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UTILIZATION OF INDONESIAN STRAIN *ACTINOBACTERIA* TO FACE THE THREAT OF BIOFILM AS THE MAIN CAUSE OF HEALTH CARE ASSOCIATED INFECTION

Pemanfaatan Aktinobakteri Strain Indonesia untuk Menghadapi Ancaman Biofilm Sebagai Penyebab Utama Health Care Associated Infection

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ABSTRACT

Health Care Associated Infection (HCAI) is one of the global infectious diseases that is expected to cause around 10 million deaths by 2050. One of the main causes is biofilm, an exopolysaccharide layer formed by bacteria, often found on medical equipment such as catheters, and has high resistance to antibiotics. Prevention efforts can be made through the search for antibiofilm compounds. Actinobacteria are known to produce potential bioactive compounds. This study utilized Actinobacteria strains from the waters of Bitung, North Sulawesi (code BT-023-026) to explore their antibiofilm potential through isolation, laboratory tests, and genetic analysis, followed by expert confirmation in the fields of intelligence, BPOM, and microbiology. The results show the great potential of BT-023-026 as an antibiofilm agent to prevent antibiotic resistance due to HCAI, while playing a strategic role in biodefense and strengthening health security through early warning and early detection.

Keywords: *Actinobacteria, Antibiofilm, Biodefense, Biofilm, Health Care Associated Infection*

ABSTRAK

Health Care Associated Infection (HCAI) merupakan salah satu penyakit infeksi global yang diperkirakan menyebabkan sekitar 10 juta kematian pada 2050. Salah satu penyebab utamanya adalah biofilm, lapisan eksopolisakarida yang dibentuk oleh bakteri, sering ditemukan pada peralatan medis seperti kateter, dan memiliki resistensi tinggi terhadap antibiotik. Upaya pencegahan dapat dilakukan melalui pencarian senyawa antibiofilm. *Actinobacteria* diketahui mampu menghasilkan senyawa bioaktif potensial. Penelitian ini memanfaatkan strain *Actinobacteria* asal perairan Bitung, Sulawesi Utara (kode BT-023-026) untuk mengeksplorasi potensi antibiofilmnya melalui isolasi, uji laboratorium, dan analisis genetik, dilanjutkan konfirmasi pakar di bidang intelijen, BPOM, dan mikrobiologi. Hasilnya menunjukkan potensi besar BT-023-026 sebagai agen antibiofilm guna mencegah resistensi antibiotik akibat HCAI, sekaligus berperan strategis dalam biodefense dan penguatan health security melalui early warning dan early detection.

Kata Kunci: *Aktinobakteri, Antibiofilm, Biodefense, Biofilm, Infeksi Terkait Perawatan Kesehatan*

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INTRODUCTION

Infections caused by biofilms, especially those formed by pathogenic bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, are one of the main causes of healthcare-associated infections (HCAI) that are difficult to treat with conventional antibiotics (Sandu et al. 2025). The problem is further exacerbated by the rise of antimicrobial resistance (AMR) globally, including in Indonesia, which has the potential to cause high mortality rates and difficulties in treating bacterial infections (Tang et al. 2023). The development of locally sourced antibiofilm products in Indonesia is still very limited and none have been officially registered. In fact, marine microorganisms such as *Actinobacteria* have great potential as sources of bioactive secondary metabolites that are stronger than terrestrial microorganisms due to their adaptation to extreme conditions such as salinity and high temperatures. These metabolites work through various mechanisms, such as inhibiting cell wall synthesis, damaging cell membranes, disrupting protein and nucleic acid synthesis, and modulating target cell signaling pathways. (Manivasagan et al. 2014). However, the development of antibiofilm products from marine *Actinobacteria* faces various technical and regulatory obstacles, ranging from the complexity of biofilm structures, low metabolite yields during mass production, to inadequate regulations to support the development of these products (Jagannathan et al. 2021). To anticipate biological threats due to biofilm infection, collaboration across agencies and the implementation of an integrated early detection system are needed to improve national preparedness. In addition, limited facilities, infrastructure and human resources in marine natural materials research are real obstacles in the exploration and development of effective antibiofilm compounds. Therefore, handling biofilm infections and AMR needs to be part of an integrated national health and biodefense strategy, supported by an early warning system and appropriate policies to effectively and sustainably control these biological threats.

