Antibacterial Screening of Marine Bacteria Isolated from Sanur Beach Sediments, Denpasar-Bali

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Abstract. In Indonesia, bacterial infections are a serious concern, exacerbated by antibiotic resistance from bacterial mutations. To combat this, discovering novel antibacterial compounds from natural sources is vital. Marine sediments, especially, represent a rich, underexplored reservoir for such compounds. Sanur Beach is a popular tourist area with diverse marine life; however, few studies have explored the diversity of marine bacteria in its sediment. This research employed a multi-faceted approach to investigate bacterial isolates from Sanur Beach marine sediments. Bacteria were initially isolated using ISP-2, Actinomycetes Isolation Agar, and Starch-M Protein Agar. Antibacterial activity was screened via the agar block method against *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* FNCC 0405, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603. Finally, 16S rRNA gene sequencing identified the isolate with the highest activity. Nine of ten isolates demonstrated antibacterial activity against at least one test bacterium. Isolate I-4 showed the largest inhibition zones: 16.28±0.73 mm (*S. aureus*), 19.37±0.23 mm (*S. mutans*), 19.79±0.06 mm (*E. coli*), and 14.92±0.85 mm (*K. pneumoniae*). Analysis of the16S rRNA gene sequence identified isolate I-4 as phylogenetically related to *Streptomyces* sp. VEL 17. This finding underscores the valuable antibacterial potential of marine-associated bacteria for synthesizing antibiotic compounds.

1 Introduction

The emergence of bacterial infections, coupled with the alarming rise of antibiotic resistance, represents a critical and escalating global health challenge. Antibiotic resistance, defined by bacteria's ability to survive and proliferate despite antimicrobial agents, primarily stems from the pervasive overuse and mismanagement of antibiotics in both clinical and agricultural

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settings. The World Health Organization has unequivocally recognized this phenomenon as a public health crisis, underscoring the urgent need to address factors contributing to antibiotic-resistant bacteria (ARB) [1].

Bacterial mechanisms for acquiring resistance are multifaceted. They may obtain resistance genes through horizontal gene transfer or develop new mutations that confer survival advantages in the presence of antibiotics. Furthermore, environments such as wastewater and agricultural settings serve as significant reservoirs for resistant bacteria and their genetic material, thereby facilitating the spread of ARB into human populations [2]. This environmental persistence profoundly complicates efforts to manage and control the dissemination of resistance.

The rise of antibiotic-resistant infections necessitates a paradigm shift toward exploring marine sources for novel antibacterial agents. As traditional antibiotics face diminishing efficacy against evolving pathogens, the search for new drug candidates from underexplored sources like marine microorganisms becomes paramount. This urgency is amplified by the realization that many potential compounds from marine microorganisms remain unassessed for their antibacterial capabilities [3]. Given their vastness and biochemical richness, marine ecosystems are uniquely positioned to provide a wealth of new antimicrobial agents to address the pressing global challenge of antibiotic resistance [4].

The imperative to search for novel antibacterial producers from marine bacteria, rather than focusing solely on terrestrial samples, is driven by several compelling factors. First, the marine environment, a vastly underexplored realm compared to terrestrial ecosystems, represents an immense trove of microbial diversity. This diversity is crucial, as many marine organisms have adapted to extreme conditions, leading to the production of unique metabolites with potent antibacterial properties. Marine bacteria, evolving in high-stress environments, often synthesize secondary metabolites that differ significantly from those produced by land-based counterparts due to their unique evolutionary adaptations and ecological niches [3, 4].

Considering the vast and unique potential of marine ecosystems as sources for novel antibacterial agents, Bali presents a particularly intriguing location for such exploration. This world-renowned tourist destination is celebrated not only for its natural beauty and rich cultural heritage but also for its abundant natural potential, including diverse microbial life [5]. Despite its recognized potential, exploration of Bali's marine bacterial wealth remains largely underexplored. Sanur beach, a popular natural tourist site, possesses significant natural resources, particularly actinobacteria. However, no specific research has yet investigated the marine bacterial community within its marine sediments. Given the critical importance of antibacterials for health, discovering potent natural antibacterial compounds from this unique environment is essential to address the negative impacts associated with synthetic alternatives.

