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Antithrombotic Activity and Hemolysis Pattern of Fibrinolytic Protease-Producing Bacterial Isolates from the Coast of Tanjung Dewa, South Kalimantan

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Abstract: Cardiovascular disease is a leading cause of death worldwide, with thrombosis playing a key role in the pathological process. Conventional antithrombotic therapy often causes side effects, necessitating alternatives based on biological resources. Marine microorganisms are known to produce fibrinolytic protease enzymes that can degrade fibrin and prevent blood clot formation. This study aimed to evaluate the differences in antithrombotic activity and hemolysis patterns of fibrinolytic protease-producing bacterial isolates obtained from the coast of Tanjung Dewa Beach, South Kalimantan. A total of 15 isolates were obtained from seawater, beach sand, mollusks, crabs, and barnacles, and then subjected to colony morphology identification, Gram staining, and spore observation. Proteolytic activity testing using Skim Milk Agar media showed that all isolates had proteolytic activity with varying indices, with PSR1 showing the highest index (3.4). Furthermore, fibrinolytic testing using the fibrin plate assay method showed that 10 isolates were capable of degrading fibrin, with AL8 and SP2 showing the highest fibrinolytic indices (3.12 and 3.11, respectively). Antithrombotic testing using the clot lysis method revealed that AL7 and SP1 exhibited the highest lysis percentages (82.05% and 88.88%, respectively). Anticoagulant activity, as determined by the Lee-White method, showed that SP2 significantly prolonged the coagulation time (142 seconds, 49.65%). Hemolysis pattern testing revealed variations in activity, with AL8 and PSR1 classified as gamma-hemolytic and therefore potentially safer. Statistical analysis showed no significant differences between isolates in terms of antithrombotic activity, anticoagulant activity, or hemolysis pattern ($p > 0.05$). These findings suggest that coastal bacterial isolates from Tanjung Dewa Beach, particularly AL8 and SP2, have the potential to serve as safe, natural antithrombotic agents, supporting the development of biomedical therapies for cardiovascular disease.
Keywords: Anticoagulant; antithrombosis; fibrinolytic bacteria; hemolysis; Tanjung Dewa Beach.

INTRODUCTION

Thrombosis is a condition in which a blood clot forms in a blood vessel, restricting blood flow. Acute venous and arterial thrombosis is the most common cause of death in both developed and developing countries, with mortality rates varying depending on the location and severity of the thrombosis. Myocardial infarction and Cerebrovascular Accident (CVA) are reported to account for the highest proportion of thrombosis-related deaths (Deniyati, 2016). With the aging population and changing lifestyles, thrombotic diseases are increasingly becoming a serious health problem (Roth et al., 2020). Cardiovascular disease, including stroke and heart disease, is

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listed as the leading cause of death globally, including in Indonesia. This disease is categorized as the most common non-communicable disease worldwide and is responsible for 20.5 million deaths in 2021 (World Heart Federation, 2023). In Southeast Asia, the number of deaths from cardiovascular disease in 2023 is estimated to reach 3.9 million per year (WHO, 2023). National data also shows a high burden of cardiovascular disease. According to the 2023 Indonesian Health Survey (SKI), the prevalence of hypertension among people aged 18 and over reached 30.8%. The prevalence of stroke was reported at 8.3%, while the prevalence of coronary heart disease was recorded at 0.85% (SKI, 2023). In South Kalimantan, the prevalence of cardiovascular disease was reported at 0.66%, with an estimated 28,380 sufferers by 2023 (SKI, 2023).

Deaths from cardiovascular disease are often associated with blood clot formation (Kartal, 2014). Thrombosis therapy is a priority in the treatment of cardiovascular disease (Marder, 2009). However, the use of currently available antithrombotic drugs is known to cause various side effects, including allergic reactions (Altaf et al., 2021). In the search for alternatives, proteolytic enzymes with fibrinolytic activity have begun to receive attention. Proteolytic enzymes function to break down proteins, including components that form blood clots, thus effectively dissolving thrombi (Boon, 2020). Several studies have demonstrated that fibrinolytic enzymes from microorganisms can dissolve blood clots without causing significant side effects, offering advantages such as low production costs, mass availability, and stable activity under various environmental conditions (Kotb, 2014; Sharma et al., 2020; Vijayaraghavan & Prakash Vincent, 2015).

