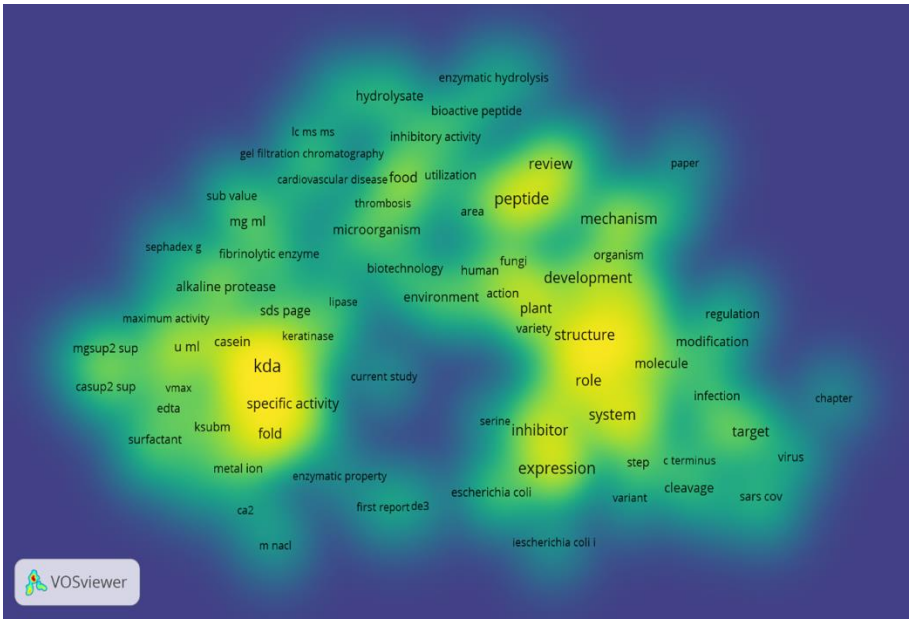

Fig. 3. Index Density Visualization "Purification enzyme protease" (Source: VOSviewer and http://app.dimensions.ai/).

In the past ten years, many studies have documented the purification of protease enzymes using many methods. Studies reporting the purification of protease enzymes in microorganisms are summarized in this review specifically. Using supporting references that have been published in the last ten years, this study attempts to analyze the purification possibilities of protease enzymes, particularly ultrafiltration methods.

Table 1 lists the various purification methods using ammonium sulfate with different grades and devices over the past ten years [17-20, 23-25]. Based on various reports listed in Table 1, purification involves conventional methods such as precipitation with ammonium sulfate or organic solvents to concentrate the enzyme and remove other unwanted proteins. Based on reports, protease enzyme purification is a process that involves several steps to isolate and purify protease enzymes from biological sources. Purification of protease enzymes is a complex process involving various methods to obtain enzymes with high purity and activity. This process is important to ensure that the enzymes produced are of a quality that meets industrial and scientific standards and can be used in various commercial and research applications.

Table 1 shows that studies discussing the purification of protease enzymes in *Metabacillus* sp. bacteria have not been found. In other words, in the last ten years, no studies that discussed the ultrafiltration of protease enzymes as debridement agents This indicates that there is still a significant novelty in research that discusses the purification of *Metabacillus* sp. bacterial enzymes, especially with the ultrafiltration method.

Table 1. Purification method conducted to develop bacterial enzyme as health commodities in the last 10 years (2015-2024).

No	Enzyme	Method	Source of microorganisms	Country	Reference
1	Protease	Purification not specified	*Bacillus subtilis* FBL-1	Korean	[14]
2	Protease	Purification not specified	*B. subtilis* K-5	Brazil	[15]
3	Protease	Ammonium sulfate precipitation and Sephadex G75-120	*B. subtilis* KT004404	Pakistan	[17]
4	Protease	Ammonium sulfate precipitation and Sephadex G-100	*Bacillus* sp. SB12	Tunisia	[18]
5	Protease	Ammonium sulphate precipitation and dialysis	*B. thuringiensis*	Turki	[19]
6	Alkaline Protease	Ammonium sulfate precipitation [50%]	*B. cereus* strain S8	India	[20]
7	Protease	Purification not specified	*Bacillus cereus* LS2B	Indonesia	[21]
8	Protease	Purification not specified	*B. safensis* strain PRN1	India	[22]
9	Protease sulfihidryl	Ammonium sulfate precipitation 20% to 80%	*Bacillus cereus* TD5B	Indonesia	[23]
10	Cellulase	Ammonium sulfate fractionation and dialysis	*B. subtilis* ITBCCB148	Indonesia	[24]
11	Protease	Ammonium sulfate fractionation and dialysis	*B. thuringiensis* HSFI-12	Indonesia	[25]

Another finding that can be drawn from Table 1 is that Asian countries, especially Indonesia, dominate research that discusses bacterial protease purification as a health commodity. Indonesia along with India, Korea, Turkey, Pakistan, and countries from other continents such as Tunisia and Brazil. Table 1 also shows the potential of renewing the purification method using ultrafiltration in these countries. To obtain a pure protease enzyme using the ultrafiltration method in Indonesia has not been widely studied. Therefore, research on ultrafiltration of protease enzymes from the bacterium *Metabacillus* sp. CS-2 can further help in improving the purity and concentration of these enzymes as debridement agents are also very important. In the past ten years, research investigating the purification of enzymes mostly was conducted using ammonium precipitation followed by dialysis and other non-specified methods [17-20, 23-25].