2 Material and methods

2.1. Sampling

Sampling was conducted at Matahari Terbit Beach, Sanur Kauh Village, Sanur, Denpasar City, Bali Province (8°40'15.68" S, 115°15'42.93" E). At low tide, 20 grams of marine sediment were collected at a depth of 10 cm using a sterilized shovel. Sample was immediately transferred to a sterile 50 mL falcon tube, tightly sealed with parafilm, and stored in an icebox with an ice pack. Sample was transported to the Laboratory of Agriculture, Faculty of Agriculture, Warmadewa University, Denpasar, Bali for further analysis.

2.2. Bacterial isolation and cultivation

Initially, a 1-gram marine sediment sample from the sampling location underwent pre-treatment. This involved drying the sample in a laminar airflow for 16 hours, followed by heating in an oven at 60°C for four hours. This pre-treatment aimed to eliminate other bacteria present in the sediment. The pre-treated sediment samples were then transferred to 50 mL falcon tubes, to which 5 mL of sterile artificial seawater was added. The resulting suspension was homogenized using a vortex. A 1 mL aliquot of the homogenized marine sediment suspension was serially diluted in 9 mL of sterile artificial seawater, creating dilutions from 10^{-1} to 10^{-5}. From the 10^{-3} to 10^{-5} dilutions, 200 µL was spread onto prepared growth media. Three distinct media were used for actinobacteria isolation: ISP-2 (4 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose, 20 g/L bacto agar), Actinomycetes Isolation Agar (Himedia), and Starch M Protein Agar (Himedia). To mimic the osmotic pressure of seawater, ISP-2 media and Actinomycetes Isolation Agar were dissolved in artificial seawater (33 g/L). All three-agar media were sterilized in an autoclave at 1 atm and 121°C for 15 minutes. Before pouring into Petri dishes, each agar medium was supplemented with nystatin (25 µg/mL) and nalidixic acid (10 µg/mL) to prevent growth of fungi and Gram-negative bacteria, respectively. Each Petri dish containing the agar medium and sample was wrapped with parafilm to prevent contamination and incubated at 28°C. Bacterial observations, including colony counting, were performed every three days for two weeks.

2.3. Morphological characterization

From each growth medium, colonies displaying unique macroscopic morphologies were selected for identification based on colony morphology code and Gram staining.

2.4. Antibacterial screening

Agar block method was employed to assesses the antimicrobial activity of each bacterial isolates from marine sediments against test bacteria namely *Streptococcus mutans* FNCC 0405, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922. Initially, all test bacteria were first refreshed in LB liquid medium, then aseptically streaked onto LB agar. Concurrently, pure bacterial isolates that had been grown on ISP-2 agar for 10 days, were cut into 1x1 cm agar blocks using a sterile scalpel. These blocks were then aseptically transferred to Petri dishes containing the streaked test bacteria. After co-incubation at 37°C for two days, marine bacterial isolates demonstrating inhibitory activity showed a clear zone of inhibition around the agar block, resulting from the diffusion of active compounds. The diameter zone of inhibition was precisely measured using a digital calliper as a direct quantitative measure of the isolate's antibacterial activity. A larger diameter unequivocally indicated a greater inhibitory effect against the target bacteria.

2.5. Molecular analysis

The bacterial isolate exhibiting the most potent antibacterial activity was selected for molecular characterization. For DNA isolation, cell mass was propagated in 1.5 mL of ISP-2 broth. Genomic DNA was then extracted using a commercial Bacteria DNA Preparation Kit (Jena Bioscience, Germany). The extracted DNA served as template for Polymerase Chain Reaction (PCR) targeting the 16S rRNA gene fragment, following established protocols and PCR cycling parameters[6]. Amplified PCR products were subsequently analysed via 1% agarose gel electrophoresis, stained with SYBR Safe, and visualized under

UV light using a GelDoc system. Finally, the confirmed PCR product was subjected to Sanger sequencing. Sequencing results were analysed using nucleotide BLAST (BLASTn) against the NCBI database. A phylogenetic tree was then constructed using MEGA X (https://www.megasoftware.net/software).