Several bacteria from aquatic environments have been successfully identified as producers of fibrinolytic enzymes, including: *Bacillus subtilis* A26, *B. subtilis* ICTF-1, *B. subtilis* HQS-3 (Agrebi et al., 2009; Huang et al., 2013; Mahajan et al., 2012), and *Bacillus aureus* from Papuma Beach, Jember (Sri Pananjung et al., 2016). Significant thrombolytic activity was also reported in the enzyme extract of the isolate *Staphylococcus of man* HSFT-2, a product derived from fermented sand sea cucumber muscle tissue, which slows blood clotting by 12% compared to the control (Afriansyah, 2024). Other isolates, such as FD-09, FD-13, and FD-14 from fermented sea cucumber muscle tissue, also slow blood clotting by 12% compared to the control (Afriansyah, 2024). *Dictyota sp.*, showing a fibrinolytic index ranging from 2.0–2.9 (Afriansyah & Ethica, 2023). In fact, isolates WU 021055 and WU 021012 from Papuma Beach were reported to have very high fibrinolytic indexes, 11 and 10, respectively (Setiawan et al., 2016).

Tanjung Dewa Beach, South Kalimantan, is a rocky coastal area known for its high microbial density. The physical and chemical conditions of the waters, such as a salinity of 24.8‰, a temperature of 28.5°C, a pH of 7.5, a DO of 5.6 mg/L, and a depth of 10.9 cm, support the growth of organisms, including microbes (Abdurrahman et al., 2020). The biodiversity in this area has the potential to be a source of new bacterial isolates with important biological activities, including fibrinolytic enzymes.

However, not all fibrinolytic bacteria are safe for use as antithrombotic agents because some also produce hemolysin toxins. Hemolysin is a toxic compound capable of destroying red blood cells and is closely related to the expression of virulence factors (Rahmi et al., 2015; Monod, 2008). Therefore, hemolytic screening is necessary to assess the safety of fibrinolytic bacteria before they are developed as therapeutic candidates (Asril & Leksikowati, 2019).

To date, information on fibrinolytic enzyme-producing bacteria from aquatic ecosystems in South Kalimantan remains very limited. A comprehensive study that

assesses not only fibrinolytic capacity but also the bacteria's hemolysis patterns is a crucial step in obtaining isolates that are both potent and safe for use. Therefore, this study aims to determine this. Differences in antithrombotic activity and hemolysis patterns of fibrinolytic protease-producing bacterial isolates originating from Tanjung Dewa Beach, South Kalimantan.

MATERIALS AND METHODS

This study was an in vitro cross-sectional laboratory study. The research stages included sampling and isolating bacteria from the coastal environment, testing proteolytic and fibrinolytic activity, and further testing of antithrombotic, anticoagulation, and hemolysis patterns of selected isolates. The research was conducted at the Microbiology Laboratory of the Medical Laboratory Technology Department, Ministry of Health Polytechnic of Banjarmasin, from January to December 2025, and received ethical approval from the Health Research Ethics Committee of the Ministry of Health Polytechnic of Banjarmasin under number 257/KEPK-PKB/2025.

The research samples consisted of beach sand, marine biota, and seawater collected from the Tanjung Dewa Beach area in South Kalimantan. Sampling was carried out using purposive sampling. Bacterial isolation was performed using a serial dilution method, followed by cultivation on Nutrient Agar media and incubation at 37°C for 24–48 hours to obtain pure colonies (Cappuccino & Sherman, 2014). Initial identification of isolates was performed through colony morphological characterization and Gram staining, while confirmation of cellular morphology used the Schaeffer-Fulton spore staining method (Amaliah et al., 2018; Cahyaningrum et al., 2021; Schaeffer & Fulton, 1933).

Proteolytic activity testing was performed by growing the isolates on Skim Milk Agar (SMA) media containing 1% skim milk. Incubation was carried out at 37°C for 48 hours, and then the formation of a clear zone around the colony was observed. Proteolytic activity was determined by calculating the proteolytic index, which is the ratio of the diameter of the clear zone to the diameter of the colony (Hidayati et al., 2021).

