Quality : Jurnal Kesehatan
Volume 18, Nomor 2 Tahun 2024, pp 131-142
pISSN : 1978-4325, eISSN : 2655-2434, DOI: 10.36082/qjk.v18i2.2016

BIOPROSPECT OF BACTERIAL FIBRINOLYTIC PROTEASE FROM BEKASAM OF LONGTAIL TUNA AS ANTITHROMBOTIC AGENT: LITERATURE REVIEW AND BIBLIOGRAPHY STUDY

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Information of Article:

Received: 12 November 2024
Revised:27 November 2024
Accepted: 29 November 2024
Available online: 30 November 2024

Keywords:
Bacterial protease, Bekasam, cardiovascular disease, fibrinolytic protease, VOS viewer

Abstract

Introduction:Cardiovascular disease (CVD) is the leading cause of death worldwide, with thrombosis being a significant risk factor. Fibrinolytic proteases have potential as antithrombotic agents and could be developed into CVD drugs. Indonesia's traditional fermented products, such as fish Bekasam, are rich in microorganisms, including fibrinolytic protease-producing bacteria, yet their potential for CVD treatment is underexplored. Fish paste in Indonesia serves as a protein source and a reservoir of bacterial diversity, which could aid in discovering new antithrombotic agents. Objective: This literature review examines research trends over the past decade on fibrinolytic protease-producing bacteria in traditional fermented products, with a focus on Bekasam made from Longtail Tuna. This fermented food is an alternative source for obtaining bacteria with antithrombotic properties. Methods: Data for this review were sourced from Google Scholar, PubMed, and the dimension.ai database, using the keyword "bacterial fibrinolytic protease" from 2015 to 2024. Visualization of global research trends was performed using VOS viewer software. Results: The review found a scarcity of studies on fibrinolytic proteases from Bekasam bacteria. Lactic acid bacteria involved in Bekasam fermentation possess proteolytic enzymes that degrade fish protein into peptides and amino acids, potentially offering antithrombotic properties. This suggests natural protease sources from traditional fermented foods have significant biomedical potential. Research on fibrinolytic protease-producing bacteria from Bekasam in Indonesia is limited and requires further development. Exploring these local fermented products could yield innovative sources for thrombosis treatment.

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Introduction

Fibrinolytic proteases are essential because they can break down fibrin, a protein that plays an important role in the blood clotting process (R. Singh et al., 2023)(Hazare et al., 2024). Fibrin is formed as part of the body's response to injury, helping to stop bleeding by forming a blood clot (Zou et al., 2023)(Litvinov & Weisel, 2023). However, if fibrin production is excessive or its dissolution is inefficient, abnormal blood clot formation, known as thrombosis, can occur (Ząbczyk et al., 2023). Thrombosis can cause various serious health problems, including cardiovascular disease (CVD) which is the leading disease of death in the world (Alkarithi et al., 2021)(Litvinov & Weisel, 2023). CVD is a disease or disorder that occurs in the cardiovascular system. Therefore, fibrinolytic proteases have great potential as therapeutic agents in the treatment of thrombosis (Diwan et al., 2021)(Hidayati et al., 2021).

Indonesia has a diversity of traditional fermented products in the form of Bekasam (Pfefferkorn et al., 2021) (Song et al., 2023). Bekasam is one of the traditional fermented products that is popular in various regions in Indonesia (Al-baarri & Afifah, 2023)(Setiarto & Herlina, 2024). Made from fish fermented with salt and rice, Bekasam is known to have a distinctive flavor and is rich in microorganisms (Rachmawati et al., 2024). The fermentation process that takes place in making Bekasam can produce various enzymes, including fibrinolytic proteases that are potential for the treatment of CVD but have not been optimally developed (Fitri et al., 2022)(Kurnianto & Aulia, 2023)(Miftachurrochmah, 2024). Fish Bekasam in Indonesia not only serves as a source of protein, but also as a great economic potential as the discovery of anti-thrombosis agents, one of which is Bekasam from Longtail Tuna (Suharto et al., 2024)(Perttola & Kallio, 2024).