The study aimed to isolate and culture *Actinobacteria* from the waters of Bitung, North Sulawesi, to obtain potential sources of secondary metabolites. Metabolite extracts were tested for their antimicrobial and antibiofilm activity against Gram-positive and Gram-negative pathogenic bacteria. Genomic analysis, particularly of isolate BT-23-026, identified biosynthetic compounds with potential antibiofilm activity, which were then validated through in vitro and in silico tests. This research also explored stakeholder perceptions regarding the development of antibiofilm products from marine *Actinobacteria* and provided strategic recommendations for research and product development within the national health system. The benefits include the provision of data and isolates of marine *Actinobacteria* with antibiofilm and antimicrobial potential for the development of biofilm-based infection control agents, genomic evidence support for the biosynthesis of active compounds, and a scientific basis for policy, cross-sector collaboration, and advanced technology development for the production of antibiofilm compounds in order to strengthen national health resilience against antimicrobial resistance and biofilm infections.

This research hypothesis states that marine *Actinobacteria* isolates from Bitung waters, particularly BT-23-026, are capable of producing secondary metabolites that effectively inhibit the growth of Gram-positive pathogenic bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. These metabolites are also thought to inhibit biofilm formation, thus having the potential to act as antibiofilm agents. The presence of biosynthetic genes such as NRPS and PKS in these isolates is thought to be positively correlated with their ability to produce antibiofilm and antimicrobial compounds (Rao et al. 2016). The antibiofilm activity of this isolate is predicted to be more effective against Gram-positive bacteria than Gram-negative, which is influenced by differences in bacterial cell wall structure. Marine *Actinobacteria* in general are thought to have greater potential in producing bioactive secondary metabolites than *Actinobacteria* from terrestrial environments, due to adaptation to extreme marine environmental stress (Al-Shaibani et al. 2021). The development of antibiofilm products from marine *Actinobacteria* has the potential to make a significant contribution to

controlling biofilm-related infections in healthcare facilities and overcoming antimicrobial resistance. Cross-sector collaboration and strengthening of the health security system through the establishment of a Fusion Center or Command Center is believed to increase the effectiveness of early detection and research on biological threats from biofilm-forming microorganisms.

Biofilms formed by pathogenic bacteria are one of the major causes of intractable Healthcare-Associated Infection (HCAI) and contribute significantly to the increase in antimicrobial resistance (AMR) globally. A number of international studies have demonstrated the potential of *Actinobacteria* as a promising source of antibiofilm secondary metabolites (Goel et al. 2021; Wibowo et al. 2023). However, these studies have mostly focused on *Actinobacterial* isolates from terrestrial environments or geographical regions outside Indonesia, so the contribution of Indonesia's rich marine biodiversity is still very limited in scientific exploration and product development.

Previous studies have mostly emphasized the antibacterial and antibiofilm activities of *Actinobacteria* from soil or mangroves (Sengupta et al. 2015; Sangkanu et al. 2017), with few investigating marine isolates thoroughly especially against major clinical pathogens causing HCAI. This study fills a gap in the genetic characterization and antibiofilm potential of Indonesian marine *Actinobacteria*, particularly isolate BT-23-026 from Bitung, North Sulawesi. Through an integrated approach—isolation, genomic analysis (16S rRNA, NRPS/PKS genes), in silico prediction (antiSMASH), and activity testing—it was found that the BT-23-026 extract effectively inhibits *Staphylococcus aureus* biofilm at low concentrations. These findings reinforce previous scientific evidence and have clinical relevance. Additionally, this study involved stakeholders such as medical intelligence, BPOM, and researchers, emphasizing the urgency of developing antibiofilm products as part of the national biodefense strategy, with the integration of regulatory aspects and health system readiness that had not been previously discussed.

METHODS

The research uses descriptive research with an experimental quantitative approach that adopts a positivistic paradigm. The experimental method was chosen because it allows observation and manipulation of variables in a controlled environment to assess the effect of the independent variable on the dependent variable (Diel et al. 2021). The main focus of the research was the identification of *Actinobacterial* species and testing the antibiofilm activity of *Actinobacterial* extracts obtained from the waters of Bitung, North Sulawesi. The main data obtained is quantitative data from experimental results, which are then reinforced with qualitative data through interviews to increase the validity of the research results (Daruhadi and Sopiati 2024). The approach follows an explanatory research design, where qualitative data serves as an external validation of quantitative data.

The unit of analysis in this study was *Actinobacteria* extracts from the waters of Bitung, North Sulawesi. The data analyzed included the results of antibiofilm activity tests and interviews with purposively selected informants. The research instruments included bacterial cultures, *Actinobacteria* extracts, growth media, incubator shakers, and micropipettes for experiments, as well as bacterial tests (positive control, homogeneity, MIC, MBC). Genetic analysis used PCR primers and DNA amplification tools for genome isolation and 16S identification. External validation was conducted through interviews using a guide tested for validity and reliability to explore informants' knowledge about the threat of biofilm.

