A Literature Review on the Potential of the Biodiversity of Thrombolytic Protease-Producing Bacteria Isolated from Brown Seaweeds *Chnoospora* sp.

Nurhilaliyah Nurhilaliyah[1] Stalis Norma Ethica[1,*] Wikanastri Hersoelistyorini[2] Aditya Rahman Ernanto[3] Wijanarka Wijanarka[4]

[1]*Magister Study Program of Clinical Laboratory Science, Universitas Muhammadiyah Semarang, Central Java, Indonesia 50273*
[2]*Food Technology Study Program, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Central Java, Indonesia*
[3]*Diploma Program of Medical Laboratory Technology, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Central Java, Indonesia*
[4]*Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Diponegoro, Central Java, Indonesia*
[*]*Corresponding author: norma@unimus.ac.id*

ABSTRACT
Bacterial proteases with antithrombotic activities can be used to treat cardiovascular disease (CVD), one of the leading causes of death in the world. Indonesia is known for its high marine biodiversity, including its brown seaweed varieties and their symbiotic bacteria. The high biodiversity of proteolytic bacteria associated with marine organisms provides an excellent opportunity to obtain novel antithrombotic agents. This literature review aimed to highlight factors supporting the potential of the biodiversity of thrombolytic protease-producing bacteria isolated from Indonesian brown macroalgae *Chnoospora* sp. as an alternative source of antithrombotic proteases derived from its associated bacteria. Results of this integrative review targeting publications in the last decade showed that studies aiming to explore the production of antithrombotic enzymes from the tissues or bacteria associated with *Chnoospora* sp. have not been reported. Biodiversity studies related to the antithrombotic protease-producing bacteria associated with *Chnoospora* sp. are also barely found based on the collected data. Meanwhile, the relatively high protein content of brown algae, including *Chnoospora* sp. could be an indication that bacteria associated with the seaweed may actively produce proteases, some of which may have antithrombotic activities. In conclusion, the potential of the biodiversity of bacteria isolated from brown seaweeds *Chnoospora* sp. is quite high to support the finding of new sources of thrombolytic agents. Thus, it is recommended to conduct a study exploring the fibrinolytic protease-producing bacteria associated with the brown algae, which is barely studied, yet abundant in Indonesia, to support the application of protease to treat thrombosis disease.

Keywords: *Antithrombosis agent, Bacterial biodiversity, Brown algae, Chnoospora* sp.

1. INTRODUCTION

The protease enzyme is among the enzymes whose applications are very broad in the health sector. The use of protease enzymes in the health industry is very much in demand, one of which is antithrombotic enzymes. Antithrombotic enzymes, including fibrinolytic proteases, can be used to treat cardiovascular disease (CVD), which is one of the leading causes of death in the world. The main death risk of CVD comes mainly from thrombosis. Thrombosis is a blood clot within blood vessels limiting or blocking the flow of blood. Both acute venous and arterial thromboses are the most frequent cause of death in CVD. The mortality rate is diverse with the acuity and location of thrombosis [1-2].

Indonesia is known for its high marine biodiversity, including its brown seaweed varieties and their symbiotic bacteria. In particular, abundant marine macroalgal organisms, including bacteria, can be found almost in all Indonesian coastal waters [1]. Algae or seaweeds are a rich source of bioactive compounds such as polysaccharides, unsaturated fatty acids, polyphenolic compounds, peptides, vitamins, essential minerals, and enzymes, which are also known to have anticoagulant, antiviral, antioxidant, anti-cancer, and immunomodulatory activities [2]. Seaweeds are one of Indonesia's most abundant aquaculture commodities and are often associated with symbiotic microorganisms [3-4]. A group of microorganisms frequently found in seaweed is bacteria. The bacteria living in seaweed tissue are endophytic bacteria. Endophytic bacteria are known to have many secondary metabolite activities, one of which is enzymatic activity [5-8].