Fibrinolytic activity was tested using fibrin agar media prepared by dissolving fibrinogen and agar in phosphate buffer, followed by the addition of thrombin to form a fibrin matrix, as described in the methods of Muisristanto and Poernomo (2015) and Peng et al. (2003). A suspension of bacteria or crude enzymes was dropped into the wells in the media, then incubated at 37°C for 18–24 hours. Fibrinolytic activity was indicated by the formation of a clear zone around the well due to fibrin degradation. The diameter of the zone was then measured, and the fibrinolytic index was calculated as the ratio of the diameter of the clear zone to the diameter of the well, as described by Setiawan et al. (2016). Crude enzyme extraction was carried out by growing the isolate in Skim Milk Broth media, incubated at 37°C with 150 rpm agitation for 48 hours, then the culture was centrifuged at 10,000 rpm for 15 minutes to obtain the supernatant, which was used as a source of fibrinolytic enzymes (Baehaki et al., 2011).

Antithrombotic activity testing was performed using the clot lysis method, as described by Prasad et al. (2006) and Nadea et al. (2023). Fresh venous blood samples (1 mL each) were placed in sterile Eppendorf tubes and incubated at 37°C for 45 minutes until complete clot formation. The supernatant was carefully removed, and 100 µL of the extracted crude enzyme was added to each tube. Streptokinase (100 µL; 30,000 IU/mL) was used as the positive control, while phosphate-buffered

saline (PBS) served as the negative control. The tubes were incubated again at 37°C for 90 minutes, after which the percentage of clot lysis was calculated by comparing the clot weights before and after treatment. A higher percentage indicated greater fibrinolytic (antithrombotic) activity.

Anticoagulation testing is performed using two methods. In the modified Lee-White method, 1 mL of fresh blood is mixed with 100 µL of crude enzyme in a sterile test tube. The tube is then tilted every 30 seconds to observe the formation of fibrin. Clotting time is recorded from the moment of mixing until a stable clot forms (Gandasoebrata, 1992; Tangkery et al., 2013; Afriansyah, 2024). The second method utilizes blood smear examination with May-Grünwald-Giemsa staining. Fresh blood treated with crude enzyme is made into a thin smear, fixed, and then stained with May-Grünwald solution for 5 minutes and Giemsa for 15 minutes. The preparation is then observed under a light microscope to assess changes in erythrocyte morphology and platelet distribution, which can indicate an anticoagulation effect (Geneser, 1994; Tangkery et al., 2013).

Hemolysis patterns were determined by growing isolates on Blood Agar Plate (BAP) media containing 5% sheep blood. Colonies were incubated at 37°C for 18–24 hours, and then the zone of hemolysis around the colony was observed. A clear zone indicates β-hemolysis, a greenish zone indicates α-hemolysis, while the absence of a hemolysis zone indicates γ-hemolysis or non-hemolytic (Turista et al., 2019).

The research data, including proteolytic index, fibrinolytic index, percentage of antithrombotic activity, coagulation time, and hemolysis pattern, were then analyzed through editing, coding, entry, and data cleaning. Data normality was assessed using the Shapiro-Wilk test, and homogeneity was evaluated using the Levene test. Although the data were normally distributed, the variance was not homogeneous; therefore, the non-parametric Kruskal–Wallis test was used to determine significant differences between groups (Ledolter et al., 2020; Quraisy, 2020).

RESULTS AND DISCUSSION

Overview of Sampling Locations

Tanjung Dewa Beach is located in Tanjung Dewa Village, Panyipatan District, Tanah Laut Regency, South Kalimantan Province, with coordinates -4.063711 South Latitude and 114.626769 East Longitude. It is accessible by land, approximately 40 km from Pelaihari City and approximately 104 km from Banjarmasin City. Tanjung Dewa Beach is characterized by its sandy and rocky areas, featuring distinctive crevices, and is surrounded by dense mangrove vegetation. This ecosystem supports the existence of a diverse range of microorganisms that have the potential to produce bioactive metabolites.

Isolation and Purification of Coastal Bacteria of Tanjung Dewa Beach

From the initial isolation results, 15 bacterial strains were obtained based on the presence of a clear zone around the colony on Skim Milk Agar (SMA) media and macroscopic morphological differences. Isolates were coded according to sample source, including seawater (AL1–AL8), beach sand (PSR1–PSR3), sand snails (SP1–SP2), sand crabs (KP1), and barnacles (TP1). Colony morphology observations included shape, edge, color, elevation, and consistency (Table 1). In general, the observed colony shapes were circular and irregular. Most isolates had flat edges, but there were some variations, such as serrated (PSR3, AL4) or grooved (AL2) edges. Colony colors also varied, ranging from white, cream, and yellow to greenish. Most colonies were convex, except for PSR3, which was flat.

