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Antibacterial activity of Gorgonian-associated actinomycetes against diabetic foot pathogens

DEVI OKTAVIA ANJANI[1], ESTER CHRISTINA CHINTA LOSUNG[1], AGUS SABDONO[2,♥], SRI SEDJATI[2]

[1]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Jacub Rais, Tembalang, Semarang 50275, Central Java, Indonesia

[2]Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Jacub Rais, Tembalang, Semarang 50275, Central Java, Indonesia. Tel./fax.: +62-24-7474698, ♥email: agus_sabdono@yahoo.com

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Abstract. *Anjani DO, Losung ECC, Sabdono A, Sedjati S. 2025. Antibacterial activity of Gorgonian-associated actinomycetes against diabetic foot pathogens. Biodiversitas 26: 4157-4166.* Diabetic foot infection is a condition affecting the lower extremities that occurs in at least 15% of all diabetes patients. The treatment of diabetic foot infection has become increasingly challenging due to the risk of infection by microorganisms around the wound. The presence of antibiotic-resistant pathogenic bacteria further aggravates this condition. Therefore, the discovery of new sources of antibiotics is urgently needed. The aim of this study was to explore the antibacterial activity of actinomycetes, particularly those associated with marine gorgonians, to better understand their potential in the treatment of diabetic foot infection. Gorgonian samples were collected from Karimunjawa Waters, Indonesia. Antibacterial activity was screened using agar plug method against *Escherichia coli, Staphylococcus aureus, Enterococcus* sp., and *Proteus* sp.. The selected isolates were then identified through Gram staining and molecular identification. The NRPS, PKS-I, and PKS-II genes were detected through the PCR method. The results showed that 12 out of 45 isolates (26.7%) exhibited antibacterial activity against DFI pathogenic bacteria, with four isolates producing inhibition zone greater than 5 mm. The strongest antibacterial activity was exhibited by isolate UR.19.4 and EA.6.5.2 against *S. aureus* and *E. coli* with inhibition zones greater than 13 mm. Molecular identification revealed that UR.19.4 and EA.6.5.2 isolates had the closest similarity to *Streptomyces griseobrunneus* (99.76%) and *Streptomyces zhaozhouensis* (99.69%), respectively. The NRPS gene was detected in both *S. griseobrunneus* (UR.19.4 isolate) and *S. zhaozhouensis* (EA.6.5.2 isolate), while the PKS-II gene was detected only in *S. griseobrunneus* (UR.19.4 isolate). Therefore, these findings highlight the potential of actinomycetes isolated from Gorgonian as a promising antimicrobial source for treating diabetic foot infections.

Keywords: Actinomycetes, antibacterial, biosynthetic gene cluster, diabetic foot infection, Gorgonian

INTRODUCTION

Diabetic Foot Infection (DFI) is an infection affecting the lower extremities' skin, tissues, and bones, leading to severe infections in diabetic patients (Ramirez-Acuña et al. 2019). The global prevalence of DFI is approximately 6.3%, making it the leading cause of lower extremity amputation worldwide (Zhang et al. 2017). This complication is experienced by 15%-25% of diabetes patients across the world. The lifetime risk of diabetic foot is 19% to 34% with recurrence rates of 65% at 3-5 years, leading to 20% incidence of lifetime lower-extremity amputation and a 5-year mortality rate of 50-70% (McDermott et al. 2023). The treatment of DFI is complicated by several risk factors, including neuropathy, Peripheral Arterial Disease (PAD), immune system disorders, and infections in open wounds (Ramirez-Acuña et al. 2019). Wounds disrupt the skin's protective barrier, exposing underlying tissues to microbial colonization, aggravating tissue damage, and hindering wound healing (Olid et al. 2015). Abu-El-Azayem et al. (2024) reported that the most commonly isolated pathogenic bacteria in DFI include *Klebsiella* spp. (24%), *Pseudomonas* spp. (16%), *Staphylococcus aureus* (13.5%), *Escherichia coli* (12%), *Proteus vulgaris* (8.5%), and *Enterococcus* spp. (3.4%). This issue is further complicated by Antimicrobial Resistance (AMR), as many antibiotics are becoming ineffective against these pathogens. *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* isolated from diabetic foot wounds have shown high resistance to five commonly used antibiotics (Moya-Salazar et al. 2023). Continuous efforts to discover new antibiotics are essential to addressing this challenge.

Gorgonians, also known as "sea fans", belong to the family Gorgoniidae, order Alcyonacea, subclass Anthozoa, and phylum Cnidaria (Sabdono et al. 2022a). These soft corals are capable of producing a diverse array of bioactive compounds as a defense mechanism against predators (Izzati et al. 2021). Gorgonians have been reported to contain terpenoids, steroids, and alkaloids, exhibiting various biological activities, including anti-inflammatory, anticancer, analgesic, antiviral, antibacterial, and antifouling properties (Kelutur et al. 2021). Additionally, these soft corals have been shown to produce enzyme inhibitors with potential pharmaceutical applications (Córdova-Isaza et al. 2023). However, the biosynthesis of bioactive compounds from gorgonians faces significant challenges due to the limited availability of the organisms, while substantial amounts of material are required for extraction. The bacteria associated with gorogonians offer a promising alternative to overcome this limitation. Unlike their host organisms, these bacteria

can be easily cultivated and are also capable of producing various high-quality bioactive compounds (Sabdono et al. 2022b).

Actinomycetes are Gram-positive, filamentous, heterotrophic, and saprophytic bacteria known for their ability to produce a wide variety of important secondary metabolites, making them a valuable source of new bioactive compounds (De Simeis and Serra 2021). Actinomycetes are the most common bacterial source of bioactive compounds, with 182 new compounds reported from this group in 2022 alone (Carroll et al. 2024). Marine actinomycetes, particularly those associated with invertebrates such as sponges and corals, have been reported to produce various bioactive compounds with unique chemical structures, including antibiotics, anticancer agents, and immunosuppressants (Worsley et al. 2023). The harsh and competitive marine environment drives these microorganisms to develop potent bioactive compounds for survival, making them highly promising for pharmaceutical applications.

Previous studies have reported the antibacterial potential of actinomycetes isolated from sponges, marine sediments, and stony corals (Liu et al. 2019; Mondal and Thomas 2022; Sibero et al. 2023). However, actinomycetes associated with gorgonian corals remain largely underexplored despite their potential to produce novel bioactive compounds (Kelutur et al. 2021). To date, research on Gorgonian-associated bacteria has mainly centered on microbial diversity, with relatively few studies assessing their antimicrobial potential against human pathogens. The aim of this study was to explore the antibacterial activity of actinomycetes, particularly those associated with Gorgonian, to better understand their potential in supporting the treatment of diabetic foot infection.

MATERIALS AND METHODS

Sample collection sites

The Gorgonian samples used in this study were collected from Karimunjawa Island, Jepara, Central Java, Indonesia. The sampling locations included Gelean Island (05°52'56.0" S 110°21'29.5" E), Sambangan Island (05°50'27.8" S 110°34'54.8" E), Seruni Island (05°51'13.3" S 110°34'36.8" E), and Burung Island (05°53'27.9" S 110°20'46.2" E) as shown in Figure 1. The permit to access the sampling location and collect specimens was acquired from the Karimunjawa National Park Authority, with permit No. 1769/T.34/TU/SIMAKSI/07/2024. The sampling method was purposive sampling, targeting soft coral invertebrates from the Gorgonian group. Sample collection was conducted through SCUBA diving at a depth of 15-28 meters. Each Gorgonian specimen was assigned a unique code based on its collection site, such as EA (Gelean Island), BA (Sambangan Island), ER (Seruni Island), and UR (Burung Island). Environmental parameters measured included pH, temperature, Dissolved Oxygen (DO), and salinity. The collected organisms were placed in Falcon tubes and stored in a cool box before being isolated in the laboratory.