Antithrombotic/fibrinolytic enzymes can be sourced from microorganisms, bacteria [12-13]. Proteolytic bacteria are among the most potential microorganisms to produce antithrombotic proteases. Other sources of fibrinolytic proteases mostly reported include other various animal tissues such as snakes and earthworms [14]. Yet, those from marine organisms associated with brown algae with high abundance in Indonesia are barely reported. The use of microorganisms as enzyme producers has many advantages: easy and fast microbial cells to grow, production scale is easier to increase, and production conditions are not dependent of the seasons [14-15].

Chnoospora sp. (Figure 1) belongs to the Phaeophyceae group (the brown algae) of seaweeds [16]. It was reported to have a relatively high protein content compared to other brown algae found in Indonesia. This literature review aimed to highlight factors supporting the potential of the biodiversity of thrombolytic protease-producing bacteria isolated from Indonesian brown seaweeds *Chnoospora* sp. as an alternative source of fibrinolytic enzymes derived from its associated bacteria. The high biodiversity of protein in Indonesia provides a great opportunity to obtain this fibrinolytic protease enzymes through the utilization of their potentially producing bacteria associated with *Chnoospora* sp. such as fibrinolytic proteases can be developed as the candidate of an antithrombotic agent.

2. METHODS

The strategy used by this integrative literature review is by setting a specific research question: What is the potential to obtain the fibrinolytic proteases with antithrombosis activity from the producing bacteria associated with *Chnoospora* sp. from Indonesia? The next is to set the criteria of studies that could be the most helpful and would make the greatest contribution to answer the research question [18].

Figure 1. The brown seaweed *Chnoospora* sp. growing together with the green seaweed *Enteromotpha* sp (Photographed by Kariyawasam, 2016) [17].

2.1. Screening the literature

To keep up with the updates, the sources of literature were limited to articles, books, and scientific reports from the PubMed and Google Scholar databases published in the last 10 years. Search terms in the forms of words or phrases used to access relevant articles, reports and books included: "brown algae, "Chnoospora", "protein content/ level", "protease-producing bacteria", "algal-symbiont bacteria", "fibrinolytic protease" along with their synonyms and derivatives such as "Phaeophyceae", "algal endophyte bacteria", etc.

2.2 Exclusion criteria of the study

Limitations were set to the obtained literature from the screening set to focus more on the answer to the set research question. For this purpose, the exclusion criteria were set, including: (i) articles that are written related with keywords, yet not linked to the solution of the research question. For examples, studies related to the fungal association of brown algae, or protease-producing bacteria from green or brown algae. (ii) articles that are not related with the utilization of protease as antithrombosis / thrombolytic agent.

2.3. Quality assessment of literature review

The quality of the conducted literature review was assessed following the previous guidelines [18-19]. The

Table 1. Protein levels of seaweeds determined by Kjeldahl method reported in the last 5 years

No.	Algal species	Protein level (dry weight, %)	References
1.	*Sargassum wightii*	1.48	[23]
2.	*Padina gymnospora*	17.0	[23]
3.	*Turbinaria ornata*	14,68	[24]
4.	*Sargassum tenerrimum*	2.4	[23]
5.	*Turbinaria ornata*	14.6	[23]
6.	*Turbinaria conoides*	3,99	[25]
7.	*Sargassum odontocarpum*	16.0	[23]
8.	*Padina boryana*	8.32	[23]
9.	*Chnoospora* sp.	12.3	[26]
10.	*Chnoospora minima*	11.3	[23]
11.	*Sargassum classifolium*	5,19	[27]
12.	*Ericaria amentacea*	141.4	[23]

guidelines included 4 important steps: (i) Design, (ii) Conduct, (iii) Data abstraction and analysis, and (iv) Structuring and writing the review [20]

3. RESULT AND DISCUSSION

Numerous studies have reported bacteria producing antithrombotic proteases from fermented food worldwide in the last decade. This review, in particular, summarizes studies reporting such bacteria isolated only from the marine environment. This study aimed to analyze the prospect of study relate to proteolytic bacteria associated with brown algae *Chnoospora* sp. to produce novel antithrombotic protease based on supporting references published in the last decade.