Table 1. Results of Bacterial Isolate Colony Morphology

Isolate Code	Shape	Edge	Color	Elevation	Consistency
AL 1	Round	Flat	Yellowish cream	Convex	Smooth
AL 2	Round	Notched	Greenish white	Convex	Smooth
AL 3	Round	Flat	Yellowish cream	Convex	Smooth
AL 4	Round	Serrated	Cream	Convex	Smooth
AL 5	Round	Flat	Yellow	Convex	Slimy
AL 6	Round	Flat	Cream	Convex	Smooth
AL 7	Round	Flat	Greenish white	Convex	Smooth
AL 8	Round	Flat	Cream	Convex	Smooth
PSR 1	Round	Flat	Yellow	Convex	Smooth
PSR 2	Round	Flat	Creamy white	Convex	Smooth
PSR 3	Irregular	Serrated	Creamy white	Convex	Dry
SP 1	Round	Flat	Greenish white	Flat	Smooth
SP 2	Round	Flat	Creamy white	Convex	Slimy
KP 1	Round	Flat	Creamy white	Convex	Smooth
TP 1	Round	Flat	Creamy white	Convex	Smooth

Table 2. Gram Staining and Spore Staining Results

Code Isolate	Bacterial Form	Spore	Gram Properties
AL 1	Coccus	Negative (-)	Positive (+)
AL 2	Bacil	Negative (-)	Negative (-)
AL 3	Bacil	Negative (-)	Negative (-)
AL 4	Bacil	Positive (+)	Positive (+)
AL 5	Bacil	Negative (-)	Negative (-)
AL 6	Bacil	Positive (+)	Positive (+)
AL 7	Bacil	Negative (-)	Negative (-)
AL 8	Coccobacil	Negative (-)	Negative (-)
PSR 1	Polimorf	Negative (-)	Negative (-)
PSR 2	Bacil	Positive (+)	Positive (+)
PSR 3	Bacil	Positive (+)	Positive (+)
SP 1	Bacil	Negative (-)	Negative (-)
SP 2	Coccobacil	Negative (-)	Negative (-)
KP 1	Bacil	Positive (+)	Positive (+)
TP 1	Bacil	Negative (-)	Negative (-)

The results of Gram staining and spore observation are shown in Table 2. Variations in cell shape were found, including cocci, bacilli, coccobacilli, and polymorphs. Most isolates were Gram-negative, except for a few (AL1, AL4, AL6, PSR2, PSR3, KP1), which were Gram-positive. The majority of isolates did not form spores; however, some isolates exhibited the ability to sporulate.

Proteolytic Activity Test

A total of 15 isolates were tested on Skim Milk Agar (SMA) media to detect their proteolytic activity. The formation of a clear zone around the colony indicates proteolytic activity. All isolates showed proteolytic activity with varying index values Table 3. Isolate PSR1 had the highest proteolytic index of 3.4, followed by SP1 (2.75), AL7 (2.55), and AL2 (2.14). The lowest proteolytic index was recorded in TP1 at 1.45. These results indicate differences in protein degradation ability between isolates.

Table 3. Proteolytic Index Results of Bacterial Isolates

Isolate Bacteria	Diameter Clear Zone (mm)	Diameter Colony (mm)	Proteolytic Index
AL 1	14	8	1.75
AL 2	15	7	2.14
AL 3	9	6	1.5
AL 4	17	10	1.7
AL 5	18	10	1.8
AL 6	21	13	1.61
AL 7	23	9	2.55
AL 8	13	9	1.44
PSR 1	17	5	3.4
PSR 2	23	14	1.64
PSR 3	20	14	1.42
SP 1	22	8	2.75
SP 2	20	13	1.53
KP 1	23	15	1.53
TP 1	16	11	1.45

Fibrinolytic Activity Test

The fibrinolytic test was conducted using the fibrin plate assay method. Of the 15 isolates, only 10 were able to form a clear zone, indicating fibrinolytic activity (Table 4). The highest fibrinolytic index was shown by AL8 (3.12) and SP2 (3.11), surpassing the positive control (2.5). In contrast, isolates AL1, AL3, AL4, and TP1 did not show fibrinolytic activity. These results confirm that although all isolates are proteolytic, not all of them have the specific ability to degrade fibrin.