Schematic summarizing the factors contributing to the significance and potential of purification studies by ultrafiltration method of protease enzymes in *Metabacillus* sp. CS-2 bacteria is shown by **Fig 4**. Minimizing risk factors for death due to necrosis, the potential of *Metabacillus* sp. CS-2 bacteria as a source of protease enzymes, the role of proteases as debridement agents in the world of health in Indonesia, and the possibility of new things resulting from purification by ultrafiltration method of protease enzymes in *Metabacillus* sp. CS-2 bacteria. Based on this, it is further recommended to conduct new research on purification by ultrafiltration method of protease enzyme in *Metabacillus* sp. CS-2 bacteria, because it has great potential as a new method.

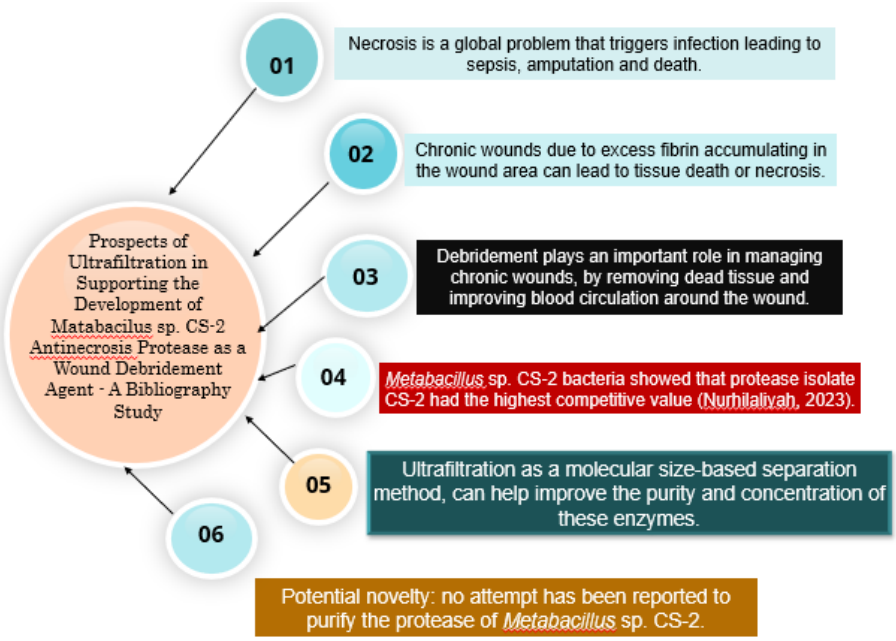

Fig. 4. The significance and potential of studying ultrafiltration purification of the bacterial enzyme protease *Metabacillus* sp. CS-2.

Despite several findings presented, there are some potential limitations of this study. This study focuses primarily on ultrafiltration methods and protease activity in *Metabacillus* sp. CS-2, which might overlook broader applications or different contexts where ultrafiltration is used for other protease enzymes. The study relies solely on three databases (Google Scholar, PubMed, and Dimensions.ai). While these are comprehensive, excluding other major databases like Scopus or Web of Science may limit the breadth of relevant literature and could miss important studies. For this reason, it is suggested to expand the scope of this study in the future by incorporating data from more databases. Also, the search in this study is constrained by specific keywords ("Ultrafiltration of protease enzymes" and related terms). This might exclude relevant studies that use different terminology or focus on closely related topics not captured by the chosen keywords.

The focus of this study on publications from 2015 to 2024 may overlook foundational research or significant earlier studies that could provide valuable context or insights into the development of ultrafiltration techniques and protease enzymes. Also, the use of VOSviewer for network visualization in this study provides a useful overview but may not capture the depth of individual studies, potentially overlooking nuances or specific findings that could influence the interpretation of the data. Hence extending the period of the reviewed literature or publications is necessary for future study in this topic.

4 Conclusion

The findings of this integrative literature review suggested that the purification by ultrafiltration can make bacterial fibrinolytic protease have more potential as a health commodity including a debridement agent by supporting the enzyme's activity. Such a study also offers novelty since it has not been reported. Therefore, to support the use of fibrinolytic proteases in overcoming the threat of necrosis diseases, research on the ultrafiltration of

protease from the *Metabacillus* group of bacteria including strain CS-2 is recommended to be done.

The entire study was planned by SNE and SGAP, with SGAP gathering, evaluating, screening, and summarizing all the articles it had acquired. NR, MSF, and ZBA assess the generated figures, tables, and schemes while examining research bias. SGAP and SNE wrote the majority of the text. NR proofread the document after SGAP initially wrote it.

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