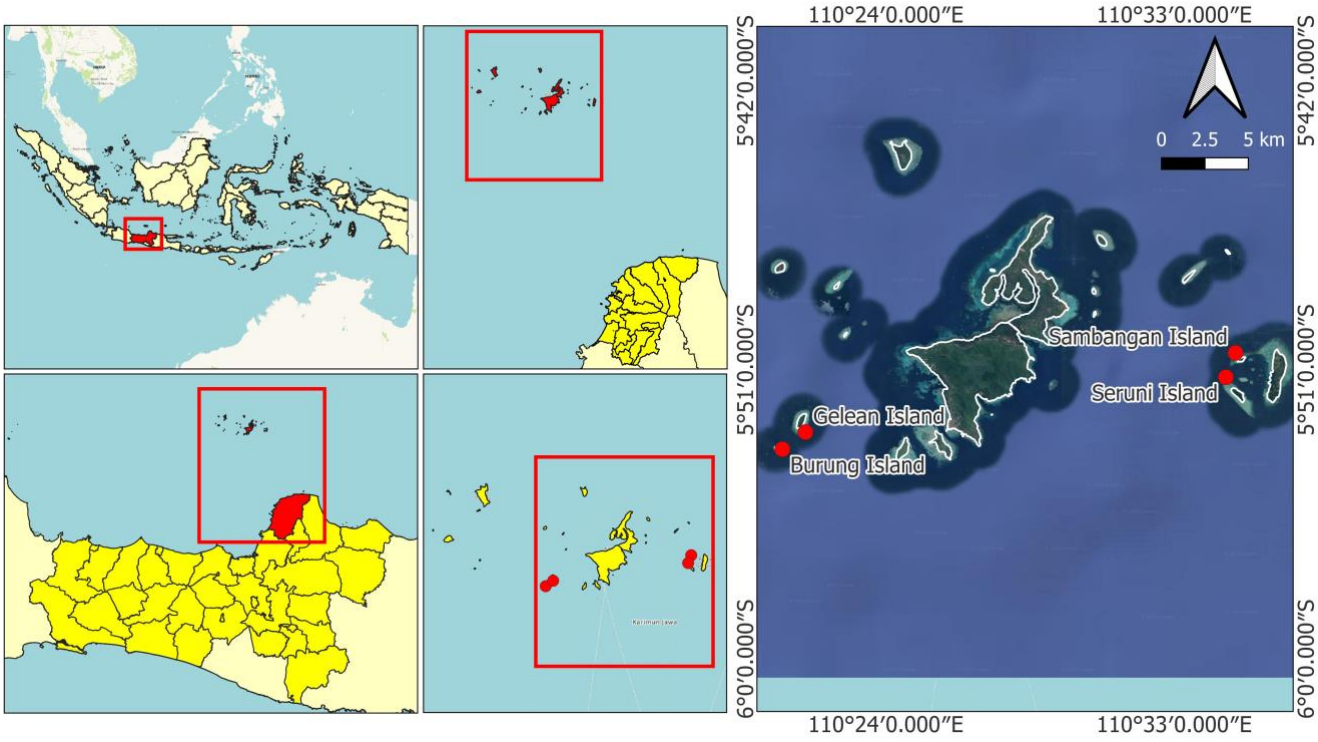


Figure 1. The sampling location in Karimunjawa, Jepara, Central Java, Indonesia

Isolation and purification

The samples were crushed using a mortar and pestle, followed by serial dilution with a dilution range of 10^{-1}-10^{-5}. Actinomycete bacteria were isolated from the sample organisms using the spread plate method on Actinomycetes Isolation Agar (AIA) media, HiMedia (de Oliveira et al. 2024). The isolation plates were incubated at room temperature for 7-21 days. The grown isolates were then purified using the four-quadrant streak method until pure colonies were obtained.

Antibacterial screening

The antibacterial assay was performed using the agar plug method based on Yi et al. (2023). The pathogens used in the assay included bacteria commonly found in diabetic foot ulcers, namely *Proteus* sp., *Enterococcus* sp., *Staphylococcus aureus*, and *Escherichia coli*. The pathogenic bacteria were inoculated into Nutrient Broth and agitated for 24 hours, then diluted to a density equivalent to 0.5 McFarland standard, with a turbidity of 1.5×10^8 CFU/mL. The bacterial suspension was then spread onto the Mueller-Hinton Agar (MHA) surface using a cotton swab. Actinomycetes isolates previously grown on an AIA medium were perforated using the base of a blue tip to form agar plugs, which were then placed onto the surface of the MHA plates inoculated with the test pathogens. Chloramphenicol (30 µg/disk) was used as a positive control by dripping it on a blank paper disc. Agar plugs from sterile MHA medium were used as a negative control. The petri dishes were incubated at 30°C, and observations were made at 24 and 48 hours. The assay was conducted in triplicate. Antibacterial activity was indicated by a clear inhibition zone surrounding the agar plug. The diameter of the Zone of Inhibition (ZOI) was measured as a quantitative indicator of the antibacterial activity exhibited by the isolates (Ulusu and Bilgic 2024; Ulusu and Ulusu 2024).

Gram-staining microscopic identification

Microscopic identification was performed using Gram staining following the method of Saryono et al. (2019). A pure actinomycetes isolate was inoculated onto a previously sterilized glass slide. The slide was sequentially treated with crystal violet, iodine, decolorizer, and safranin, each for one minute. The prepared slide was then observed under a microscope.

DNA extraction

DNA isolation from *Actinomycetes* isolates was performed using the Presto™ Mini gDNA Bacteria Kit (Geneaid Ltd., Taiwan) following the method described by Nurliana et al. (2022). Pure bacterial isolates were inoculated into 1.5 mL microtube containing Buffer Gram+ (200 µL) and lysozyme (0.8 mg/200 µL), followed by homogenization. The sample was incubated at 37°C for 30 minutes. Proteinase K (20 µL) was added, and the mixture was incubated at 60°C for 10 minutes. The cell lysis process involved the addition of Buffer GT (200 µL), homogenization for 10 seconds, and incubation at 70°C for 10 minutes. For DNA binding, absolute ethanol (200 µL) was added, followed by homogenization. The solution was then transferred to a new microtube fitted with a GD Column and centrifuged at 14,000-16,000 rpm for 2 minutes, after which the supernatant was discarded. The GD Column was then placed into a fresh 2 mL microtube. The washing process involved adding Buffer W1 (400 µL) followed by centrifugation at 14,000-16,000 rpm for 3 minutes, then discarding the supernatant. Next, 600 µL of Wash Buffer containing ethanol was added, and the solution was centrifuged at 14,000-16,000 rpm for 30 seconds before discarding the supernatant. The GD Column was transferred to a new 2 mL tube, and 100 µL of preheated Elution Buffer (70°C) was added. The solution was incubated at room temperature (27-29°C) for 3-5 minutes, followed by centrifugation at 14,000-16,000 rpm for 1 minute and the extracted DNA was stored at -20°C.

Amplification of 16S rRNA gene using PCR and DNA sequencing

PCR amplification was performed using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3'). The final PCR reaction volume was 25 µL, consisting of the following components: 1 µL Primer 27F (10 µM), 1 µL Primer 1492R (10 µM), 12.5 µL My Taq Green Mix, 9.5 µL ddH_2O, and 1 µL DNA template. The thermal cycler conditions were set as follows: pre-denaturation at 95°C for 1 minute; 35 cycles, denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds and post-extension at 72°C for 3 minutes. Electrophoresis was conducted using a 1% agarose gel at 100 V for 30 minutes. The resulting PCR products were submitted to PT Genetika Science, Indonesia for DNA sequencing. The obtained sequence data were analyzed and compared to the NCBI database using BLAST feature. A phylogenetic tree was subsequently constructed using MEGA 11.

