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Screening of indigenous methicillin-resistant *Staphylococcus aureus* (MRSA)-inhibiting actinomycetes from Sicanang Mangrove and Cermin Beach in North Sumatra Province, Indonesia

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Abstract. *Yurnaliza Y, Munir E, Gultom RO, Nasution AJ. 2024. Screening of indigenous methicillin-resistant* Staphylococcus aureus *(MRSA)-inhibiting actinomycetes from Sicanang Mangrove and Cermin Beach in North Sumatra Province, Indonesia. Biodiveritas 25: 3401-3410.* Methicillin-resistant *Staphylococcus aureus* (MRSA) is the major issue of antimicrobial resistance in medical practice. Unexplored locations like the Sicanang mangrove and Cermin beach in North Sumatra, Indonesia are expected to find indigenous actinomycetes as MRSA-inhibiting antibiotics producers. This study aims to explore the potential of actinomycetes in North Sumatra, especially in the Sicanang Mangrove forest and Cermin Beach, in inhibiting the growth of MRSA. Actinomycetes were isolated and characterized using starch casein agar and humic acid vitamin agar. Actinomycetes that inhibit MRSA and *S. aureus* (ATCC 25923) bacteria were qualitatively screened using an antagonist test. Subsequently, the inhibitory activities of methanol (MeOH) and ethyl acetate (EtOAc) actinomycetes extracts against MRSA and *S. aureus* were quantitatively monitored. The potential actinomycetes with the highest inhibitory ability were identified based on their 16S rRNA gene sequence. The research revealed 30 isolates capable of inhibiting MRSA and *S. aureus*. Among them, 10 actinomycetes isolates inhibited MRSA and *S. aureus*, with inhibition zone diameters ranging from 15 to 28 mm. Moreover, MeOH and EtOAc actinomycetes extracts produced a similar inhibition zone (7-20.8 mm against MRSA and *S. aureus*). Although a concentrated extract produced a large inhibition zone, the zone was smaller than that produced by chloramphenicol. Molecular identification showed that the potential actinomycetes, particularly SMC 9, were closely related to *Streptomyces rochei*, with a similarity of 96.02%, while SPC 9 had a high similarity (99%) with *Streptomyces antibioticus*. Based on research findings, two species of *Streptomyces* obtained from the Sicanang mangrove and Cermin Beach could be developed as producers of MRSA-inhibiting antibiotics.

Keywords: Actinomycetes, antibacterial activity, methicillin-resistant *Staphylococcus aureus*, North Sumatra, *Streptomyces*

Abbreviations: DMSO: Dimethyl Sulfoxide; HVA: Humic Acid Vitamin agar; ISP: International *Streptomyces* Project; MHA: Müller Hilton Agar; MRSA: Methicillin-Resistant *Staphylococcus aureus*; SCA: Starch Casein Agar

INTRODUCTION

Antimicrobial resistance is currently one of the major public health issues. This has affected the prevention and cure of various illnesses caused by pathogens no longer sensitive to frequently used medications (Zahra et al. 2022). The most common resistant bacterial strain causing major community-acquired infections is Methicillin-Resistant *Staphylococcus aureus* (MRSA). Methicillin, a beta-lactam antibiotic, is not actively used in healthcare caused by MRSA infection. MRSA is also resistant to other types of β-lactam antibiotics, such as penicillin and cephalosporin (Alghamdi et al. 2023). MRSA's resistance mechanism involves the production of the β-lactamase enzyme, which hydrolyzes beta-lactams and changes the binding pocket for bacterial cell wall production (Alghamdi et al. 2023). Since 1961, MRSA infections have been a major public health concern and have become important nosocomial infections done to their high prevalence and potential fatality. MRSA infections caused by *S. aureus* can lead to skin, lung, heart, regenerative soft-tissue, and bloodstream infections or bacteremia. A significant number of nosocomial bacteremia infections contribute to heightened mortality rates (Ayau et al. 2017). Seven common antibiotics were used against MRSA, including vancomycin, sulfamethoxazole, trimethoprim (TMP-SMZ), daptomycin, quinupristin-dalfopristin, tigecycline, clindamycin, and linezolid (Okwu et al. 2019). However, MRSA gradually develops antibiotic resistance by creating new clones that can resist almost all currently available antibiotics (Mahjabeen et al. 2022). The protective ability of antibiotics produced by actinomycetes against MRSA has been evaluated to overcome the resistance caused by MRSA (Bhakyashree and Kannabiran 2020). It is urgent to explore new antibiotic-producing microorganisms from nature and develop them into new medicinal ingredients to suppress the growth of drug-resistant *S. aureus*. Actinomycetes are potential candidates as continuous antibiotic producers worldwide (De Simeis and Serra 2021).