Table 4. Fibrinolytic Index Results of Bacterial Isolates

Isolate Bacteria	Diameter Clear Zone (mm)	Diameter Colony (mm)	Fibrinolytic Index
Control +	15	6	2.5
AL 1	-	12.5	-
AL 2	12.5	8	1.56
AL 3	-	5	-
AL 4	-	6	-
AL 6	19	8	2.37
AL 5	-	5	-
AL 7	17	11	1.54
AL 8	25	8	3.12
PSR 1	8	5	1.6
PSR 2	13	6	2.16
PSR 3	9	6	1.5
SP 1	18	12	1.5
SP 2	28	9	3.11
KP 1	12	8	1.5
TP 1	-	11	-

Antithrombotic Activity Test

Ten isolates with fibrinolytic activity were further tested for their ability to lyse clots*, evaluating* their potential as antithrombotic agents. The results of the percentage of blood clot lysis showed variation between isolates Table 5. Isolate AL7 showed the highest activity, with lysis reaching 82.05% in 200 µL enzyme treatment. SP1 also showed prominent results with 88.88% lysis at the same concentration. Some isolates, such as KP1, showed lower activity (maximum 52.83%). In general, increasing enzyme concentration was not always directly proportional to the percentage of lysis, indicating variations in response between isolates.

Table 5. Percentage Results Clot Lysis Bacterial Isolates

Isolate Bacteria	Percentage Clot Lysis				
	Positive Control	Negative Control	100 ul Enzyme	200 ul Enzyme	300 ul Enzyme
AL 2	68.42%	0%	78.37%	73.91%	76.74%
AL 6	47.50%	0%	40.47%	55%	79.48%
AL 7	66.66%	0%	81.08%	82.05%	77.14%
AL 8	64.10%	0%	50%	46.80%	37.14%
PSR 1	71.11%	0%	54.16%	33.33%	62%
PSR 2	60%	0%	56%	44.98%	59.67%
PSR 3	56.09%	0%	37.20%	60.78%	67.34%
SP 1	53.48%	0%	50.81%	88.88%	68.62%
SP 2	60.52%	0%	64.51%	72.22%	50%
KP 1	56.52%	0%	54.76%	52.83%	30.43%

Anticoagulant Activity Test (Lee-White Method)

Anticoagulant activity was tested in 10 fibrinolytic isolates using the Lee-White method. The results of coagulation time measurements are shown in Table 6. All isolates had prolonged coagulation time compared to the negative control. SP2 showed the most remarkable prolongation, at 142 seconds (49.65%). Other isolates with high activity were AL7 (129 seconds; 43.14%) and PSR2 (120 seconds; 41.95%).

Table 6. Anticoagulant Activity Results of Bacterial Isolates Method Lee-White

Isolate Bacteria	Time (seconds)			Time Difference (seconds)	Percentage Change Over Time
	Positive Control	Negative Control	100 ul Enzyme		
AL 2	Not Frozen	299	382	83	27.75%
AL 6	Not Frozen	298	322	24	8.05%
AL 7	Not Frozen	299	428	129	43.14%
AL 8	Not Frozen	298	364	66	22.14%
PSR 1	Not Frozen	286	366	80	27.97%
PSR 2	Not Frozen	286	406	120	41.95%
PSR 3	Not Frozen	299	348	49	16.38%
SP 1	Not Frozen	299	349	50	16.72%
SP 2	Not Frozen	286	428	142	49.65%
KP 1	Not Frozen	286	308	22	7.69%

Statistical Test of Antithrombotic and Anticoagulant Activity

Statistical analysis was performed to compare antithrombotic and anticoagulant activity between isolates. The Shapiro-Wilk normality test showed that all data were

normally distributed. The results of the homogeneity test (Levene's test) indicated that the variance of the antithrombotic group was not homogeneous, so the ANOVA test could not be used. Therefore, the non-parametric Kruskal-Wallis test was used.

Kruskal-Wallis results showed no significant difference in antithrombotic activity between isolates (p = 0.447) or anticoagulant activity (p = 0.635). Thus, although the activities of the isolates differed numerically, there was no statistically significant difference between the groups.