Longtail tuna contains omega-3 fatty acids that have the potential to provide benefits in the prevention of cardiovascular diseases, including thrombosis (Monteiro, 2024). The protein content in the digestive organs of Longtail Tuna consists of essential amino acids and contains omega-3, Longtail Tuna also contains a variety of important nutrients needed by the body, such as vitamin D, calcium, potassium, choline, vitamin B, and zinc (Betancor et al., 2022)(Das et al., 2024). The existence of superior microorganisms in Bekasam is an important factor in the process of mass production of enzymes, so efforts to find microorganisms as producers of fibrinolytic protease are very necessary in Indonesia (Herlina & Setiarto, 2024)(Nguyen et al., 2024).

The use of microorganisms from bacteria as a source of enzymes has advantages such as the scale of growth of cells that are easier to increase, faster bacterial growth, growth that is not affected by the season, and its genetic properties can be manipulated (Goforth et al., 2024)(Ruan et al., 2023). The use of enzymes from natural sources has several advantages over synthetic enzymes. Enzymes from traditional fermentation in the form of Bekasam tend to be more compatible with the human biological system, reducing the risk of allergic reactions and unwanted side effects. In addition, the enzyme production process from microorganisms found in Bekasam can be more environmentally friendly and sustainable (Leroy et al., 2023)(Kurnianto et al., 2024a)(Kurnianto et al., 2024b).

Microorganisms involved in Bekasam fermentation can produce various enzymes with diverse biological activities (Mahmud et al., 2023). Fibrinolytic protease from microorganisms found in Longtail Tuna Bekasam is very interesting because it is sourced from natural ingredients that are safer and have no side effects. The potential of fibrinolytic protease from Bekasam Tuna Longtail can be developed into innovative commercial products. The pharmaceutical and biotechnology industries can utilize these findings to produce drugs or supplements that are effective in preventing and treating thrombosis (Luu et al., 2024)(Renaud et al., 2024).

Methods

A bibliography was conducted using the search database http://app.dimensions.ai. The research was conducted using the keyword “fibrinolytic protease bacteria” published in the research range from 2015 to 2024 by searching titles and abstracts. This bibliography aims to show the development of research related to “fibrinolytic protease bacteria”. By using VOS viewer software, it can visualize the bibliometric network or scientific publication data that is needed at this time. Journal reviews were conducted using sources from the *PubMed and Google Scholar* databases published from 2014-2023 that discussed “fibrinolytic protease bacteria associated with traditional fermentation”. The article search used *Medical Subject Title Headings (MeSH)* with several combinations including “fibrinolytic protease”, “fibrinolytic protease enzyme”, “fibrinolytic protease bacteria”, “traditional fermentation bacteria”, “Bekasam”, “Bekasam bacteria”. This journal review aims to determine the traditional fermented fibrinolytic protease-producing bacteria from Bekasam.

2.1 Journal Eligibility Criteria

The determination of journal standardization was based on the inclusion criteria set as follows: (i) traditional fermentation bacteria; (ii) fibrinolytic protease enzyme; (iii) bacterial fibrinolytic protease; (iv) reported in Indonesian or English; (v) search for review journals published in 2015-2024. All journals were obtained using computerized and manual search tools from PubMed and Google Scholar. Journal exclusion criteria in this study were journals that were not related to traditional fermentation bacteria.

2.2 Journal Selections

Journal selection was based on Pigott and Polanin's (2020) guidelines (Pigott & Polanin, 2020) 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.

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 traditional fermented bacterial proteases; and (ii) selective reporting of results. The overall acceptable risk of bias was considered minimal when all requirements were met.

Results

Using the database at http://app.dimensions.ai/ which resulted in 38,402 publications of scientific articles or journals published in the data range 2015 to 2024. Figure. 1 shows the number of journals published on “fibrinolytic protease bacteria” yearly. The VOS viewer software offers a network visualization map to display the overall data. Figure 2 displays the network visualization of 104 terms. For co-occurrence, VOS viewer also offers a density visualization map. Figure 3 displays the density visualization of 104 terms. Figure 1 shows that between 2015 and 2024, there is a decrease in the number of studies conducted on the topic of fibrinolytic protease bacteria. The peaks were in 2018 and 2019. Figures 2 and 3 show the network and index of research on fibrinolytic protease bacteria, but they do not mention Bekasam bacteria capable of producing fibrinolytic protease. Therefore, the research on fibrinolytic protease bacteria related to Bekasam is still scarce.