Data Collection Technique

Interview

Explanatory design interviews are used as an effective method to explain experimental results in depth. With a structured format and targeted questions, interviews aim to increase public understanding of scientific research and its implications. In this study, the interviewees consisted of various experts and practitioners, including the Biological Hazard Sub-Directorate BIN team, doctors and intelligence experts, *Ac-*

tinobacteria experts from several universities, as well as regulatory practitioners and natural medicine development research from BPOM and BRIN. Although the interview questions will be adapted to the experimental results, interview guidelines have been developed to maintain the structure and focus of the discussion. This guideline is divided into three main parts, namely the potential of Indonesian *Actinobacteria* strains in overcoming biofilm threats, problems faced in the utilization of *Actinobacteria* extracts along with possible solutions, and input and suggestions from the speakers regarding the development of research and utilization of *Actinobacteria* as antibiofilms in the future.

Experiment

The experimental procedure begins with the preparation of Yeast Starch Agar (YSA) solid media containing 2% NaCl, made by mixing yeast extract, starch, NaCl, and agar into distilled water, then homogenized, heated, and sterilized using an autoclave at 121°C for 15 minutes. Furthermore, *Actinobacterial* rejuvenation was carried out using 10% glycerol at -80°C with thawing process, then one agar chip was taken and scratched on YSA + 2% NaCl media, then incubated at 30°C for 2-3 weeks. Liquid culture of *Actinobacterial* isolates growing on YSB media (+NaCl) was incubated for 10-14 days, then centrifuged to separate the supernatant and pellet which was stored at 4°C before use.

For genomic DNA isolation, an extraction buffer solution consisting of a mixture of Tris HCl, NaCl, EDTA, SDS, and Nuclease Free Water solutions was made according to standard procedures. DNA was extracted manually through the stages of sample preparation, cell lysis with extraction buffer, DNA separation using isopropanol, and purification with 70% ethanol, then dissolved in NFW. Genetic analysis was designed in-house in the study, which involved 16S rRNA gene amplification using universal primers and PCR detection of PKS-I, PKS-II, and NRPS genes with specific primers, followed by electrophoresis to confirm amplification success, based on the approach described in previous studies (Ayuso-Sacido and Genilloud 2005). PCR products were subsequently sent for sequencing, and the results were analyzed using BLAST and MEGA software to construct a phylogenetic tree.

Antibacterial tests were performed using the agar diffusion method against test bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. Biofilm formation and inhibition tests used the microtiter plate assay method with crystal violet staining and absorbance measurements using an ELISA reader at a wavelength of 595 nm, then calculated the percentage of biofilm inhibition and eradication based on the OD value.

The best isolate was selected based on its ability to inhibit biofilm formation by at least 50%, after which the Minimum Inhibitory Concentration (MIC) for biofilm formation and biofilm eradication were assessed using various extract concentrations. These procedures were adapted from established protocols for evaluating antibiofilm activity, in which the $MBIC_{50}$ is defined as the lowest concentration of an extract capable of inhibiting 50% of biofilm formation, as described by (Alenazy 2023) and (Teanpaisan et al. 2017). Isolate preservation was carried out by making glycerol stock from YSB media and 85% glycerol solution, then sterilized and stored aseptically at 4°C before being transferred to a deep freezer at -80°C.

Data Analysis Technique

Triangulation is used to test the validity of data by comparing information from various sources, methods, and times (Donkoh and Mensah 2023). In this study, triangulation of sources and time was used, namely by conducting interviews at the same time for all respondents and comparing the results with the literature as a form of verification.

Research on the use of Indonesian *Actinobacteria* to combat the threat of biofilms was conducted by collecting and classifying data from the literature to identify potential bioactive compounds. The results were integrated with knowledge about antibiotic resistance. This analysis forms the basis for an early warning and detection system for biofilm threats, as well as providing a reference for policymakers. The SWOT

approach was used to design effective and sustainable antibiofilm development strategies, with projections of challenges up to 2050.

RESULTS AND DISCUSSION

Results

Isolation, Secondary Metabolite Extraction, and Antimicrobial-Antibiofilm Evaluation of Actinobacterial Isolates from Bitung Marine Waters against Gram-Positive and Gram-Negative Pathogens

In 2023, ten *Actinobacteria* isolates (BT-23-019 to BT-23-078) were successfully rejuvenated from seawater in Bitung, North Sulawesi. After culturing, their secondary metabolites were extracted using ethyl acetate. The concentrated extracts obtained (65–123 mg per isolate) were tested for their antimicrobial and antibiofilm activity against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*, which are common human pathogenic bacteria.