3 Results

Ten bacterial isolates with different morphological characteristics were obtained from marine sediments collected at Matahari Terbit Beach, using three different agar media: ISP-2, Actinomycetes Isolation Agar, and Starch M Protein Agar. Morphological identification was performed based on the Colony Morphology Code (CMC) criteria, which considers the shape, surface, colour, and elevation of each bacterial colony. A summary of the morphological observations for each bacterial isolate is presented in Table 1.

Table 1. Morphological characteristics of bacterial isolates

Isolate Code	Agar Medium	Shape	Surface	Colour	Elevation	Gram Type
I-1	ISP-2	1 (Circular)	3 (Dull)	4 (Yellow)	1 (Flat)	+
I-2	ISP-2	1 (Circular)	2, 4 (Rough and Wrinkled)	2 (Turbid)	3 (Raised/ Bulge on colony)	+
I-3	ISP-2	2 (Irregular)	2 (Rough)	4 (Yellow)	2 (Raised)	+
I-4	ISP-2	2 (Irregular)	2, 4 (Rough and Wrinkled)	4 (Yellow)	1 (Flat)	+
I-5	ISP-2	1 (Circular)	3 (Dull)	3 (Translucent)	2 (Raised)	+
A-1	AIA	2 (Irregular)	2 (Rough)	4 (Yellow)	1 (Flat)	+
A-2	AIA	2 (Irregular)	2 (Rough)	2 (Turbid)	2 (Raised)	+
A-3	AIA	1 (Circular)	3 (Dull)	3 (Translucent)	1 (Flat)	+
A-4	AIA	3 (Filamentous)	3 (Dull)	4 (Yellow)	1 (Flat)	+
SM-1	Starch-M Protein	2 (Irregular)	3 (Dull)	2 (Turbid)	1 (Flat)	+

Gram staining revealed that all isolates were Gram-positive bacteria (Figure 1), exhibiting morphological characteristics typical of actinobacteria (Figure 2). On agar plates, these actinobacteria displayed predominantly irregular or circular colony shapes with wrinkled, pigmented surfaces. Microscopic examination further confirmed their actinobacterial nature by revealing the presence of spore-like structures, similar to those found in fungi.

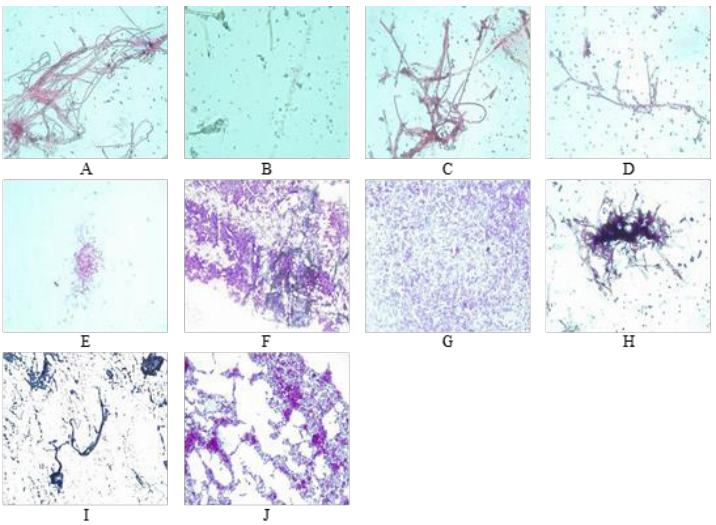

Fig. 1. Microscopic appearance of ten candidate actinobacteria isolates after Gram staining. A. Isolate I-1, B. Isolate I-2, C. Isolate I-3, D. Isolate I-4, E. Isolate I-5, F. Isolate A-1, G. Isolate A-2, H. Isolate A-3, I. Isolate A-4, J. Isolate SM-1.