Biosynthetic gene clusters of NRPS, PKS-I, and PKS-II detection

Detection of NRPS, PKS-I, and PKS-II genes was performed through PCR amplification using the following primers: A2gamForward (5′-AAGGCNGGCGSBGCSTAYSTGCC-3') and A3gamReverse (5'-TTGGGBIKBCCGGTSGINCCSGAGGTG-3') primers for NRPS, KS-F (5'-TSGCSTGCTTGGAYGCSATC-3') and KS-R (5'-TGGAANCCGCCGAABCCGCT-3') primers for PKS-I, IIPF6 (5'-TSGCSTGCTTCGAYGCSATC-3') and IIPR6 (5'-TGGAANCCGCCGAABCCGCT-3') primers for PKS-II. The final PCR reaction volume was 25 µL, consisting of 1 µL Primer Forward (10 µM), 1 µL Primer Reverse (10 µM), 12.5 µL My Taq Green Mix, 9.5 µL DDH_2O, and 1 µL DNA template. The Thermal Cycler conditions were set as follows: pre-denaturation at 95°C for 1 minute; 30 cycles, denaturation at 95°C for 1 minute, annealing at 58.4°C (NRPS) and 59.4°C (PKS-I and PKS-II) for 1 minute, extension at 72°C for 1 minute, and post-extension at 72°C for 3 minutes.

Statistical analysis

All experiments were performed in triplicate to ensure statistical reliability. The data were presented as the mean diameter of the inhibition zone±Standard Deviation (SD). Kruskal-Wallis test (non-parametric One-Way Analysis of Variance (ANOVA)) was conducted using SPSS to evaluate significant differences in the mean of the inhibition zones among the isolates.

RESULTS AND DISCUSSION

Isolation of actinomycetes

A total of five Gorgonian hosts were obtained from the sampling locations. The hosts were identified through their morphological characteristics. Morphological identification showed that the hosts were *Ellisella* sp., *Viminella* sp., *Astrogorgia* sp., *Junceella* sp., and *Siphonogorgia*. The waters of Karimunjawa are known as a habitat for various species of hard corals, soft corals, echinoderms, and other invertebrates (Sabdono et al. 2022c; Sibero et al. 2023; Hendrawati et al. 2024). Sabdono et al. (2022a) reported the presence of soft corals belonging to the gorgonian group in these waters, including the genera *Viminella* sp., *Ellisella* sp., *Antipathes* sp., *Melithaea* sp., *Astrogorgia* sp., and *Junceella* sp.. In another study by Sabdono et al. (2022c), at least 12 genera of Gorgonians were identified in the same waters, belonging to five families, namely Ellisellidae, Plexauridae, Melithaeidae, Acanthogorgidae, and Isididae. The diversity of Gorgonian species in the Karimunjawa waters is presumed to be due to the relatively uncontaminated and protected water quality (Sabdono et al. 2022c).

A total of 45 actinomycetes isolates were successfully obtained from various Gorgonian hosts in Karimunjawa. Previous studies have reported that Gorgonian corals host a wide range of microorganisms (van de Water et al. 2017; Sabdono et al. 2022b). This was further supported by Sabdono et al. (2022a), who found that bacterial isolates from Gorgonian corals belonged to the phyla Proteobacteria, Actinobacteria, and Firmicutes. Similar results were reported by Larasati et al. (2023), in which bacteria associated with *Plexaura* sp. were dominated by *Bacillus* sp., *Virgibacillus* sp., and *Nitrareductor* sp. The interaction between corals and their associated bacterial communities, encompassing both intracellular and extracellular associations, plays a crucial role in coral health and survival. These coral-associated bacteria contribute significantly to various metabolic processes, including carbon, nitrogen, and sulfur cycling, and are also implicated in the protection of the host from pathogenic organisms (Maire et al. 2021). Previous studies have identified genes within these bacterial communities that are involved in key functional pathways, such as nitrification, denitrification, Dimethylsulfoniopropionate (DMSP) transformation, and the biosynthesis of fixed carbon and amino acids, all of which potentially benefit the coral host (Li et al. 2022). In turn, the coral provides essential nutrients and a favorable ecological niche for the bacterial symbionts (Alsharif et al. 2023). This mutualistic relationship is believed to enhance coral defense mechanisms through physiological adaptation and the induction of secondary metabolite production.

Antibacterial activity

The results of antibacterial activity screening showed that 12 (26.7% of the total) isolates out of 45 exhibited antibacterial activity against pathogenic bacteria commonly found in diabetic foot infections. The number of active isolates can be seen in Table 1, while the antibacterial activity is presented in Table 2. Isolates exhibiting antibacterial activity were predominantly derived from the hosts *Ellisella* sp. (5 isolates) and *Viminella* sp. (4 isolates). In contrast, only one isolate with antibacterial activity was obtained from each of the hosts *Astrogorgia* sp., *Junceella* sp., and *Siphonogorgia* sp. This finding suggests a potential correlation between host species and the biosynthetic capabilities of their associated microbiota.

Previous studies have found that certain marine host organisms harbor more diverse and metabolically active microbial communities compared to other hosts (Srinivasan et al. 2021). Differences in bioactive compounds in microbial communities among hosts can be caused by the differences in microbial diversity, host-specific biochemical characteristics, and ecological conditions such as habitat and symbiotic interactions (Tincu and Taylor 2004; Petersen et al. 2020; Sabdono et al. 2022a). Sabdono et al. (2022a) reported that microbial diversity differs among gorgonian hosts, resulting in varying antibacterial activity of the associated isolates. This is further supported by findings that marine actinobacteria produce various levels of secondary metabolites depending on the symbiotic relationship and environmental niches (Jagannathan et al. 2021; Ngamcharungchit et al. 2023). The predominance of active isolates from *Ellisella* sp. and *Viminella* sp. suggests that these gorgonian corals offer favorable microenvironment for bioactive compound-producing microorganisms.

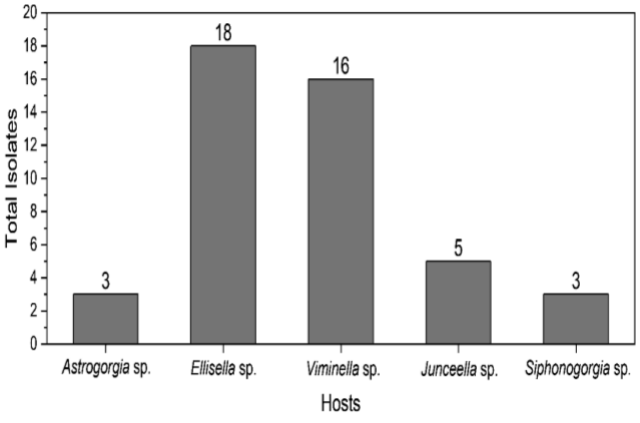


Figure 2. Total of Gorgonian-associated actinomycetes isolates from each host

The limited antibacterial activity observed (26.7%) can be attributed to various factors, including environmental conditions, isolation source, methodology, genetic diversity, and target pathogen bacteria (Ahmed et al. 2020; Mondal and Thomas 2022; Gitari et al. 2023; Ulusu et al. 2024). A similar percentage of antibacterial activity of bacteria isolated from Karimunjawa waters was observed in multiple studies, ranging from 14%-20% (Sabdono et al. 2022a, 2022b; Larasati et al. 2023; Hendrawati et al. 2024). Furthermore, Nurhikmayani et al. (2019) explained that the inactivity of bacterial isolates does not necessarily mean they do not exhibit antibacterial activity, however, it can also be due to the inability of antibacterial compounds to diffuse in an agar medium. A total of 6 (50%) isolates exhibited antibacterial activity against more than one pathogenic bacterium, while the remaining 6 (50%) showed activity against only one type of pathogenic bacterium. Among these, four isolates demonstrated significant antibacterial activity, being active against more than one pathogenic bacterium with an inhibition zone >5 mm. No antibacterial activity was detected against the pathogen *Proteus* sp. among the four selected isolates. The antibacterial activity of the potentially selected isolates can be seen in Table 3.