Table 1. Weight of Viscous Extracts from 10 *Actinobacterial* Isolates of Bitung Sea Waters and Antimicrobial Activity Tests

No	Isolat Code	Viscous Extract Weight (mg)
1	BT-23-078	123
2	BT-23-069	78
3	BT-23-065	65
4	BT-23-056	89
5	BT-23-055	97
6	BT-23-047	102
7	BT-23-028	84
8	BT-23-026	88
9	BT-23-025	103
10	BT-23-019	99

As shown in Table 1, the 10 *Actinobacterial* isolates from Bitung sea waters produced viscous extracts with varying weights, ranging from 65 mg to 123 mg. All extracts were subjected to antimicrobial activity tests against both Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and Gram-negative bacteria, *Escherichia coli*, which are commonly associated with human infections.

Results of Genomic Test, Antimicrobial Potential Test and Antibiofilm Test

Genomic Analysis and Potential Production of Antibiofilm Compounds in Marine Actinobacterial Isolates from Bitung Waters, North Sulawesi

Genomic analysis of *Actinobacterial* isolates from the marine waters of Bitung, North Sulawesi, revealed that out of ten isolates tested, three failed to yield results in the 16S rRNA gene assay, possibly due to suboptimal sample preparation. The remaining seven isolates were successfully identified, with the majority belonging to the genus *Streptomyces*, a group well-known for producing secondary metabolites, including streptomycin, which possesses antimicrobial activity.

Table 2. *Actinobacterial* Species Analysis Results Based on NCBI Database

No.	Isolate Number/Code	Source	Weight (mg)	Closest Taxonomy (Species)	NCBI ACC Number
1	BT-23-078	Sea	123	*Streptomyces tendae* (99,33%)	JQ819729.1
2	BT-23-069	(NA)	78	(NA)	(NA)
3	BT-23-065	(NA)	65	(NA)	(NA)

No.	Isolate Number/Code	Source	Weight (mg)	Closest Taxonomy (Species)	NCBI ACC Number
4	BT-23-056	Sea	89	*Streptomyces globisporus* (99,32%)	OP271851.1
5	BT-23-055	(NA)	97	(NA)	(NA)
6	BT-23-047	Sea	102	*Advenella mimigardefordensis* (99,35%)	MZ22945.1
7	BT-23-028	Sea	84	*Streptomyces parvulus* (99,77%)	KT206996.1
8	BT-23-026	Sea	88	*Streptomyces parvulus* (99,04%)	MK392063.1
9	BT-23-025	Sea	103	*Streptomyces globisporus* (99,64%)	JX535034.1
10	BT-23-019	Sea	99	*Streptomyces cavourensis* (100%)	KR906446.1

Table 2 shows the identification of ten *Actinobacteria* isolates from Bitung waters based on 16S rRNA analysis and NCBI data. Seven isolates were successfully identified to the species level, mostly from the genus *Streptomyces*, while the other three could not be identified, possibly due to suboptimal DNA quality. All seven isolates were detected to have NRPS/PKS genes with DNA bands of approximately 1,500 bp, indicating the potential for secondary metabolite synthesis.

Isolate BT-23-026 was selected for further analysis with antiSMASH, which revealed the presence of antimicrobial biosynthesis genes, including Actinomycin D (82% similarity) and Germicidin (100%). These results indicate the isolate's great potential in producing antimicrobial and antibiofilm compounds. Electrophoresis confirmed the successful amplification of the 16S rRNA gene (≈500 bp) and NRPS/PKS (≈1,500 bp), although three isolates without DNA bands were suspected to be related to low DNA quality.