Hemolysis Pattern Test

Ten fibrinolytic isolates were then tested for hemolysis patterns using Blood Agar Plate (BAP) media. The results of the observations are shown in Table 7. Isolates AL8 and PSR1 exhibited gamma hemolysis, indicating a lack of hemolytic activity. Conversely, some isolates exhibited alpha hemolysis (partial), while others exhibited beta hemolysis (complete).

Table 7. Hemolysis Pattern Test Results of Fibrinolytic Protease Bacterial Isolates

Code Isolate	Spora	Gram Properties	Hemolisis Type
AL2	Negative (-)	Negative (-)	Alpha Hemolisis (α)
AL6	Positive (+)	Positive (+)	Beta Hemolisis (β)
AL7	Negative (-)	Negative (-)	Alpha Hemolisis (α)
AL8	Negative (-)	Negative (-)	Gamma Hemolisis (γ)
PSR1	Negative (-)	Negative (-)	Gamma Hemolisis (γ)
PSR2	Positive (+)	Positive (+)	Beta Hemolisis (β)
PSR3	Positive (+)	Positive (+)	Beta Hemolisis (β)
SP1	Negative (-)	Negative (-)	Alpha Hemolisis (α)
SP2	Negative (-)	Negative (-)	Alpha Hemolisis (α)
KP1	Positive (+)	Positive (+)	Beta Hemolisis (β)

The normality test for hemolysis patterns revealed that the data were not normally distributed; therefore, the Kruskal-Wallis test was employed. The analysis showed no significant difference between the hemolysis pattern groups (p = 0.782).

The morphology of bacterial colonies observed in this study is consistent with the descriptions of Cappuccino & Sherman (2013) and Suyasa (2019), which state that bacterial colonies are generally circular, irregular, filamentous, or rhizoidal. Colony elevation can be flat, raised, convex, umbonate, or crateriform, while the colony margin can be entire, lobate, curled, undulate, serrate, or filamentous.

The Gram staining results in this study indicate that the bacterial isolates obtained are predominantly bacillus-shaped. Tortora et al. (2015) explained that bacillus-shaped bacteria generally possess flagella as a means of locomotion, allowing them to move to more favorable environments. In contrast, coccus-shaped bacteria typically lack flagella and survive by adhering to surfaces in aquatic environments. This phenomenon aligns with the findings of Berlanga (2010) and Azam & Malfatti (2007), who stated that marine bacteria tend to associate with solid surfaces, such as the shells of marine organisms, macroalgae, coral reefs, and mangrove roots, as a colonization strategy.

Most isolates in this study were Gram-negative, while some, such as AL1, AL4, AL6, PSR2, PSR3, and KP1, were Gram-positive. Tortora et al. (2015) explained that Gram-positive bacteria have cell walls with a thick peptidoglycan layer and a high teichoic acid content, which plays an important role in maintaining cell wall integrity and resistance to autolysis. Meanwhile, the complex structure of the Gram-negative

cell wall, with its outer membrane composed of lipopolysaccharide, makes the bacteria more resistant to extreme environmental conditions (Johnson et al., 1968; Yang et al., 2022). This is in accordance with the fact that approximately 95% of marine bacteria are Gram-negative, with most being motile and having pigments.

Spore observations revealed that most isolates did not form spores, except for a few Gram-positive isolates, such as Bacillus. These results align with the findings of Schaeffer & Fulton (1933) and Berlanga (2010), who stated that only certain Gram-positive bacteria are capable of forming spores as a form of adaptation to unstable environmental conditions. Abecia (2015) also reported that some marine bacterial isolates can form spores to survive extreme changes in temperature and salinity.

Proteolytic activity testing showed that all bacterial isolates were capable of degrading casein, with varying proteolytic indices. Isolate PSR1 showed the highest index (3.4), while TP1 had the lowest index (1.45). These results are higher than those compared to Afriansyah's findings on Dictyota sp. fermentation isolates with an index <1, indicating the potential of isolates from Tanjung Dewa Beach as protease producers. The resulting clear zone is the result of casein hydrolysis into peptides and amino acids, which are then utilized by the bacteria (Cappuccino & Sherman, 2013; Panicker et al., 2022). This proves that coastal bacteria from Tanjung Dewa Beach have the potential to be a source of proteolytic enzymes with industrial and medical value (Muisristanto et al., 2015; Arnosti, 2011).