According to Davis and Stout (1971), antibacterial activity is categorized based on the inhibition zone diameter into four groups: weak (<5 mm), moderate (5-10 mm), strong (11-20 mm), and very strong (>20 mm). A zone of inhibition indicates that the microorganism cannot multiply around the isolate-grown plug, thus resulting in a clear zone around the plug (Ulusu 2024). Based on this classification, the antibacterial activity of the four potential isolates ranged from moderate to strong. Notably, isolates UR.19.4 and EA.6.5.2 exhibited the most promising antibacterial activity, with inhibition zones >5 mm and activity against three pathogenic bacteria. The results of variance in antibacterial activity between isolates against different pathogens showed a significant difference (p-value<0.05, at a 95% confidence level). This indicates that the antibacterial activity of the isolates differs significantly from each other when tested against the different pathogens. Previous studies have highlighted the potential of marine invertebrate-associated bacteria as antibacterial agents (Anteneh et al. 2021; Majithiya and Gohel 2022; Wijaya et al. 2022).

Gorgonian-associated bacteria have been recorded to exhibit antibacterial activity against urinary tract infections and nosocomial pathogens (Wizendro et al. 2022; Larasati et al. 2023). Sabdono et al. (2022a) reported that actinomycetes from the genus *Streptomyces* have been identified as Gorgonian-associated bacteria with antibacterial activity against *E. coli*. In another study, Zhang et al. (2013) reported that several *Streptomyces* sp. isolated from gorgonian corals exhibited antibacterial activity against *Bacillus subtilis, E. coli, Vibrio alginolyticus, Pseudoalteromonas piscicida,* and *Micrococcus luteus* with varying degrees of strength, from weak to strong. This shows that bacteria associated with gorgonian, particularly *Streptomyces,* exhibit great antibacterial activity against various pathogens. In line with previous findings, the present study demonstrates comparable results, wherein the antibacterial activity varied among isolates depending on the target pathogens and environmental conditions influencing the secondary metabolite production.

Marine-derived *Streptomyces* species are recognized as prolific producers of bioactive secondary metabolites, including polyketides, alkaloids, macrolactams, peptides, and other structurally diverse compounds with potent antibacterial properties (Zhao et al. 2024; Zhu et al. 2024; Pan et al. 2025). These metabolites have demonstrated varying degrees of antibacterial activity against human pathogenic bacteria, with reported Minimum Inhibitory Concentrations (MIC) ranging from as low as 1 µg/mL to 500 µg/mL, making them promising candidates for antibiotic development (Pan et al. 2025). Majithiya and Gohel (2022) reported that actinomycetes could produce a wide range of complex biopolymers, including polysaccharides, extracellular and intracellular enzymes, antibiotics, inhibitors, and various metabolic products.

Table 1. Total active gorgonian-associated actinomycetes isolates

Hosts	Σ Isolates	Σ Active isolates	Percentage (%)
Astrogorgia sp.	3	1	33.3
Ellisella sp.	18	5	27.8
Viminella sp.	16	4	25.0
Junceella sp.	5	1	20.0
Siphonogorgia sp.	3	1	33.3
Total	45	12	26.7

Table 2. Antibacterial activity of Gorgonian-associated actinomycetes isolates

Hosts	Isolates code	*Escherichia coli*	*Staphylococcus aureus*	*Proteus* sp.	*Enterococcus* sp.
Astrogorgia sp.	UR.14.6	-	-	-	+
Ellisella sp.	UR.19.4	+	+	-	+
Ellisella sp.	UR.19.1	+	+	-	+
Ellisella sp.	EA.9.1	-	+	-	+
Viminella sp.	EA.12.1.5	+	+	-	-
Junceella sp.	EA.13.1.1	-	+	-	+
Ellisella sp.	EA.17.1.8	-	+	-	-
Siphonogorgia sp.	EA.6.5.2	+	+	-	+
Viminella sp.	EA.6.5.1	+	-	-	+
Viminella sp.	EA.7.3	-	+	-	-
Ellisella sp.	EA.9.2	-	+	-	-
Viminella sp.	ER.12.4	-	+	-	-

Note: +: Presence of antibacterial activity, -: Absence of antibacterial activity

Table 3. Inhibition zone of selected active isolates

Isolates code	Diameter of inhibition zone (mm)							
	*Escherichia coli**		*Staphylococcus aureus**		*Proteus* sp.		*Enterococcus* sp.*	
	24h	48h	24h	48h	24h	48h	24h	48h
UR.19.4	13.55±0.35[a]	13.15±0.07[a]	13.15±0.92[a]	13.00±1.27[a]	0.00±00	0.00±00	7.90±1.27[b]	5.90±0.71[b]
EA.13.1.1	0.00±00	0.00±00	7.69±0.21[b]	7.94±0.07[b]	0.00±00	0.00±00	0.00±00	6.70±0.7[b]
EA.6.5.1.	5.88±0.88[b]	5.70±0.81[b]	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	10.15±1.33[a]
EA.6.5.2.	7.65±0.49[b]	7.65±0.64[b]	9.24±0.91[b]	9.28±0.59[b]	0.00±00	0.00±00	6.65±0.21[b]	6.40±0.01[b]
Control (+)	24.35±0.92[a]	23.5±0.71[a]	21.55±0.78[a]	18.05±1.06[a]	21.15±0.35[a]	20.2±0.42[a]	26.95±1.20[a]	28.1±3.82[a]
Control (-)	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00	0.00±00

Note: Values show the average diameter of the inhibition zone±standard deviation. Chloramphenicol (30 µg/disc) was used as control (+). *: p-value<0.05, [a]: Strong antibacterial activity, [b]: Moderate antibacterial activity

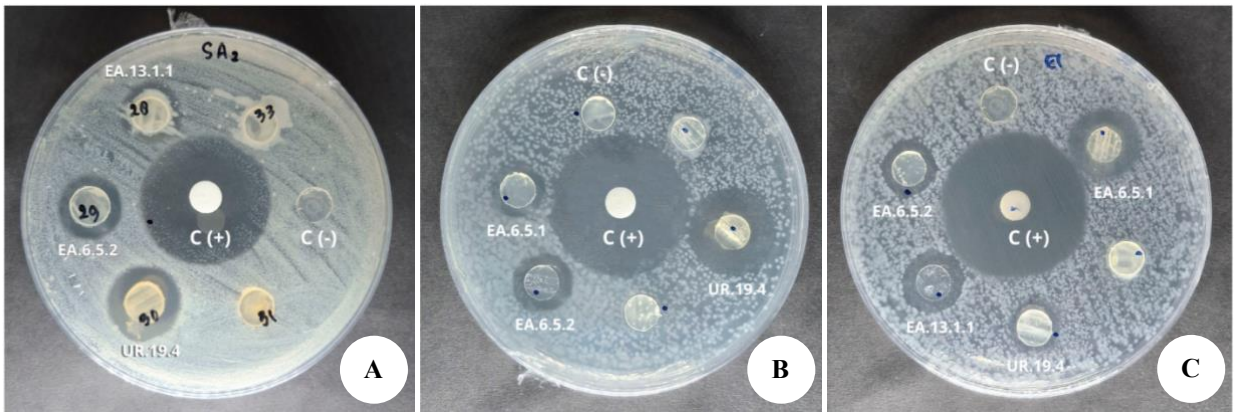


Figure 4. Antibacterial activity of isolates UR.19.4, EA.13.1.1, EA.6.5.1, and EA.6.5.2 against: A. *Staphylococcus aureus*, B. *Escherichia coli*, and C. *Enterococcus* sp.