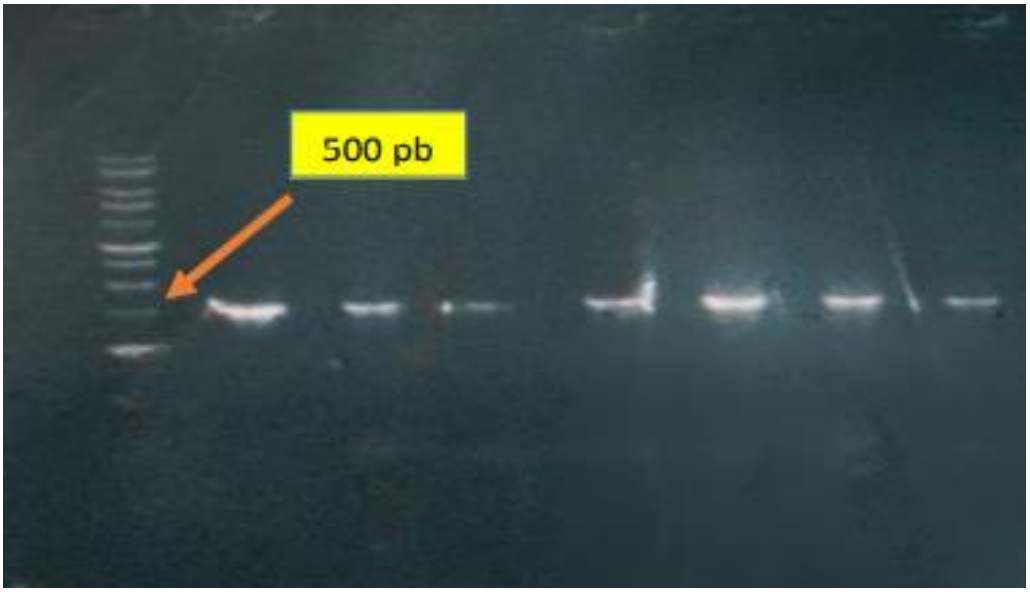
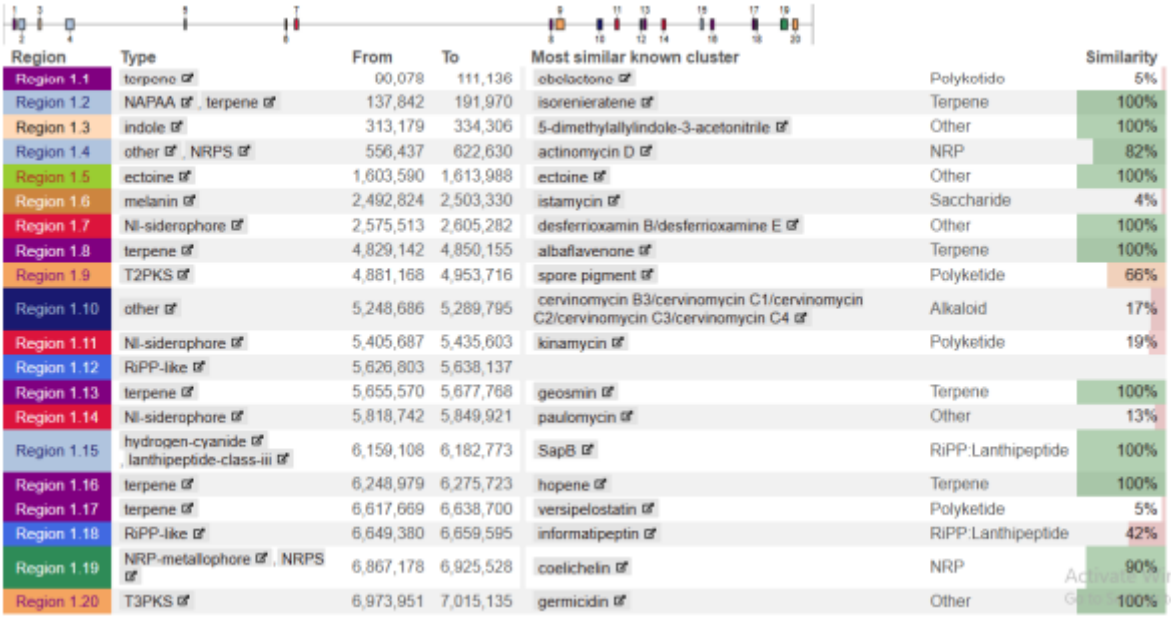


Figure 1. Electrophoresis results using GelDoc

Figure 1 shows the results of DNA gel electrophoresis for several *Actinobacterial* isolates. A clear DNA band of approximately 500 bp is visible, corresponding to the expected size of the target fragment. This band confirms successful amplification and suggests that the isolate possesses the genetic potential to produce secondary metabolites. Variations in band intensity among

the wells reflect differences in DNA concentration across the isolates. The DNA size was estimated using the same molecular marker (ladder) run alongside the samples.