Brown algae have been reported as one of the highly rich protein sources containing all essential amino acids and minerals. It was reported that among brown, red, and green seaweeds, the maximum protein levels were recorded in brown seaweeds members and a minimum in green seaweeds members [21]. Interestingly, brown algae contain essential metabolites such as proteins, carotenoids, polysaccharides, and phlorotannins, which have been associated with multiple health benefits for different diseases, including cardiovascular diseases (CVD). CVD is known as one of the main causes of death globally [22].

This review highlighted protein contents (dry weight) of brown algae reported worldwide in the last five years (Table 1) determined using the Kjeldahl method. Data in Table 1 shows that the highest protein content reported from brown seaweeds is *Ericaria amentacea* collected from the middle-east with 141.4 g/Kg protein content. The highest protein content in red algae group is 58.5 g/Kg reported in *Corallina elongate*. Meanwhile, in the green algae group, the highest protein level belongs to *Caulerpa racemosa* with 18.3 mg/g [23].

Table 2. Studies reported the use of marine bacteria producing antithrombotic proteases in the last 5 years

No.	Bacterial species	Source of marine bacteria	Country	References
1.	*Bacillus sphaericus*	Mangrove ecosystem	Indonesia	[31]
2.	*Bacillus subtilis*	Unspecified	Indonesia	[32]
3.	*Serratia marcescens*	Seawater	India	[33]
4.	*Streptomyces radiopugnans*	Sea sponge	India	[34]
5.	*Fictibacillus* sp.	Seawater	India	[35]
6.	*Bacillus flexus*	Algal sediment	India	[36]
7.	*Bacillus megaterium*	Seawater	India	[37]
8.	*Serratia rubidaea*	Seawater	India	[38]
9.	*Bacillus invictae*	Seawater	Tunisia	[39]
10.	*Pseudomonas aeruginosa*	Seawater	India	[40]
11.	*Enterococcus gallinarum*	Sardine	Pakistan	[41]
12.	*Bacillus licheniformis*	Sea sponge	India	[42]
13.	*Staphylococcus hominis*	Sea cucumber	Indonesia	[43]
14.	*Bacillus aryabhattai*	Sea cucumber	Indonesia	[43]
15.	*Staphylococcus saprophyticus*	Sea cucumber	Indonesia	[43]
16.	*Bacillus flexus*	Saltpan	India	[44]
17	*Bacillus tequilensis*	Sea cucumber	Indonesia	[45]

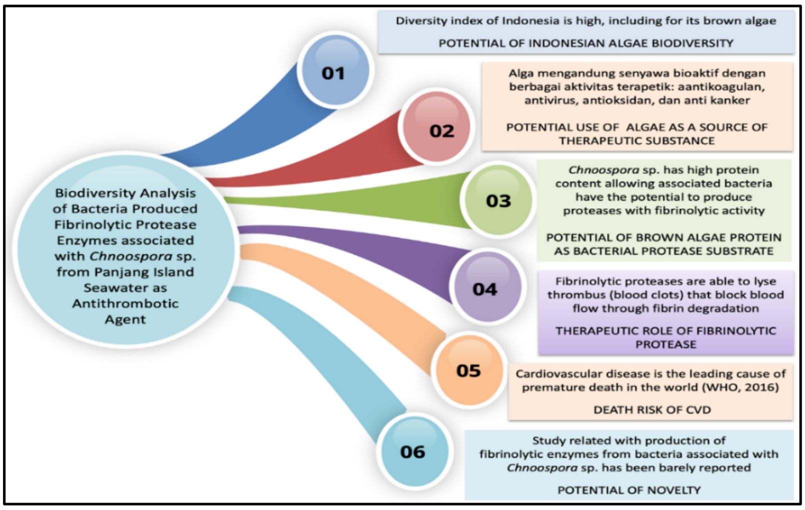

Figure 2. Factors contributing the importance and prospect of biodiversity study on antithrombotic protease producing bacteria associated with brown seaweed *Chnoospora* sp

In the brown algae group, *Chnoospora* sp. is abundantly available in Indonesia yet is barely studied in the world in terms of its associated bacteria and their potential in producing therapeutic enzymes [23-25]. The high index of Indonesian marine biodiversity infers that high variety of bacteria could be obtained from Indonesian marine algae such as *Chnoospora* sp. The protein content of this brown macroalgae has been reported, ranging from 11.3-12.3 % mg/g [23, 26]. Such relatively high protein content of *Chnoospora* sp. offered potential that bacteria associated with the seaweed may produce proteases. Such bacterial proteases may belong to serine or metalloproteases known to have therapeutic activities, particularly those beneficial for treating thrombosis. Antithrombotic protease enzymes can lyse thrombus (blood clots) formed in the thrombosis process through fibrin degradation [28].