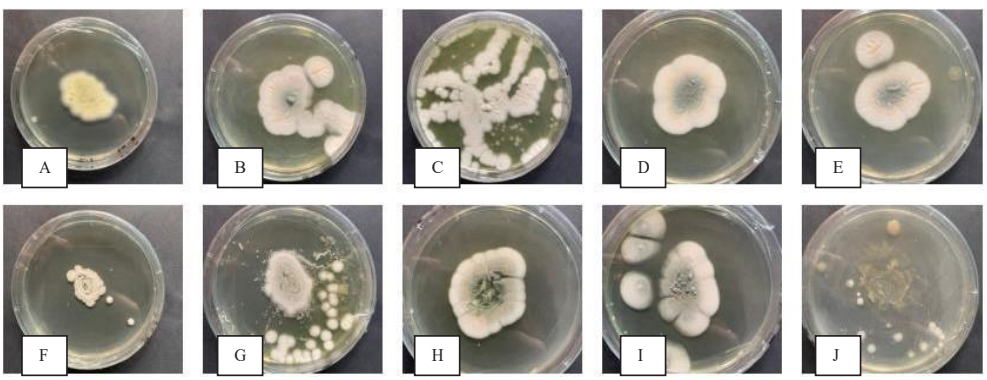

Fig. 2. Colony morphology of candidate actinobacteria isolates from Matahari Terbit beach marine sediments grown on agar media

The agar block method revealed that the ten isolates exhibited varying degrees of antimicrobial activity against different test bacteria. Nine isolates (I-1, I-2, I-3, I-4, I-5, A-2, A-3, A-4, SM-1) showed activity against *S. aureus* ATCC 25923 (Table 2). Five isolates (I-2, I-3, I-4, I-5, A-4) demonstrated activity against *S. mutans* FNCC 0405. Only isolate A-1 showed no inhibitory activity against any of the four test bacteria.

Table 2. Diameter zone of inhibition (mm±SD) of bacterial isolates against bacterial test

No	Isolate Code	*S. aureus* ATCC 25923	*S. mutans* FNCC 0405	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 700603
1	I-1	8.06 ± 6.9	0 ± 0	0 ± 0	12.52 ± 1.01
2	I-2	12.29 ± 1.47	18.04 ± 0.32	19.78 ± 0.20	12.20 ± 0.36

3	I-3	13.99 ± 0.31	19.57 ± 1.12	13.46 ± 2.23	15.45 ± 1.29
4	I-4	16.28 ± 0.73	19.37 ± 0.23	19.79 ± 0.06	14.92 ± 0.85
5	I-5	15.32 ± 1.13	21.17 ± 0.70	19.82 ± 1.22	10.66 ± 0.41
6	A-1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
7	A-2	11.66 ± 1.029	0 ± 0	0 ± 0	0 ± 0
8	A-3	11.50 ± 1.44	0 ± 0	12.91 ± 1.74	14.66 ± 0.74
9	A-4	15.73 ± 0.95	16.02 ± 0.99	19.16 ± 0.19	10.48 ± 0.11
10	SM-1	12.57 ± 0.49	0 ± 0	0 ± 0	0 ± 0

Based on the pre-screening results presented in Table 2, isolate I-4 exhibited the highest inhibitory activity against *S. aureus* ATCC 25923, with a mean inhibition zone diameter of 16.28 ± 0.73 mm. Isolate I-5 showed the strongest inhibition against *S. mutans* FNCC 0405, producing a zone diameter of 21.17 ± 0.70 mm. For *E. coli* ATCC 25922, isolate I-4 again demonstrated the strongest inhibitory effect, with a diameter of 19.79 ± 0.06 mm (Figure 3). Lastly, isolate I-3 was most effective against *K. pneumoniae* ATCC 700603, yielding an inhibition zone of 15.45 ± 1.29 mm.