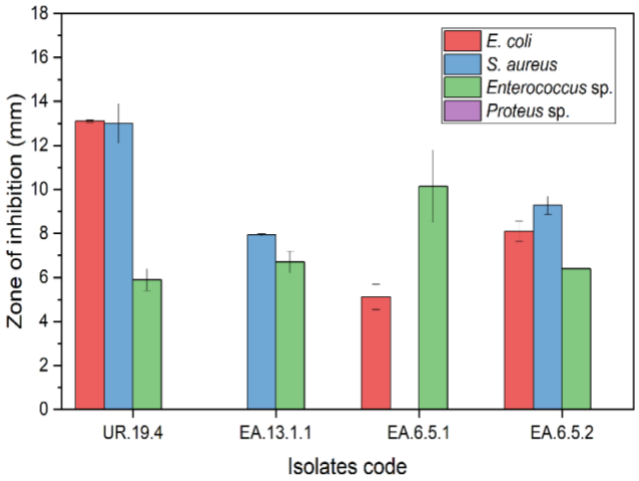


Figure 3. Bar graph representing antibacterial activity of the four selected isolates at the end of the study (48 hours)

Gram staining and 16s rRNA identification

Molecular identification results showed that out of four potential isolates, only two had the closest similarity to actinomycete bacteria. Isolates UR.19.4 and EA.6.5.2 had the closest similarity to *Streptomyces griseobrunneus* (99.76%) and *Streptomyces zhaozhouensis* (99.69%), respectively, while isolates EA.13.1.1 and EA.6.5.1 had the closest similarity to *Bacillus* sp. and *Bacillus paramycoides* (100% each). The phylogenetic tree constructed (Figure 6) showed that isolate UR.19.4 is closely related to *S. griseobrunneus* strain S1, while isolate EA.6.5.2. is closely related to *S. zhaozhouensis* strain RFCAC01D. Microscopic observation results are presented in Table 4, while the molecular identification results of the selected isolates are presented in Table 5. Previous studies have reported that *S. zhaozhouensis* has been isolated from marine sediments and sponges (Lacret et al. 2014; Dhaneesha et al. 2019; Heo et al. 2023). Several studies have also identified *Streptomyces* strains from marine environments that exhibit high sequence similarity and close phylogenetic relationships with *S. griseobrunneus*. Hong et al. (2009) reported that isolates 219820 and 219808, isolated from mangrove sediments, exhibited high sequence identity (>97%) with *S. griseobrunneus* NBRC 12775. Similarly, another study identified isolate MNM-1400 from marine sediment as a marine *Streptomyces* with high sequence similarity to several *Streptomyces* species, including *S. griseobrunneus* (percent identification of 99%) (Gozari et al. 2016).

The microscopic morphological observation further supports the molecular identification result in this study. Isolates UR.19.4 and isolate EA.6.5.2, identified as members of the phylum Actinobacteria, exhibited a branched filamentous morphology, while the two other

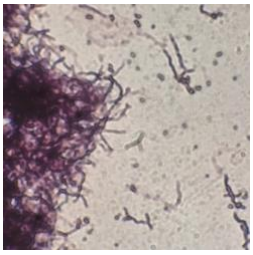
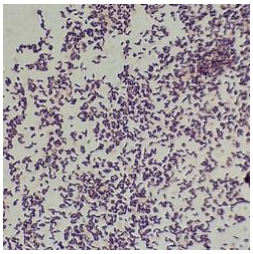
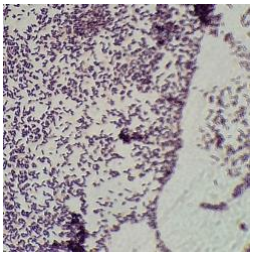
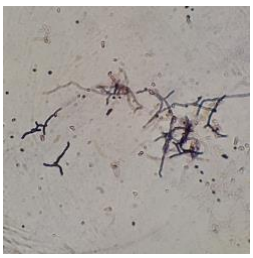
isolates, belonging to the genus *Bacillus* sp., displayed a rod-shaped (*Bacillus*) morphology. Members of the *Streptomyces* genus are typically characterized by their purple staining in Gram tests and by their filamentous, branching structures that resemble fungal hyphae. These structures often develop into long chains of spores, which are another characteristic feature of the genus (Ambarwati et al. 2023). These morphological features align with the findings of Malviya et al. (2013) who reported that under the microscope, actinomycetes, such as *Streptomyces* sp. and *Nocardiopsis* sp. were observed to be Gram-positive bacteria with branched filaments.

The presence of *Bacillus* sp. and *Bacillus paramycoides* from the potential isolates suggests the diverse microbial ecology associated with gorgonian and the variability of the *Bacillus* sp. colony. Devi et al. (2019) reported that diverse *Bacillus* species thrive in marine environments and could produce compounds that demonstrate antibacterial activity. The growth of *Bacillus* sp. upon isolation, even when targeting actinomycetes, has also been reported in other studies due to the wide range of *Bacillus* colony morphology depending on the growth medium used; some colonies resemble actinomycetes characteristics until further identification using 16s rRNA sequencing (Duraipandiyan et al. 2010; Daquioag and Penuliar 2021). The results in this study revealed that *Bacillus* sp. and *Bacillus paramycoides* exhibited inhibitory effects against *S. aureus, E. coli,* and *Enterococcus* sp. Sabdono et al. (2022a) reported that various *Bacillus* species exhibiting antimicrobial activity were found in Karimunjawa waters.

Biosynthetic gene cluster of NRPS, PKS-I, and PKS-II detection

The gene detection results indicated that *Streptomyces griseobrunneus* possessed both NRPS and PKS-II genes, while *Streptomyces zhaozhouensis* only possessed the NRPS gene. The PKS-I gene was not detected in any of the isolates. The gene detection results are presented in Table 6 and Figure 7. The presence of NRPS and PKS-II genes could explain the antibacterial activity observed in these isolates, as these gene clusters are known to drive the biosynthesis of diverse secondary metabolites with antimicrobial, anticancer, and antitumor properties (Gong et al. 2018). Yim et al. (2014) reported that the majority of antibiotics produced by Actinomycetes are associated with NRPS biosynthetic pathways. The presence of NRPS, PKS-I, and PKS-II genes has been previously detected in actinomycetes isolated from mangrove soils, corals, and nudibranchs (Gong et al. 2018; Sabdono et al. 2022b; Wizendro et al. 2022).