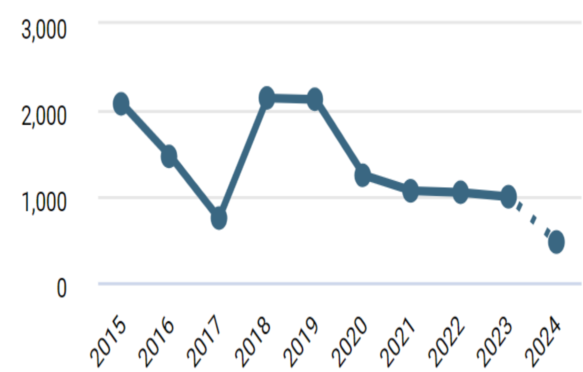

Figure 1. Total of publications on “fibrinolytic protease bacterial” from 2015 to 2024 (source: http://app.dimensions.ai/)

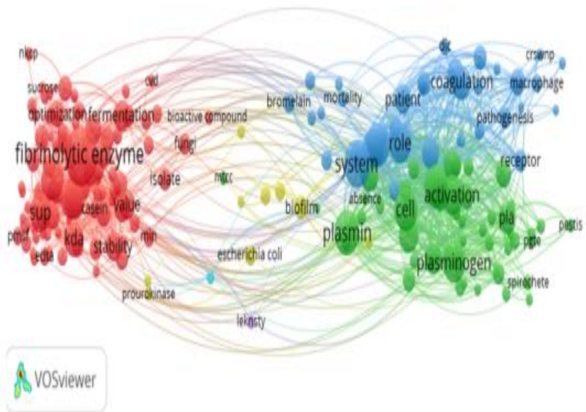

Figure 2. Network Visualization of " fibrinolytic protease bacterial from fermentation Bekasam" Source: VOSviewer and http://app.dimensions.ai/

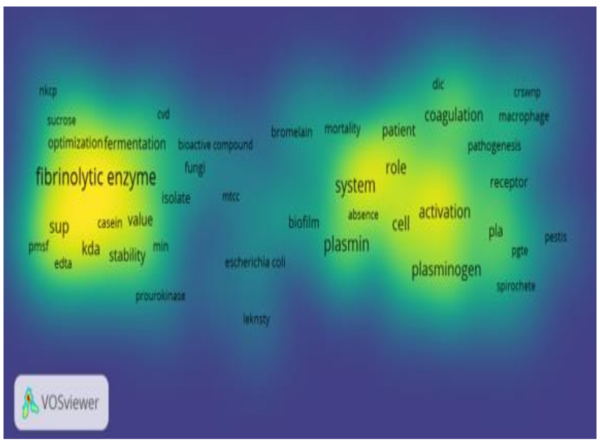

Figure 3. Index Density Visualization "fibrinolytic protease bacterial"
(Source: VOSviewer and http://app.dimensions.ai/)

Table 1. Protein content of various tuna species reported in the last 7 years

Latin Name	Protein content (g)/100 g (wet weight)	Bacterial Species	References
Thunnus thynnus	23-28	*a. Enterobacter cloacaeand* *b. Enterobacter aerogenes,* *c. Klebsiella oxytoca,* d. *Klebsiella pneumoniae* e. *Bacillus* sp. *f. Proteus mirabilis* *g. Serratia liquefaciens*	Astuti et al., 2023; Lopez-Sabater *et al.*, 2020
T. tonggol	24-26	a. *Bacillus* sp. *b. Enterococcus faecalis* *c. Enterobacter aerogenes* d. *Pseudomonas* sp. *e. Klebsiella pneumoniae* *h. Lactobacillus* sp.	Hongpattarakere *et al.*, 2016(Hongpattarakere et al., 2016)
T. alalunga	24-26	*a. Enterobacter,* *b. Klebsiella* sp. *c. Lactobacillus* sp. *d. Proteus* sp. *e. Pseudomonas* sp.	Mahamudin *et al.*, 2016(Mahamudin et al., 2016)
T. albacores	23-25	*a. Pseudomonas* sp. b. *Enterobacteriaceae* sp.	Jääskeläinen *et al.*, 2019
Katsuwonus pelamis	22-25	*a. Pseudomonas* sp. *b. Enterobacteriaceae* sp.	Suzuki *et al.*, 2021
T. obesus	22-25	a. *Acinetobacters* sp. b. *Pseudomonas* sp. c. *Enterococcus faecalis*	Wang & Xie, 2020