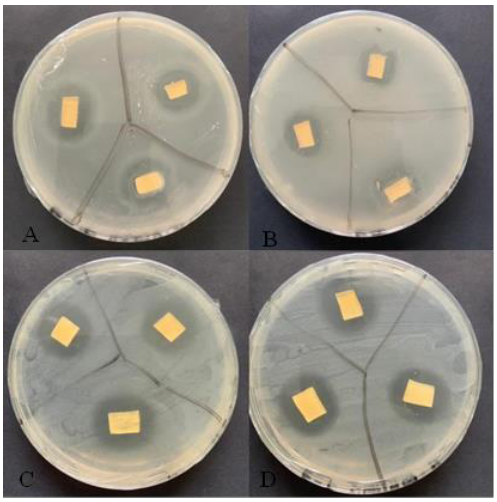

Fig. 3. Agar block assay observations of Isolate I-4 against test bacteria. A. *S. aureus* ATCC 25923, B. *K. pneumoniae* FNCC 0405, C. *E. coli* ATCC 25922, and D. *S. mutans* ATCC 700603.

Isolate I-4 is a bacterial isolate exhibiting actinobacteria-like characteristics with the highest observed antibacterial activity. Specifically, isolate I-4 possesses the morphological features

of a Gram-positive bacterium with coccus-shaped cells and the presence of hyphae (Figure 4). Isolate I-4 formed colonies with an irregular, rough, and dull surface, appearing powdery and adhering firmly to the medium (Figure 5). As seen from the top, the colony surface (Figure 5A) exhibited a greyish-yellow pigmentation. When viewed from the back of the plate, the submerged colony showed a light greenish-yellow pigmentation (Figure 5B). Considering these observations, isolate I-4 was selected for further molecular characterization as the most promising actinobacterial candidate. This decision was based on its ability to inhibit the growth of all four test bacteria, consistently demonstrating the largest inhibition zone among all suspected actinobacterial isolates.

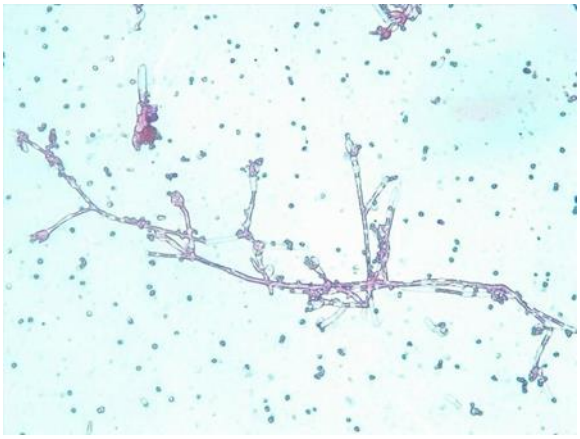

Fig. 4. Gram staining result of isolate I-4.

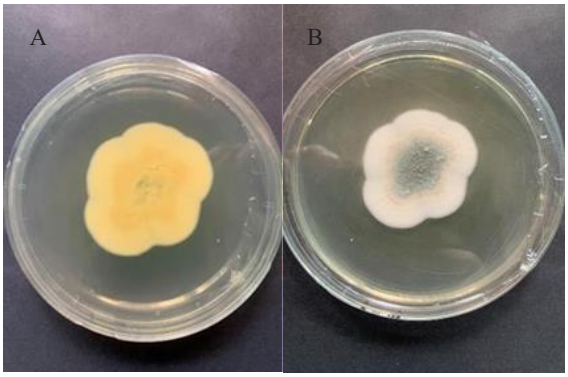

Fig. 5. Morphology of isolate I-4 on ISP-2 agar medium. Image A shows substrate mycelium, and Image B shows aerial mycelium.

Table 3 illustrates the alignment of the 16S rRNA gene sequence from Isolate I-4 with existing data in the NCBI database. The top ten results from this BLAST sequence alignment indicate that isolate I-4 is closely related to the species *Streptomyces* sp. VEL17.

Table 3. Molecular Identification of Isolate I-4 Based on 16S rRNA Gene BLASTn Analysis.