Table 4. Microscopic observations of selected isolates

Hosts	Isolates code	Shape	Microscopic view
Ellisella sp.	UR.19.4	Branched filaments, Gram positive	
Junceella sp.	EA.13.1.1	Rod shape, Gram positive	
Viminella sp.	EA.6.5.1	Rod shape, Gram positive	
Siphonogorgia sp.	EA.6.5.2.	Branched filaments, Gram positive	

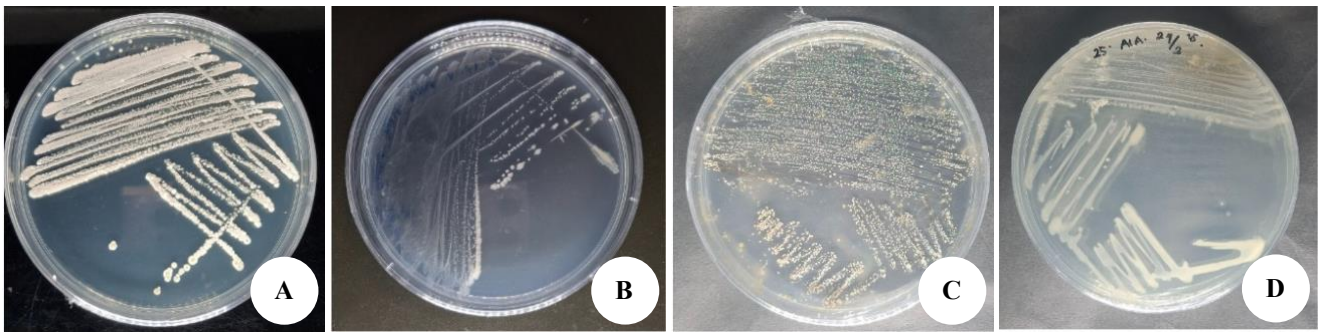


Figure 5. Colony morphology isolates of: A. UR.19.4, B. EA.13.1.1, C. EA.6.5.2, and D. EA.6.5.1

Table 5. Molecular identification of selected isolates by 16s rRNA gene

Hosts	Isolates code	Closest similarity	Percentage identify	Query cover	Accession no.
Ellisella sp.	UR.19.4	*Streptomyces griseobrunneus*	99.76%	100%	PV563089
Siphonogorgia sp.	EA.6.5.2	*Streptomyces zhaozhouensis*	99.69%	100%	PV563088
Junceella sp.	EA.13.1.1	*Bacillus* sp.	100%	99.29%	PV563087
Viminella sp.	EA.6.5.1.	*Bacillus paramycoides*	100%	99.93%	PV563090

Table 6. Detection of NRPS, PKS-I, and PKS-II genes

Isolates code	NRPS	PKS-I	PKS-II
UR.19.4	+	-	+
EA.6.5.2	+	-	-
EA.13.1.1	-	-	-
EA.6.5.1	-	-	-

Note: +: Detected, -: Non-detected

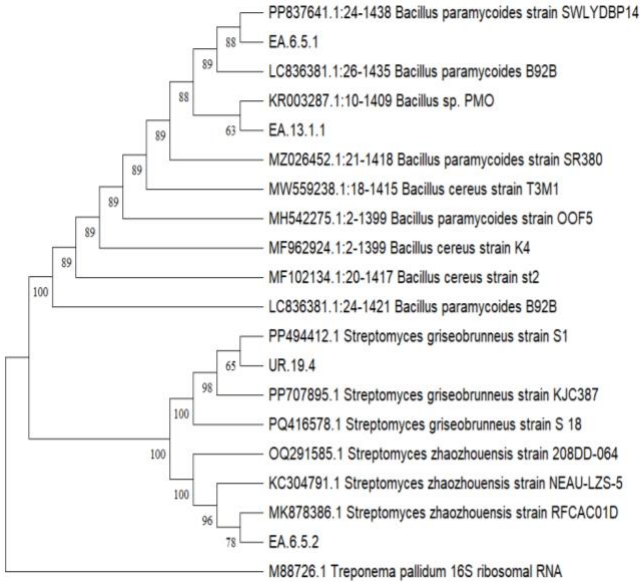


Figure 6. Phylogenetic tree of gorgonian-associated actinomycetes and bacteria. *Treponema pallidum* was used as an outer group. Bootstrap 1000 replication in MEGA 11 was used to construct the phylogenetic tree

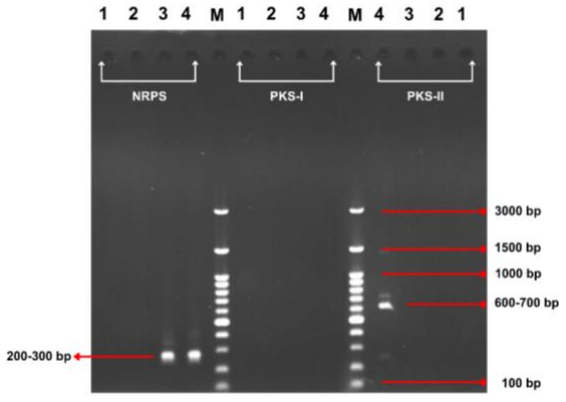


Figure 7. Detection of NRPS, PKS-I, and PKS-II gene in isolate: 1. EA.13.1.1, 2. EA.6.5.1, 3. EA.6.5.2, 4. UR.19.4 with DNA Ladder 100 bp (M) as marker in 1% agarose gel

In conclusion, this study identified *S. griseobrunneus* (UR.19.4) and *S. zaozhouensis* (EA.6.5.2) as Gorgonian-associated actinomycetes exhibiting antibacterial activity against *E. coli, S. aureus*, and *Enterococcus* sp. The detection of NRPS genes in both isolates and PKS-II genes in UR.19.4 indicates their biosynthetic potential for producing antimicrobial secondary metabolites. This study demonstrated the potential of actinomycetes associated with marine gorgonian as a promising antimicrobial source against diabetic foot pathogens. Further studies should be conducted focusing on compound extraction, metabolite profiling, and Minimum Inhibitory Concentration (MIC) determination for therapeutic application.

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REFERENCES

Abu-El-Azayem AK, Nashaat N, Dwedar RA, Fekry KM, Bassyouni RH, Hegab AS. 2024. Microbiological profile of diabetic foot infections. Microbes Infect Dis 5: 1530-1540. DOI: 10.21608/mid.2024.295059.1977.

Ahmed RN, Daniel F, Gabla ID, Sanni A. 2020. Potentials of actinomycetes from reserved environments as antibacterial agents against drug-resistant clinical bacterial strains. Ethiop J Health Sci 30 (2): 251-258. DOI: 10.4314/ejhs.v30i2.13.

Alsharif SM, Waznah MS, Ismaeil M, El-Sayed WS. 2023. 16S rDNA-based diversity analysis of bacterial communities associated with soft corals of the Red Sea, Al Rayyis, White Head, KSA. J Taibah Univ Sci 17 (1): 2156762. DOI: 10.1080/16583655.2022.2156762.

Ambarwati A, Santoso B, Sofyan A. 2023. Phylogenetic analysis of *Streptomyces* producing antimicrobial agent isolated from Kukup Beach sand, Yogyakarta, Indonesia. Biodiversitas 24 (4): 2374-2383. DOI: 10.13057/biodiv/d240452.

Anteneh YS, Yang Q, Brown MH, Franco CMM. 2021. Antimicrobial activities of marine sponge-associated bacteria. Microorganisms 9 (1): 171. DOI: 10.3390/microorganisms9010171.

Carroll AR, Copp BR, Grkovic T, Keyzers RA, Prinsep MR. 2024. Marine natural products. Nat Prod Rep 41 (2): 162-207. DOI: 10.1039/d3np00061c.

Córdova-Isaza A, Jiménez-Mármol S, Guerra Y, Salas-Sarduy E. 2023. Enzyme inhibitors from Gorgonians and soft corals. Mar Drugs 21 (2): 104. DOI: 10.3390/md21020104.

Daquioag JEL, Penuliar GM. 2021. Isolation of actinomycetes with cellulolytic and antimicrobial activities from soils collected from an urban green space in the philippines. Intl J Microbiol 2021: 6699430. DOI: 10.1155/2021/6699430.

Davis WW, Stout TR. 1971. Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. Appl Microbiol 22: 666-670. DOI: 10.1128/am.22.4.666-670.1971.

de Oliveira RC, Diniz FV, Peters LP, Carvalho CM. 2024. Antimicrobial activity of actinomycetes isolated from soils in the Brazilian Amazon. Braz Arch Biol Technol 67 (5): e24230213. DOI: 10.1590/1678-4324-2024230213.