Fibrinolytic activity testing revealed that only 10 of the 15 isolates were capable of degrading fibrin. Isolates AL8 and SP2 showed the highest fibrinolytic index (3.12 and 3.11), even higher than the positive control (2.5). These results are consistent with a report by Setiawan et al. (2016), which showed that certain marine bacteria have varying fibrinolytic abilities even though all are proteolytic. This indicates that fibrinolytic activity is more specific to the fibrin substrate (Khikmah et al., 2024).

Antithrombotic activity was assessed using the clot lysis *method, which* revealed variations between isolates. Isolates AL7 and SP1 showed the best results with lysis reaching more than 80%, exceeding several previous studies that reported lysis percentages of <70% (Krishnamurthy & Belur, 2018; Fuad et al., 2020; Sompalli & Malaviya, 2024). These results indicate that the crude enzyme from the Tanjung Dewa Beach isolate has high potential as a natural thrombolytic agent. The phenomenon of the enzyme saturation point was also observed, where increasing enzyme concentration does not always increase the percentage of lysis (Westlund & Andersson, 1985; Islamiyah et al., 2022). This can be attributed to the limited penetration of the enzyme into dense blood clots, which is hindered by platelet contraction (Rijken & Sakharov, 2000; Tutwiler et al., 2018).

Anticoagulant activity, as determined using the Lee-White method, demonstrated that the crude enzyme isolates were capable of prolonging blood coagulation time. Isolate SP2 provided the most excellent prolongation time (142 seconds), followed by AL7 and PSR2. These results are higher than those of studies by Afriansyah (2024) and Ferdiani et al. (2023), which showed lower prolongation of coagulation time. The mechanism of action of this anticoagulant is believed to be through the degradation of fibrin, the primary component of blood clotting, thereby resembling the effects of chemical anticoagulants such as heparin or EDTA (Nugraha & Badrawi, 2024).

Statistical analysis revealed that the antithrombotic and anticoagulant data were normally distributed but not homogeneous; therefore, the Kruskal-Wallis test was employed. The results showed no significant differences between the isolates, although numerical variations in activity were observed. This indicates that all tested

isolates possess antithrombotic potential, although the levels of activity varied.

Hemolysis patterns vary between isolates. Two isolates (AL8 and PSR1) exhibit gamma hemolysis and are therefore considered non-pathogenic, while the others exhibit alpha or beta hemolysis. This finding is consistent with Afriansyah & Ethica (2023), who also reported variations in hemolysis patterns in marine fibrinolytic isolates. Isolates AL8 and PSR1 are considered the most promising because they exhibit high fibrinolytic and proteolytic activity while being non-pathogenic. Therefore, these two isolates have great potential to be developed as natural antithrombotic therapeutic agents.

This study has limitations in the use of crude enzymes, so the specific activity of the enzymes has not been fully characterized. In addition, bacterial identification was performed solely based on morphological, Gram-positive, and spore characterization, without the use of molecular analysis, such as 16S rRNA gene sequencing. Other factors, such as environmental conditions and culture media, can also influence the enzyme activity results obtained.

Based on the research results, the AL8 and PSR1 isolates have great potential as sources of non-pathogenic fibrinolytic enzymes for further development in antithrombotic therapy. Further research is needed to purify the enzymes, analyze their toxicity, and identify them molecularly to ensure their safety and effectiveness. Furthermore, biotechnological applications such as genetic engineering and large-scale fermentation could be potential development directions for the clinical and industrial use of these marine microbial fibrinolytic enzymes.

CONCLUSION

Based on the research results, bacterial isolates from the Tanjung Dewa coast have the potential as a source of fibrinolytic protease enzymes with varying antithrombotic and anticoagulant activities. Of the 15 isolates obtained, 10 isolates exhibited fibrinolytic activity, with isolates AL8 and SP2 displaying the highest index, and isolates AL7 and SP1 demonstrating the highest percentage of blood clot lysis in the antithrombotic test. The anticoagulant test using the Lee-White method showed that isolate SP2 was able to prolong coagulation time the most. Analysis of the hemolysis pattern revealed that AL8 and PSR1 were classified as non-pathogenic (γ-hemolysis), indicating they may be safer candidates for developing natural antithrombotic agents. These findings confirm that coastal bacteria from Tanjung Dewa have important prospects in the development of biomedical therapies for cardiovascular diseases.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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