Description	Accession number	Query cover %	Percentage identity %	E-value	Max score

Streptomyces sp. VEL17 gene for 16S ribosomal RNA, partial sequence	AB914463.2	99	99.72	0.0	2614
Streptomyces sp. MAH25 gene for 16S ribosomal RNA, partial sequence	AB899157.1	99	99.44	0.0	2595
Streptomyces indiaensis strain IF 5 16S ribosomal RNA gene, partial sequence	FJ951435.1	99	99.37	0.0	2586
Streptomyces sp. VEL27 gene for 16S ribosomal RNA, partial sequence	AB909959.1	99	99.65	0.0	2584
Streptomyces sp. VITTKGB 16S ribosomal gene, partial sequence	GU358071.1	99	99.30	0.0	2580
Streptomyces exfoliatus strain MMA1033 16S ribosomal RNA gene, partial sequence	KY580807.1	99	98.74	0.0	2531
Streptomyces sp. UrEPI15 16S ribosomal RNA gene, partial sequence	MH553633.1	99	98.47	0.0	2516
Streptomyces sp. TIR12 16S ribosomal RNA, partial sequence	AB899158.1	99	98.13	0.0	2505
Streptomyces sp. HmuEPI22 gene for 16S ribosomal RNA gene, partial sequence	MH553624.1	99	97.78	0.0	2473
Streptomyces exfoliatus strain A156716S ribosomal RNA gene, partial sequence	MH547398.1	99	99.21	0.0	2460

4 Discussion

The initial phase of this study successfully yielded ten bacterial isolates from various selective agar media. These isolates displayed a wide array of colony morphologies, which were meticulously documented on agar plates. Crucially, all isolates were confirmed as Gram-positive bacteria through detailed staining. This combination of diverse morphology and Gram-positive nature, particularly when coupled with the observation of spore-like structures under the microscope, strongly suggested the presence of a rich and varied actinobacterial population within these marine sediments. *Actinobacteria* are globally renowned for their remarkable capacity to produce a vast array of diverse secondary metabolites, including many compounds with significant pharmaceutical applications [7]. This inherent biosynthetic prowess positions marine sediments as an invaluable frontier in the ongoing global search for novel bioactive compounds.

The primary aim of this research was to screen candidate actinobacteria isolates obtained from marine sediments of Sanur's Matahari Terbit Beach for antibacterial activity. Among the ten isolates, isolate A-1 did not produce any zone of inhibition, indicating a complete absence of antibacterial activity against the tested bacteria. The varying antibacterial activities, as observed through different inhibition zones, are likely dependent on the secondary metabolites produced by each bacterial isolate. Generally, production of secondary metabolite is highly influenced by culture conditions and the environment [8]. Therefore, it is likely that the nutrient composition of the LB agar medium used for the antibacterial activity test was not optimal for the actinobacteria isolate's growth. This suboptimal

environment might have prevented the bacterial isolate from synthesizing its secondary metabolites. Furthermore, the composition of antibacterial compounds produced by *Actinobacteria* varies depending on their specific type [8]. In this study, we could not determine the exact factor that caused isolate A-1 to show no antibacterial activity against any of the four tested bacteria. It is possible that isolate A-1 does not produce antibacterial compounds at all, but instead synthesizes other types of secondary metabolites, such as antifungal, antioxidant, or other beneficial compounds.

All nine presumed actinobacterial isolates were more effective at inhibiting the growth of Gram-positive bacteria (*S. aureus* ATCC 25923 and *S. mutans* FNCC 0405) compared to Gram-negative bacteria (*E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603). This finding aligns with previous research, which also demonstrated that actinobacteria isolated from marine ecosystems exhibit better antibacterial activity against Gram-positive bacteria [9]. Generally, Gram-negative bacteria possess a more robust defence system against antibacterial compounds than Gram-positive bacteria[10]. This difference is attributed to the distinct components of their cell walls. In addition to peptidoglycan, Gram-negative bacteria have an outer membrane composed of lipopolysaccharides that covers 90% of the cell surface. This outer membrane acts as an additional protective system, making it difficult for lipophilic substances to penetrate [9].