De Simeis D, Serra S. 2021. Actinomycetes: A never-ending source of bioactive compounds—An overview on antibiotics production. Antibiotics 10 (5): 483. DOI: 10.3390/antibiotics10050483.

Devi S, Kiesewalter HT, Kovács R, Frisvad JC, Weber T, Larsen TO, Kovács ÁT, Ding L. 2019. Depiction of secondary metabolites and antifungal activity of *Bacillus velezensis* DTU001. Synth Syst Biotechnol 4 (3): 142-149. DOI: 10.1016/j.synbio.2019.08.002.

Dhaneesha M, Hasin O, Sivakumar KC, Ravinesh R, Naman CB, Carmeli S, Sajeevan TP. 2019. DNA binding and molecular dynamic studies of Polycyclic Tetramate Macrolactams (PTM) with potential anticancer activity isolated from a sponge-associated *Streptomyces zhaozhouensis* subsp. *mycale* subsp. *nov*. Mar Biotechnol 21 (1): 124-137. DOI: 10.1007/s10126-018-9866-9.

Duraipandiyan V, Sasi AH, Islam VIH, Valanarasu M, Ignacimuthu S. 2010. Antimicrobial properties of actinomycetes from the soil of Himalaya. J Mycol Méd 20: 15-20. DOI: 10.1016/j.mycmed.2009.11.002.

Gitari JM, Muraya MM, Onyango BO, Maingi JM. 2023. Characterization and antibacterial activity of soil actinomycetes from diverse land use systems in Meru South, Eastern Kenya. J Adv Microbiol 23 (10): 93-108. DOI: 10.9734/jamb/2023/v23i10760.

Gong B, Chen S, Lan W, Huang Y, Zhu X. 2018. Antibacterial and antitumor potential of actinomycetes isolated from mangrove soil in the Maowei Sea of the Southern Coast of China. Iran J Pharm Res 17 (4): 1339-1346.

Gozari M, Mortazavi MS, Bahador N, Jahromi ST, Rabbaniha M. 2016. Isolation and screening of antibacterial and enzyme producing marine actinobacteria to approach probiotics against some pathogenic vibrios in shrimp *Litopenaeus vannamei*. Iran J Fish Sci 15 (1): 630-644.

Hendrawati A, Ayuningrum D, Sabdaningsih A, Amalia R. 2024. Exploring the antibacterial potential of tunicate-associated bacteria (Ascidiacea) at the Shipwreck Site of Menjangan Kecil Waters, Karimunjawa. Asia Pac J Mol Biol Biotechnol 32: 127-136. DOI: 10.35118/apjmbb.2024.032.2.14.

Heo C-S, Kang JS, Kwon J-H, Anh CV, Shin HJ. 2023. Pyrrole-containing alkaloids from a marine-derived actinobacterium *Streptomyces zhaozhouensis* and their antimicrobial and cytotoxic activities. Mar Drugs 21 (3): 167. DOI: 10.3390/md21030167.

Hong K, Gao A-H, Xie Q-Y, Gao H, Zhuang L, Lin H-P, Yu H-P, Li J, Yao X-S, Goodfellow M, Ruan J-S. 2009. Actinomycetes for marine drug discovery isolated from mangrove soils and plants in china. Mar Drugs 7 (1): 24-44. DOI: 10.3390/md7010024.

Izzati F, Warsito MF, Bayu A, Prasetyoputri A, Atikana A, Sukmarini L, Rahmawati SI, Putra MY. 2021. Chemical diversity and biological activity of secondary metabolites isolated from Indonesian marine invertebrates. Molecules 26: 1898. DOI: 10.3390/molecules26071898.

Jagannathan SV, Manemann EM, Rowe SE, Callender MC, Soto W. 2021. Marine actinomycetes, new sources of biotechnological products. Mar Drugs 19 (7): 365. DOI: 10.3390/md19070365.

Kelutur FJ, Saptarini NM, Mustarichie R, Kurnia D. 2021. Bioactive compounds profile of Gorgonian corals and their pharmacological activities: A review. Rasayan J Chem 14 (3): 1773-1789. DOI: 10.31788/rjc.2021.1436406.

Lacret R, Oves-Costales D, Gómez C, Díaz C, de la Cruz M, Pérez-Victoria I, Vicente F, Genilloud O, Reyes F. 2014. New ikarugamycin derivatives with antifungal and antibacterial properties from *Streptomyces zhaozhouensis*. Mar Drugs 13: 128-140. DOI: 10.3390/md13010128.

Larasati SJH, Trianto A, Radjasa OK, Sabdono A. 2023. Bacterial diversity of the Gorgonian coral *Plexaura* sp.: Screening for anti-pathogenic property against nosocomial pathogenic *Acinetobacter baumanii*. Intl J Conserv Sci 14 (1): 361-370. DOI: 10.36868/ijcs.2023.01.24.

Li J, Zou Y, Yang J, Li Q, Bourne DG, Sweet M, Liu C, Guo A, Zhang S. 2022. Cultured bacteria provide insight into the functional potential of the coral-associated microbiome. mSystems 7 (4): e0032722. DOI: 10.1128/msystems.00327-22.

Liu T, Wu S, Zhang R, Wang D, Chen J, Zhao J. 2019. Diversity and antimicrobial potential of actinobacteria isolated from diverse marine sponges along the Beibu Gulf of the South China Sea. FEMS Microbiol Ecol 95 (7): fiz089. DOI: 10.1093/femsec/fiz089.

Maire J, Blackall LL, van Oppen MJH. 2021. Intracellular bacterial symbionts in corals: Challenges and future directions. Microorganisms 9: 2209. DOI: 10.3390/microorganisms9112209.

Majithiya VR, Gohel SD. 2022. Actinobacteria associated with marine invertebrates: Diversity and biological significance. In: Hozzein WNN (eds). Actinobacteria - Diversity, Applications and Medical Aspects. IntechOpen, London. DOI: 10.5772/intechopen.106642.

Malviya MK, Pandey A, Sharma A, Tiwari SC. 2013. Characterization and identification of actinomycetes isolated from 'fired plots' under shifting cultivation in Northeast Himalaya, India. Ann Microbiol 63: 561-569. DOI: 10.1007/s13213-012-0504-x.

McDermott K, Fang M, Boulton AJM, Selvin E, Hicks CW. 2023. Etiology, epidemiology, and disparities in the burden of diabetic foot ulcers. Diabetes Care 46 (1): 209-221. DOI: 10.2337/dci22-0043.

Mondal H, Thomas J. 2022. Isolation and characterization of a novel actinomycete isolated from marine sediments and its antibacterial activity against fish pathogens. Antibiotics 11 (11): 1546. DOI: 10.3390/antibiotics11111546.

Moya-Salazar J, Chamana JM, Porras-Rivera D, Goicochea-Palomino EA, Salazar CR, Contreras-Pulache H. 2023. Increase in antibiotic resistance in diabetic foot infections among peruvian patients: A single-center cross-sectional study. Front Endocrinol 14: 1267699. DOI: 10.3389/fendo.2023.1267699.

Ngamcharungchit C, Chaimusik N, Panbangred W, Euanorasetr J, Intra B. 2023. Bioactive metabolites from terrestrial and marine actinomycetes. Molecules 28 (15): 5915. DOI: 10.3390/molecules28155915.

Nurhikmayani R, Daryono BS, Retnaningrum E. 2019. The isolation and molecular identification of antimicrobial-producing lactic acid bacteria from Chao, South Sulawesi (Indonesia) fermented fish product. Biodiversitas 20 (4): 1063-1068. DOI: 10.13057/biodiv/d200418.