Region	Type	From	To	Most similar known cluster		Similarity
Region 1.1	terpene	00,078	111,136	obolactone	Polyketide	5%
Region 1.2	NAPAA , terpene	137,842	191,970	isorenieratene	Terpene	100%
Region 1.3	indole	313,179	334,306	5-dimethylallylindole-3-acetonitrile	Other	100%
Region 1.4	other , NRPS	556,437	622,630	actinomycin D	NRP	82%
Region 1.5	ectoine	1,603,590	1,613,988	ectoine	Other	100%
Region 1.6	melanin	2,492,824	2,503,330	istamycin	Saccharide	4%
Region 1.7	NI-siderophore	2,575,513	2,605,282	desferrioxamin B/desferrioxamine E	Other	100%
Region 1.8	terpene	4,829,142	4,850,155	albaflavenone	Terpene	100%
Region 1.9	T2PKS	4,881,168	4,953,716	spore pigment	Polyketide	66%
Region 1.10	other	5,248,686	5,289,795	cervinomycin B3/cervinomycin C1/cervinomycin C2/cervinomycin C3/cervinomycin C4	Alkaloid	17%
Region 1.11	NI-siderophore	5,405,687	5,435,603	kinamycin	Polyketide	19%
Region 1.12	RiPP-like	5,626,803	5,638,137			
Region 1.13	terpene	5,655,570	5,677,768	geosmin	Terpene	100%
Region 1.14	NI-siderophore	5,818,742	5,849,921	paulomycin	Other	13%
Region 1.15	hydrogen-cyanide , lanthipeptide-class-iii	6,159,108	6,182,773	SapB	RiPP:Lanthipeptide	100%
Region 1.16	terpene	6,248,979	6,275,723	hopene	Terpene	100%
Region 1.17	terpene	6,617,669	6,638,700	versipelostatin	Polyketide	5%
Region 1.18	RiPP-like	6,649,380	6,659,595	informatipeptin	RiPP:Lanthipeptide	42%
Region 1.19	NRP-metallophore , NRPS	6,867,178	6,925,528	coelichelin	NRP	90%
Region 1.20	T3PKS	6,973,951	7,015,135	germicidin	Other	100%

Figure 2. Gel electrophoresis results visualized with GelDoc

Figure 2 illustrates the genomic region analysis of isolate BT-23-026 performed using antiSMASH. A total of 20 biosynthetic regions were identified, encompassing diverse gene types such as polyketides, terpenes, NRPS, and others. Each region spans specific positions on the genome and exhibits varying degrees of similarity to known biosynthetic gene clusters. Several regions, including 1.3, 1.5, 1.13, 1.15, 1.16, and 1.20, show high similarity of up to 100%, indicating the potential for the production of bioactive compounds such as isorenieratene, actinomycin D, geosmin, SapB, and germicidin.

Antimicrobial and Antibiofilm Activity Test of Extract Isolate BT-26 against Staphylococcus aureus, Bacillus subtilis, and Escherichia coli Bacteria

Based on antimicrobial activity, the extract of isolate BT-26 showed a very strong ability to inhibit the growth of Gram-positive bacteria, namely *Staphylococcus aureus* and *Bacillus subtilis*, with a minimum inhibitory concentration (MIC) value of 0.0125 µg/mL at dilution up to 20 times. This MIC value indicates that the BT-26 extract was able to suppress the growth of both bacteria at a very low concentration, which indicates a very high antimicrobial potential. When compared to other studies, other plant extracts or antibacterial compounds generally require much higher concentrations to achieve the same effect. *Polygonum chinense L.* extract showed an MIC of 4 mg/mL against S. aureus (Liu et al. 2023), while *Dryopteris erythrosora* extract was only able to inhibit S. aureus at an MIC of 0.125 mg/mL (Yun and Bai 2023). The plicacetin compound was reported to have an MIC of 3.8 µg/mL against S. aureus and B. subtilis (Devi et al. 2023), while fractions purified from several crude extracts showed MICs between 0.5-16 µg/mL against S. aureus (Chalasani et al. 2015) and 0.312-0.624 mg/mL against B. subtilis (Hellany et al. 2024). In fact, certain medicinal plant extracts require concentrations of up to 12.5 mg/mL to inhibit the growth of various pathogens (Rekha et al. 2018). So compared to the various extracts and antibacterial compounds that have been reported, BT-26 extract shows much higher antimicrobial potential, being able to work at very low concentrations. However, it should be noted that these comparisons remain influenced by the extraction methods, chemical composition of the extracts, and assay conditions used in each study, so further evaluation is

needed to thoroughly confirm the effectiveness of BT-26 extracts.

In contrast, in the test against *Escherichia coli*, a Gram-negative bacterium, BT-26 extract showed no inhibitory activity up to 11 times dilution, indicating that this extract is less effective or inactive against E. coli. The antibiofilm test using a modified method from (Haney et al. 2021) showed that BT-26 crude extract was able to inhibit biofilm formation with a high percentage of inhibition in the concentration range of 240 to 3.75 µg/mL. At these concentrations, the BT-26 extract showed excellent antibiofilm activity, with a significant reduction in biofilm formation compared to the control, indicating that BT-26 has the potential to be developed as an effective antibiofilm agent.