A substantial number of these strains exhibited potent antibacterial activity, specifically against common human pathogens like *S. aureus* and *S. mutans*. This broad-spectrum activity is a pivotal finding, as it directly indicates the capability of compounds derived from these marine bacteria to combat a wide range of bacterial infections, addressing an urgent need in public health. Among these promising candidates, isolate I-4 stood out significantly. It demonstrated exceptionally robust inhibitory effects against multiple test bacteria, effectively targeting both Gram-positive pathogens like *S. aureus* and medically important Gram-negative pathogens such as *E. coli* and *K. pneumoniae*. This broad-spectrum efficacy is a key indicator of isolate I-4's high promise as a subject for further investigation into its therapeutic applications. Furthermore, its specific morphological traits, including coccus-shaped cells with distinct hyphae and unique colony pigmentation, provided additional compelling evidence supporting its initial classification as an actinobacterium.

Marine *Streptomyces* have garnered significant attention in the field of antimicrobial compound discovery due to their prolific capacity to synthesize a diverse range of bioactive secondary metabolites [6]. Recent studies indicate that *Streptomyces* strains derived from marine environments produce unique chemical structures effective against various pathogens, including those resistant to conventional treatments [4, 11]. The genetic backgrounds of marine *Streptomyces* also support their antibiotic-producing capabilities. For instance, a comparative genomics analysis revealed that many marine strains possess unique bioactive gene clusters adapted to the marine environment [12, 13]. Previous research also identified a marine *Streptomyces* strain that capable of producing angucyclinone antibiotics, indicating that marine-derived *Streptomyces* can contribute significantly to the discovery of new classes of antibiotics [14, 15]. These findings collectively underscore the immense untapped potential of marine actinobacteria from this specific environment as a vital source for discovering novel antibacterial compounds, which is crucial in the ongoing global effort to combat antimicrobial resistance.

Conclusion

Overall, this research has successfully isolated ten Gram-positive bacterial strains exhibiting actinobacteria-like morphology from marine sediments of Sanur's Matahari Terbit Beach, in Bali, Indonesia. Among these, Isolate I-4 demonstrated the most potent antibacterial activity, yielding significant inhibition zones against bacterial test. Molecular identification further

revealed that isolate I-4 shares a 99.72% sequence similarity with Streptomyces sp. VEL17, highlighting its potential as a source of novel antibacterial compounds.

Moving forward, the primary focus should be on the comprehensive characterization of the antibacterial compounds produced by the isolate I-4. Future research directions include: isolation and purification of the active compounds, followed by their structural elucidation using advanced analytical techniques. Investigating the mechanism of action of these compounds against target pathogens will also be critical. Furthermore, optimizing fermentation conditions to enhance the yield of bioactive metabolites and exploring the potential for other therapeutic properties, such as antiviral, antifungal, or anticancer activities, would be valuable next steps. Finally, genome sequencing of the isolate I-4 could provide deeper insights into its biosynthetic gene clusters, paving the way for targeted genetic manipulation to potentially enhance compound production or discover new derivatives.

Acknowledgment

This research was financially funded by the fundamental research grant no 163/Unwar/FKIK/Unit Penelitian/PD-13/VIII/2022 provided by Faculty of Medicine and Health Sciences, Warmadewa University awarded to Anak Agung Gede Indraningrat.