Table 2. Fermented foods source of fibrinolytic enzymes reported from around the world in 2011-2020

Fermented Foods	Fibrinolytic Enzyme	Refrences
Shiokara	Katsuwokinase	Purwaeni *et al.*, 2020
Jotgal Korean	Fibrinolitik Enzim 36 kDa (JP-I)	Kim *et al.*, 2020
Tempeh	Tpase	Purwaeni *et al.*, 2020
Moromi	Subtilisin	Syahbanu *et al.*, 2020
Cheonggukjang	AprE176	Raveendran *et al.*, 2018
Ba-bao Douchi	Subtilisin FS33	Ashipala dan He, 2018
Dosa batter	CFR15	Devaraj *et al.*, 2018
Natto	Nattokinase	Biji *et al.*, 2016
Thua nao	Subtilisin NAT	Inatsu *et al.*, 2016
Douche	DFE	Nailufar *et al.*,2016
Pigeon pea	Nattokinase	Nailufar *et al.*, 2016
Kimchi	BsfA	Ahn *et al.*, 2015
Cheonggukjang	NatTK, NatOC, NatWT, dan DFEG169A	Jeong *et al.*, 2015
Kishk	KSK-II	Kotb, 2014
Bovine milk	Streptokinase	Vijayaraghavan, 2014
Keju Sayur	Nattokinase	Seo dan Lee, 2014
Gembus	Fibrinolitik Enzim 20 kDa	Afifah *et al.*, 2014
Doenjang	Subtilin DJ-4	Bhargavi dan Prakasham, 2012
Meju	AprE5-41	Jo *et al.*, 2011
Da Jang	Subtilisin DJ-4	Wei *et al.*, 2011

Table 3. Bacteria produced from Protease-producing Fermented Food Products from Around the World 2015-2022

Fermented Foods	Bacterial Species	Country
Shrimp paste	*Bacillus flexus*	Indonesia
Bekasam *(Oreochromis niloticus)*	*Lactobacillus acidophilus*	Indonesia
Rusip *(Scabra holothurian)*	*a. Staphylococcus hominis* *b. Bacillus aryabhattai* *c. Staphylococcus saprophyticus*	Indonesia
Sea Cucumber *(Holothuria Scabra)*	*Bacillus tequilensis*	Indonesia
Fermentative food preparations (Milks, Fruits, Vegetables and Sea Food)	*a. Bacillus subtilis* *b. Pseudomonas aeruginosa* *c. Alkaligen* sp.	India
Soybean Paste	*Bacillus* sp.	India
Hwangseokae jeotgal (Fish, Shellfish, Shrimp)	*Bacillus* sp.	Korea
Douchi (Black Bean)	*Bacillus* sp.	China
Funazushi and salted crucian carp (Goldfish)	*Bacillus* sp.	Japan
Jotgal (Small shrimp)	*Bacillus* sp.	Korea
Sardines	*a. Entroccocus* sp. *b. Bacillus* sp. *c. Citrobacter* sp.	Pakistan
Douchi (Blak bean)	*B.subtillis*	China
Sea Squirt Jeotgal	*a. Bacillus amyloliquefaciens* *b. Escherichia coli* *c. B. subtilis*	Korea
Sugokai	*Bacillus* sp.	Korea
Shad Fish Soy Sauce *(Trichogaster pectoralis)*	*Pediococcus halophilus*	Indonesia
Sauce Fish	*Bacillus* sp.	Malaysia
Medicinalmushroom/ *Cordyceps militaris*	*Arthrobacter* sp.	China
Doenjang (Soy Beans)	*a. Bacillus* sp. *b. Escherichia coli*	Korea
Kimchi (Korean radish)	*a. Bacillus, Leuconostoc* *b. Propionibacterium, Weissella,* *c. Staphylococcus* sp. *d. Bifidobacterium* *e. E.coli*	Korea
Heshiko and narezushi (Sushi)	*Bacillus* sp.	Japan