Other members of brown algae abundant in Indonesia include *Padina* sp., *Turbinaria* sp., and *Sargassum* sp. They were reported to have higher protein content than *Chnospora* sp. However, bacteria associated with Padina sp., *Turbinaria* sp., and Sargassum have been extensively studied [20, 29-30]. *Ericaria amentacea*, on the other hand, is known to have the highest level of protein in the group of brown algae, even when compared to those of green and red algae [23]. Nevertheless, *E. amentacea*, formerly known as *Cystoseira stricta*, is hardly found in Indonesia. Table 2 shows various marine bacteria and their origin as sources of antithrombotic proteases worldwide in the last 5 years. As seen in Table 2 no studies explicitly listed mentioned whether any of these marine bacteria were isolated from brown algae [31-45]. It means that the originality of a study isolating marine bacteria producing antithrombotic proteases from brown algae is still high. Another finding, which can be generated from Table 2 is that studies reporting the diversity of marine bacteria in relation to antithrombotic agent development are dominated by Asian countries, particularly Indonesia, followed by India and China. It shows that the potential of Indonesian marine bacterial diversity in producing antithrombotic potential is quite promising. However, the high biodiversity of brown algae in Indonesia has not been adequately explored to obtain antithrombotic enzymes by utilizing potential brown algal-associated microorganisms/ bacteria. Thus, it is also important to study how the biodiversity of brown algae in Indonesia could contribute more to the discovery of antithrombotic protease-producing bacteria. This integrative review targeting publications in the last decade showed that studies aiming to explore the production of antithrombotic enzymes from the tissues or bacteria associated with brown algae, including *Chnoospora* sp., are barely found [31-45]. Biodiversity studies related to the antithrombotic protease-producing bacteria associated with *Chnoospora* sp. are also hardly found based on the collected data. Meanwhile, the relatively high protein content of brown algae, including *Chnoospora* sp., could indicate that bacteria associated with the seaweed may actively produce proteases, which some of them could have antithrombotic activities.

Figure 2 shows a scheme summarizing factors contributing to the importance and prospect of biodiversity study on antithrombotic protease-producing bacteria associated with brown seaweed *Chnoospora* sp. The factors include death risk factor of thrombosis, the potential of brown algae as a source of therapeutic metabolites including proteins, the therapeutic role of antithrombotic proteases, potential of Indonesian algal biodiversity, and potential of novelty coming from the discovery of new antithrombotic protease-producing bacteria associated with brown algae, in particular *Chnoospora* sp. Based on these (Figure 2), a study to investigate the biodiversity of bacteria producing antithrombotic proteases isolated from Indonesian brown seaweeds *Chnoospora* sp. should be done as it offers a high chance of leading to the discovery of novel antithrombotic agents.

It could be concluded from this integrative literature review that the potential of the biodiversity of bacteria isolated from brown seaweeds *Chnoospora* sp. is quite high to support the finding of new sources of thrombolytic proteases. Such a study offers novelty during efforts in seeking new sources of antithrombotic bacteria to treat CVDs. Thus, it is recommended to conduct a study exploring the fibrinolytic protease-producing bacteria associated with the brown algae, which is barely studied, yet abundant in Indonesia, to support the application of protease to threaten thrombosis disease.

AUTHORS' CONTRIBUTIONS

NN and SNE brought the original review idea, WW collected all of obtained references, while ARE and WH summarised and selected the literatures based on exclusion criteria. SNE and WW evaluated the tables and schemes generated, as well as analysed the bias of the study. The main text was prepared by NN and SNE. The manuscript was initially written by NN, and then proofread by SNE.

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