References

1. R Iseppi, M Mariani, C Condò, C Sabia, and P Messi,Essential Oils: A Natural Weapon Against Antibiotic-Resistant Bacteria Responsible for Nosocomial Infections. Antibiotics 10 (4), 417 (2021). https://doi.org/10.3390/antibiotics10040417.
2. J Kim and J Ahn,Emergence and Spread of Antibiotic-Resistant Foodborne Pathogens From Farm to Table. Food Science and Biotechnology 31 (12), 1481-1499 (2022). https://doi.org/10.1007/s10068-022-01157-1.
3. A M Thawabteh, Z Swaileh, M Ammar, W Jaghama, M Yousef, R Karaman, S A Bufo, and L Scrano,Antifungal and Antibacterial Activities of Isolated Marine Compounds. Toxins 15 (2), 93 (2023). https://doi.org/10.3390/toxins15020093.
4. D Bharathi and J Lee,Recent Advances in Marine-Derived Compounds as Potent Antibacterial and Antifungal Agents: A Comprehensive Review. Mar Drugs 22 (8), 348 (2024). https://doi.org/10.3390/md22080348.
5. N K D Cahyani, N Kasanah, D S Kurnia, and M T Hamann,Profiling Prokaryotic Communities and Aaptamines of Sponge Aaptos suberitoides from Tulamben, Bali. Mar Biotechnol (NY) 25 (6), 1158-1175 (2023). https://doi.org/10.1007/s10126-023-10268-7.
6. K Zhang, W Ding, C Han, L Long, H Yin, and J Yin,Investigation on taxonomy, secondary metabolites and antibacterial activity of Streptomyces sediminicola sp. nov., a novel marine sediment-derived Actinobacteria. Microbial Cell Factories 23 (1), 285 (2024). https://doi.org/10.1186/s12934-024-02558-z.
7. D A van Bergeijk, B R Terlouw, M H Medema, and G P van Wezel,Ecology and genomics of Actinobacteria: new concepts for natural product discovery. Nature Reviews Microbiology 18 (10), 546-558 (2020). https://doi.org/10.1038/s41579-020-0379-y.
8. D De Simeis and S Serra,Actinomycetes: A Never-Ending Source of Bioactive Compounds-An Overview on Antibiotics Production. Antibiotics 10 (5), (2021). https://doi.org/10.3390/antibiotics10050483.
9. M C De La Hoz-Romo, L Díaz, and L Villamil,Marine Actinobacteria a New Source of Antibacterial Metabolites to Treat Acne Vulgaris Disease-A Systematic Literature

Review. Antibiotics (Basel) 11 (7), (2022). https://doi.org/10.3390/antibiotics11070965.
10. Z Breijyeh, B Jubeh, and R Karaman,Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules 25, (2020). https://doi.org/10.3390/molecules25061340.
11. J T Wibowo, A Bayu, W D Aryati, C Fernandes, A Yanuar, A Kijjoa, and M Y Putra,Secondary Metabolites From Marine-Derived Bacteria With Antibiotic and Antibiofilm Activities Against Drug-Resistant Pathogens. Mar Drugs 21 (1), 50 (2023). https://doi.org/10.3390/md21010050.
12. A Sedeek, H Elfeky, A Hanora, and S Solyman,Genomic insights into biosynthesis and adaptation in the bioactive marine bacterium Streptomyces albidoflavus VIP-1 from the Red Sea. BMC Microbiology 25, (2025). https://doi.org/10.1186/s12866-025-04109-x.
13. Y-H Chung, H Kim, C-H Ji, H-W Je, D Lee, S H Shim, H-S Joo, and H-S Kang,Comparative Genomics Reveals a Remarkable Biosynthetic Potential of the Streptomyces Phylogenetic Lineage Associated with Rugose-Ornamented Spores. mSystems 6 (4), (2021). https://doi.org/10.1128/msystems.00489-21.
14. X Xu, X Huang, and W Xu,Marine actinomycetes-derived angucyclines and angucyclinones with biosynthesis and activity--past 10 years (2014–2023). European Journal of Medicinal Chemistry 283, 117161 (2025). https://doi.org/10.1016/j.ejmech.2024.117161.
15. E M Sabido, C P Tenebro, A F L Suarez, S D C Ong, D J V L Trono, D S Amago, J E Evangelista, A M Q Reynoso, I G M Villalobos, L D D Alit, C F Surigao, C A Villanueva, J P Saludes, and D S Dalisay,Marine Sediment-Derived Streptomyces Strain Produces Angucycline Antibiotics against Multidrug-Resistant Staphylococcus aureus Harboring SCCmec Type 1 Gene. Journal of Marine Science and Engineering 8 (10), 734 (2020). https://doi.org/10.3390/jmse8100734.