Discussion

In the past ten years, many studies have documented fibrinolytic proteases produced by bacteria found in fermented foods, soil, and marine environments around the world (N. Singh & Shera, 2023). Using the supporting references that have been published in the past ten years, this study attempts to analyze the possibility of studying fibrinolytic protease bacteria associated with the traditional fermentation of Bekasam, specifically to produce fibrinolytic protease.

According to reports, Longtail Tuna Bekasam is among rich sources of protein having all the necessary minerals and amino acids. Based on reports, of the five species of Tuna fish, Longtail Tuna has the highest protein content compared to Longfin, Yellowfin, Skipjack, and Bigeye Tuna species. It is interesting to note that the essential metabolites found in Longtail Tuna fish scat, such as proteins, omega-3s, nutrients, and amino acids have been linked to many health benefits for various diseases, including CVD. One of the leading causes of death worldwide is CVD (Astuti et al., 2023)(Lopez-Sabater et al., 2020)(Hongpattarakere et al., 2016)(Mahamudin et al., 2016)(Jääskeläinen et al., 2019).

The protein content of Tuna species reported globally over the past seven years was reviewed in this study (Table 1). The highest protein content of 23-28 g/100g was contained in Bluefin Tuna, which contained the bacterial species Enterobacter sp., *Klebsiela oxytoca*, Bacillus sp., and *Proteus mirabillis*, having the highest protein content among all other reported tunas (Table 1). Longtail and Longfin Tuna were the Tuna species with a high protein content of 24-26 g/100g. Yellowfin Tuna, Skipjack Tuna, and Bigeye Tuna had the highest protein content of 22-25 g/100g (Astuti et al., 2023)(Lopez-Sabater et al., 2020)(Hongpattarakere et al., 2016)(Mahamudin et al., 2016)(Jääskeläinen et al., 2019).

In the group of Tuna fish species widespread throughout Indonesia are processed into traditional fermented products in the form of Bekasam, but the bacteria associated with them and their potential to produce therapeutic enzymes are still very little researched worldwide (Leroy et al., 2023)(Kurnianto et al., 2024b). Given that Tuna fish species have a high diversity index of Indonesian traditional fermented products, it is possible to obtain a wide variety of fibrinolytic protease bacteria from them. Since Tuna species, both Bluefin Tuna, Longtail Tuna, and Longfin Tuna groups have relatively high protein content, it is possible that the bacteria associated with Bekasam from Tuna fish can produce fibrinolytic proteases. The bacterial proteases in question can be classified as serine or metalloproteases, which are well known for their therapeutic properties, especially in the treatment of thrombosis (Song et al., 2023). The blood clot called thrombus formed during the thrombosis process can be lysed by the antithrombotic protease enzyme (Akhtar et al., 2023)(Song et al., 2023).

Table 2 provides information on different types of fermented foods that contain fibrinolytic proteases along with scientific references that support these findings. Some examples of fermented foods mentioned include Shiokara, Jotgal Korean, Tempeh, and Moromi. Shiokara is known to contain the fibrinolytic enzyme Katsuwokinase according to research by Purwaeni et al. (2020) (Purwaeni et al., 2020). Jotgal Korean contains a fibrinolytic enzyme with a molecular weight of 36 kDa (JP-I) [44]. Meanwhile, Tempeh contains the enzyme Tpase (Purwaeni et al., 2020) and Moromi contains Subtilisin (Syahbanu et al., 2020).