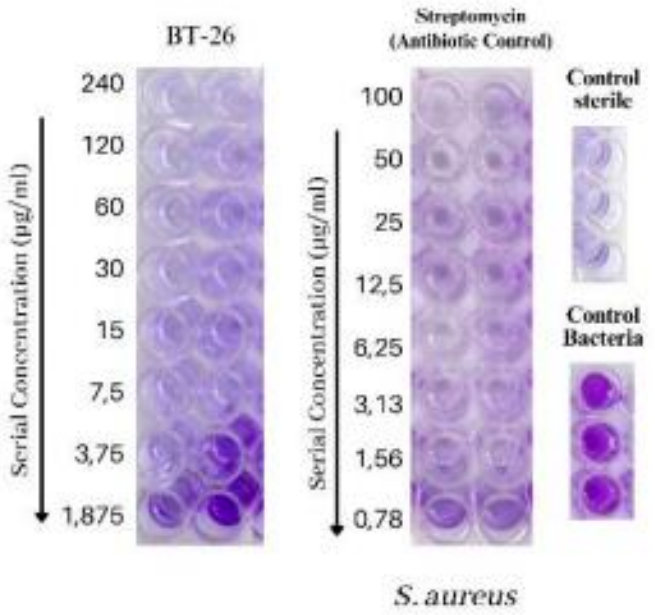


Figure 3. *Staphylococcus aureus* Biofilm Inhibition Activity by BT-26 Extract at Various Concentrations

Figure 3 shows the results of the biofilm inhibition test of *Staphylococcus aureus*by BT-26 extract visualized through the microtiter test (left) and the graph of the percentage of biofilm inhibition (right). In the microtiter assay, the reduction of purple color intensity in the test wells indicates the reduction of biofilm formation as the extract concentration increases. It can be seen that at high concentrations, i.e. 240 and 120 µg/mL, biofilm formation is completely inhibited, while at intermediate concentrations (60-7.5 µg/mL) the inhibition is still high although starting to decrease. The graph on the right side reinforces this observation by showing 100% inhibition percentage at 240-120 µg/mL, a gradual decrease at 60-7.5 µg/mL (86-74%), and much reduced inhibition at 3.75 µg/mL (37%), until no inhibition at 1.875 µg/mL. Based on the results, these data indicate that BT-26 has high effectiveness in inhibiting S. aureus biofilm formation at medium to high concentrations, with effectiveness decreasing dramatically at concentrations below 3.75 µg/mL.

Multi-Stakeholder Review of Indonesian Marine Actinobacterial Potential and Antibiofilm Product Development Challenges to Address the Threat of Biofilm-Related Infections in Healthcare Facilities

The interview revealed that biofilms pose a serious biological threat that can trigger healthcare-associated infections (HCAIs). BIN emphasized the importance of national preparedness against microbial threats, including through early detection systems and cross-sector collaboration. An integrated command center needs to be established to strengthen health security and accelerate the detection of biological threats.

Research findings during the symposium in Bali showed that Indonesian marine *Actinobacteria* have great potential as

antibiofilm agents, with metabolites that are stronger than those derived from terrestrial sources. However, biofilms are complex, requiring comprehensive research up to the clinical stage.

From a regulatory perspective, BPOM emphasized that there are no registered antibiofilm products in Indonesia, but stated its readiness to support the registration process for local products. BRIN also acknowledged that research is already underway but has not been intensive. The threat of antimicrobial resistance and low compliance by health facilities with antibiotic policies demonstrate the urgency of strengthening research, policies, and antibiofilm innovation based on local research.

Table 3. Interview Findings Related to *Actinobacterial* Utilization and Biofilm Threat in Indonesia

Aspect	Key Findings
The New Pandemic Threat Paradigm	Biological threats from microorganisms have the potential to reoccur, need collaboration and early detection for preparedness
Strengthening the Health Security System	The importance of the Fusion Center / Command Center between agencies for early detection and research development related to biological threats
Potential of *Actinobacteria* as Antibiofilm Agents	Marine *Actinobacteria* have potent metabolites, but the development of antibiofilms is complex and should be comprehensive to clinical aspects
Product Utilization of Indonesian Strain Natural Materials	No antibiofilm products registered in Indonesia yet, BPOM ready to support, resistance challenges to local products
Utilization of Marine *Actinobacteria*	Research on antibiofilms from marine *Actinobacteria* exists but is less massive, great potential for indigenous Indonesian products
AMR and Biofilm Threats	AMR and biofilms are real threats, healthcare facility compliance is low, antibiofilm policy and research is needed