Nurliana N, Siregar BH, Sari WE, Helmi TZ, Sugito S. 2022. Identification of cellulolytic lactic acid bacteria from the intestines of laying hens given akbisprob based on 16S ribosomal ribonucleic acid gene analysis. Vet World 15 (7): 1650-1656. DOI: 10.14202/vetworld.2022.1650-1656.

Olid AS, Solà I, Barajas-Nava LA, Gianneo OD, Cosp XB, Lipsky BA. 2015. Systemic antibiotics for treating diabetic foot infections. Cochrane Database Syst Rev 2015 (9): cd009061. DOI: 10.1002/14651858.cd009061.pub2.

Pan C, ul Hassan SS, Ishaq M, Yan S, Jin H. 2025. Marine actinomycetes: A hidden treasure trove for antibacterial discovery. Front Mar Sci 12: 1558320. DOI: 10.3389/fmars.2025.1558320.

Petersen L-E, Kellermann MY, Schupp PJ. 2020. Secondary metabolites of marine microbes: From natural products chemistry to chemical ecology. In: Jungblut S, Liebich V, Bode-Dalby M (eds). YOUMARES 9 - The Oceans: Our Research, Our Future. Springer, Cham. DOI: 10.1007/978-3-030-20389-4_8.

Ramirez-Acuña JM, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, Flores-Morales V, Martinez-Fierro ML. 2019. Diabetic foot ulcers: Current advances in antimicrobial therapies and emerging treatments. Antibiotics 8 (4): 193. DOI: 10.3390/antibiotics8040193.

Sabdono A, Lestari ES, Sibero MT. 2022a. Biogeographic assessment of gorgonian-associated bacteria with antipathogenic urinary tract infections (utis) in Karimunjawa Marine National Park, Java Sea, Indonesia. Nat Conserv 49: 137-151. DOI: 10.3897/natureconservation.49.84825.

Sabdono A, Radjasa O, Trianto A, Korshunova T, Martynov A, Sibero M. 2022b. Diversity and antimicrobial activity of marine nudibranch associated bacteria against tropical human skin pathogens. F1000Res 11: 421. DOI: 10.12688/f1000research.108857.2.

Sabdono A, Radjasa OK, Trianto A, Sibero MT, Kristiana R, Jessica S, Larasati H. 2022c. Comparative assessment of Gorgonian abundance and diversity among islands with different anthropogenic stressors in Karimunjawa Marine National Park, Java Sea. Intl J Conserv Sci 13 (1): 341-348.

Saryono, Finna P, Usman P, Wahyu PN, Aulia A. 2019. Isolation and identification of bacteria and actinomycetes isolated from wilting banana plants (*Musa* sp.). IOP Conf Ser: Mater Sci Eng 532: 012028. DOI: 10.1088/1757-899x/532/1/012028.

Sibero MT, Frederick EH, Wijayanti DP, Haryanti D, Siswanto AP, Igarashi Y. 2023. Antimicrobial and cytotoxic properties of actinobacteria associated with a stony coral *Fungia* sp. from Karimunjawa National Park, Indonesia. J Appl Pharm Sci 14 (1): 132-140. DOI: 10.7324/japs.2024.139056.

Srinivasan R, Kannappan A, Shi C, Lin X. 2021. Marine bacterial secondary metabolites: A treasure house for structurally unique and

effective antimicrobial compounds. Mar Drugs 19 (10): 530. DOI: 10.3390/md19100530.

Tincu JA, Taylor SW. 2004. Antimicrobial peptides from marine invertebrates. Antimicrob Agents Chemother 48 (10): 3645-3654. DOI: 10.1128/aac.48.10.3645-3654.2004.

Ulusu F, Bilgic A. 2024. Preparation of pillar[5]arene functionalized silica gel hybrid material: Investigation of biological (antimicrobial, antioxidant and anti-cancer) properties. J Sol-Gel Sci Technol 2024: 1. DOI: 10.1007/s10971-024-06580-w.

Ulusu F, Sarilmaz A, Ulusu Y, Ozel F. 2024. Quaternary nanorods: Promising versatile agents for cancer therapy, antimicrobial strategies and free radical neutralization. Particul Sci Technol 42 (6): 944-952. DOI: 10.1080/02726351.2023.2299869.

Ulusu F, Ulusu Y. 2024. Biosynthesis and characterization of silver nanoparticles mediated by *Cistus salviifolius* L. and *Ferula communis* L. extracts and evaluation of their antioxidant, antibacterial, and cytotoxic potentials. Biol Bull 51: 845-856. DOI: 10.1134/s1062359023603634.

Ulusu F. 2024. Exploring the therapeutic potential of microwave-assisted biosynthesized silver nanoparticles using *Erica manipuliflora* Salisb.: A comprehensive study on anticancer and antibacterial potentials. Particuology 95: 212-222. DOI: 10.1016/j.partic.2024.09.018.

van de Water JAJM, Melkonian R, Voolstra CR, Junca H, Beraud E, Allemand D, Ferrier-Pagès C. 2017. Comparative assessment of mediterranean Gorgonian-associated microbial communities reveals conserved core and locally variant bacteria. Microb Ecol 73: 466-478. DOI: 10.1007/s00248-016-0858-x.

Wijaya AP, Sabdono A, Sibero MT, Trianto A, Radjasa OK. 2022. Antimicrobial activity of nudibranch *Chromodoris lineolata* associated bacteria against skin diseases pathogens from Jepara coastal waters, Indonesia. Biodiversitas 23: 1911-1919. DOI: 10.13057/biodiv/d230425.

Wizendro W, Trianto A, Lestari ES, Sibero MT, Sabdono A. 2022. Antibacterial activity of Gorgonian coral junceella associated bacteria against Catheter Associated Urinary Tract Infection (CAUTI) pathogens isolated from Karimunjawa Marine National Park. AACL Bioflux 15 (6): 3271-3279.

Worsley AL, Lui DH, Ntow-Boahene W, Song W, Good L, Tsui J. 2023. The importance of inflammation control for the treatment of chronic diabetic wounds. Intl Wound J 20: 2346-2359. DOI: 10.1111/iwj.14048.

Yi KH, Othman NFA, Rahim AA, Mokhtar SI, Pahirulzaman KAK. 2023. Antimicrobial activities of actinomycetes isolated from flooded and unflooded soils. Malays J Med Health Sci 19 (S9): 42-49. DOI: 10.47836/mjmhs.19.s9.7.

Yim G, Thaker MN, Koteva K, Wright G. 2014. Glycopeptide antibiotic biosynthesis. J Antibiot 67 (1): 31-41. DOI: 10.1038/ja.2013.117.

Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. 2017. Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. Ann Med 49 (2): 106-116. DOI: 10.1080/07853890.2016.1231932.

Zhang X-Y, He F, Wang G-H, Bao J, Xu X-Y, Qi S-H. 2013. Diversity and antibacterial activity of culturable actinobacteria isolated from five species of the South China Sea Gorgonian corals. World J Microbiol Biotechnol 29 (6): 1107-1116. DOI: 10.1007/s11274-013-1279-3.

Zhao Y, Chen H, Yue L, Dong Y, Su D, Lyu J, Li W, Li H. 2024. Heronamides with unreported skeletons from deep-sea *Streptomyces*: Discovery and biosynthesis. Org Chem Front 11 (4): 1175-1183. DOI: 10.1039/d3qo01837g.

Zhu X, Wang S, Song Y, Chen T, Yan Y. 2024. LC-MS guided discovery of a new type of abyssomicin, glycoabyssomicin a, from a deep-sea derived *streptomyces*. Nat Prod Res 23: 1-6. DOI: 10.1080/14786419.2024.2417839.