Other types of fermented foods also contain different fibrinolytic enzymes. For example, Cheonggukjang contains AprE176 (Raveendran et al., 2018) and Ba-bao Douchi contains Subtilisin FS33 (Ashipala & He, 2018). Fermented foods such as Dosa batter contain CFR15 (Devaraj et al., 2018) and the famous Natto from Japan contains Nattokinase (Biji et al., 2016). Other studies have also found that Thua nao contains Subtilisin NAT (Inatsu et al., 2016) and Kimchi contains BsfA (Ahn et al., 2015). The discovery of these fibrinolytic enzymes shows the great potential of fermented foods in supporting health, especially in helping to break down fibrin in the blood.

Table 3 lists various bacteria from traditional fermented products that have been used as sources of biological activities such as antibacterial, anti-dengue, and enzyme-producing globally over the past ten years (Rinto et al., 2021)(Fuad et al., 2021)(Hidayati, 2021)(Keziah, 2021)(Rajaselvam et al., 2021) Table 3 shows that there are very few studies that address whether there are fibrinolytic protease bacteria from traditional fermented products isolated from Longtail Tuna Bekasam. This indicates that there is still a significant novelty in studies isolating fibrinolytic protease-producing bacteria from Longtail Tuna Bekasam. Another conclusion that can be drawn from Table 3 is that Asian countries, especially Indonesia, dominate research describing the bacterial diversity of traditional fermented products in the development of medicines and health fields. Indonesia followed by Korea, China, India, and Japan show the potential of bacterial diversity from traditional fermented products that have the potential to produce antithrombotic agents.

To obtain fibrinolytic protease using microorganisms or bacteria that have great economic potential with high diversity of traditional fermentation products in Indonesia has not been widely studied. Therefore, research on how Indonesia's diversity of traditional fermented products in the form of Bekasam can further assist in the identification of bacteria that produce fibrinolytic proteases as antithrombotic agents is also very important. In the past ten years, very limited research investigating the synthesis of fibrinolytic proteases from tissues or bacteria associated with the Bekasam product of Longtail Tuna Bekasam has been reported (Kim et al., 2020) (Meng et al., 2021) (Ito, 2020) (Nawaz et al., 2020) (Hu et al., 2019) (Yao et al., 2019)(Sari, 2018).

A schematic summarizing the factors contributing to the significance and potential of the study of Indonesian product diversity in the form of traditional fermentation rich in microorganisms and producing fibrinolytic protease-producing bacteria from Longtail Tuna Bekasam is depicted in Figure 4. Minimizing risk factors for death from thrombosis, the potential of Longtail Tuna Bekasam as a source of therapeutic metabolites such as proteins, the role of antithrombotic fibrinolytic proteases in medicine, the potential of Indonesian diversity in the form of fermented food products, and the possibility of new things resulting from the identification of new antithrombotic fibrinolytic protease-producing bacteria from Longtail Tuna Bekasam. Based on this (Figure 4), it is further recommended to research to explore the diversity of food fermentation products from fibrinolytic protease-producing bacteria that have been isolated from Bekasam Longtail Tuna, because it has great potential to produce identification of new antithrombotic agents.

According to this integrative literature review, there is a high probability that the bacteria isolated from Longtail Tuna fish Bekasam have high potential diversity of traditional fermented products, which may help the discovery of new sources of fibrinolytic proteases. Investigating new sources of antithrombosis bacteria to treat CVD is made possible by studies such as this. Therefore, to support the use of fibrinolytic proteases in overcoming the threat of thrombosis diseases, research on fibrinolytic protease-producing bacteria associated with traditional fermented products from Bekasam Longtail Tuna in Indonesia has not received much attention and is recommended to be carried out.

Conclusions and Recommendations

The diversity of fermented products from Indonesia that produce fibrinolytic protease bacteria isolated from Bekasam Longtail Tuna has significant potential to contribute to the identification of new sources of fibrinolytic protease for antithrombosis that have never been reported. For the utilization and development of fibrinolytic protease to treat thrombosis, it is recommended to research fibrinolytic protease bacteria from Bekasam Longtail Tuna which is still very limited in research.

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