Based on Table 2, biological threats from microorganisms have the potential to reemerge, making interagency collaboration and early detection systems crucial for national preparedness. Strengthening the health system through the establishment of a Fusion Center is key to accelerating detection and supporting research on biological threats. Marine *Actinobacteria* show promise as antibiofilm agents, but their development requires a comprehensive clinical approach. Currently, there are no registered antibiofilm products in Indonesia, although BPOM is ready to provide support amid the challenges of resistance. Research on antibiofilms from marine *Actinobacteria* is still limited and needs to be improved in order to become an indigenous Indonesian product. In addition, the threat of antimicrobial resistance (AMR) and biofilm formation is becoming more serious due to low compliance by health facilities with prevention programs, making antibiofilm research and stronger policies a strategic solution.

Discussion

Research has revealed the significant potential of marine *Actinobacterial* strain BT-23-026 isolated from the waters of Bitung, North Sulawesi, as an effective antibiofilm agent. The antibiofilm activity of the isolate was evident in the concentration range of 240-3.75 µg/ml that the secondary metabolites it produced had the ability to inhibit bacterial biofilm formation. The findings are in line with previous studies which mentioned that *Actinobacteria* from marine environments tend to produce more potent bioactive compounds than *Actinobacteria* from land, this is thought to be due to extreme marine environmental pressures such as high salinity, temperature, and biological competition which force the microbes to produce secondary metabolites as a survival

mechanism (Ullah et al. 2021; Mahmood et al. 2022). In particular, marine *Actinobacteria* have been reported to be effective against test bacteria such as *Escherichia coli*, *Bacillus subtilis*, and Micrococcus luteus, which strengthens the validity of the results of this study.

Confirmation from intelligence experts, BPOM regulators, and *Actinobacterial* experts support the relevance of these findings in the development of antibiofilms as part of the national biodefense strategy. The development of antibiofilm compounds is considered important to strengthen the health security system, especially in early warning and early detection mechanisms against the threat of antibiotic resistance associated with Healthcare-Associated Infections (HCAIs). This is in accordance with the medical intelligence approach that emphasizes the importance of early detection and strengthening surveillance systems to anticipate health crises (Mudra and Rofii 2025).

The threat of antimicrobial resistance is indeed an increasingly serious global problem that is predicted to cause more than 700,000 deaths per year by 2050 if there is no effective intervention (Endale et al. 2023). In Indonesia, this situation is exacerbated by several factors such as non-compliance with antibiotic use, overuse of antibiotics in the livestock sector, and high dependence on imports of medicinal raw materials up to 90% (Kurnianto and Syahbanu 2023). In addition, the national antimicrobial resistance diagnosis and reporting system is still not comprehensively integrated, resulting in less than optimal strategic decision-making.

To address the challenges of biofilm and antimicrobial resistance, synergy is needed between raising public awareness, effective health policies, and natural medicine research. Although Indonesia has the strength of evidence of Actinobacteria's antibiofilm activity, this research is still hampered by limitations in human resources, equipment, and low metabolite production. Significant opportunities exist from technological advances such as LC-HRMS, genome mining, and in silico screening, as well as stakeholder support and biodiversity wealth, especially in the marine environment. However, threats such as rapidly increasing resistance, high research costs, and the absence of specific regulations remain challenges. Medical intelligence plays a crucial role in processing data into strategic recommendations, including early warning systems, MDR monitoring, and strengthening local raw materials. Greater research investment, cross-sector collaboration, and public education are needed to support the development of effective and sustainable antibiofilm drugs in Indonesia.

CONCLUSSION

Theoretically, research has proven that the BT-23-026 actinobacteria isolate from Bitung waters has strong antibiofilm activity at concentrations of 240–3.75 µg/ml and has the potential to be an effective antibiofilm agent. This finding reinforces the theory that marine actinobacteria produce stronger metabolites than those originating from land.

In practice, BT-23-026 extract has the potential to be used as an alternative therapy for antibiotic-resistant biofilm infections, but further clinical trials are needed to ensure its safety and effectiveness. Cross-sector collaboration is essential for the development of natural medicines that are effective against healthcare-associated infections.

Further research needs to focus on the molecular mechanisms of biofilm formation and the exploration of other marine bioactive compounds, supported by government policies and funding to strengthen national health security in the face of the threat of antimicrobial resistance.

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