Actinomycetes are Gram-positive filamentous bacteria with a high G+C content in their DNA (Barka et al. 2016). Actinomycetes inhabit various ecosystem settings, including

soil, rhizosphere, marine ecosystems, freshwater, volcanic caves, hot spots, bug guts, animal feces, and as endophytes in plants (Selim et al. 2021). Actinomycetes can be isolated from mangrove soil (Asnani et al. 2020; Fatimah et al. 2022). Environmental factors, such as extreme pH, salinity, and temperature, can affect actinomycetes populations found in soils (Devanshi et al. 2021). Nevertheless, alkaline soil with large amounts of organic matter is the predominant habitat for these organisms (Barka et al. 2016). The largest genus of actinomycetes, *Streptomyces*, is abundant and widely distributed in soils. *Streptomyces* contribute to the earthy odorant of soil and water because of their geosmin compound (Jiang et al. 2007). Most known antimicrobials were originally isolated from the genus *Streptomyces* (Donald et al. 2022). Some rare actinomycetes, all actinomycetes genera except for the *Streptomyces* group, were also reported to be able to produce antibiotics, such as *Amycolatopsis orientalis*, *Micromonospora purpurea*, *Saccharopolyspora erythraea*, *Actinoplanes teichomyceticus*, *A. rifamycinica* and *Dactylosporangium aurantiacum* subsp. *hamdenesis* consecutively as producer of vancomycin, gentamicin, erythromycin, teicoplanin, rifamycin and fidaxomicin (Parra et al. 2023). They produce all-important drug classes used in clinics today, such as β-lactams, tetracyclines, macrolides, or glycopeptides. However, in recent years, their effectiveness has been endangered by the increased resistance of life-threatening pathogenic bacteria (Mast and Stegmann 2019). Exploring actinomycetes or *Streptomyces* from nature to overcome MRSA resistance has been carried out by many researchers, such as Asnani et al. (2020), Siddharth et al. (2021) and Awad et al. (2024). Asnani et al. (2020) reported *Streptomyces clavuligerus* strain A-ZN-05 from sediment mangrove in Cilacap, Indonesia, potential as anti-MRSA. In contrast, Awad et al. (2024) reported that *Streptomyces* collected from the soil of the Suez governorate produce secondary metabolites that effectively inhibit MRSA. Besides *Streptomyces*, *Nocardiopsis* sp. from the marine environment can produce diketopiperazine derivative as metabolites and is also effective against MRSA (Siddharth et al. 2021).

Exploration of unique actinomycetes from the natural environment is expected to find a new MRSA-inhibiting antibiotic producer. Two locations in North Sumatra, the Sicanang Mangrove area and Cermin Beach sediment are selected as sources of actinomycetes. Actinomycetes from these mangrove forests and beaches remain largely unexplored. Therefore, we aim to isolate actinomycetes capable of inhibiting MRSA from this understudied environment.

MATERIALS AND METHODS

Samples location

The purposive sampling method collected water and sandy sediment samples from two locations at Sicanang Mangrove (A) forest of Medan District and Cermin Beach (B) of Serdang Bedagai District, North Sumatra, Indonesia. The water and sandy sediment were composite from three sampling points from each location, with approximately 100 g for sediment and 100 mL for water. Coordinate of sampling sites for the Sicanang Mangrove were 3˚45'29", 98˚39'5" (A1), 3˚45'30" 98˚39'5" (A2), 3˚45'29" 98˚39'4" (A3) while sampling site in Cermin Beach were 3˚39' 16.33" 98˚ 59' 1.00"(B1), 3˚39' 8.61" 98˚ 59' 21.54"(B2), 3˚38' 55.18" 98˚59' 36.70"(B3) (Figure 1). These samples were then transported to the laboratory for actinomycetes isolation. Environmental parameters, such as point coordinates, temperature, salt content, and pH of the water/ sandy sediment, were also measured during sampling.

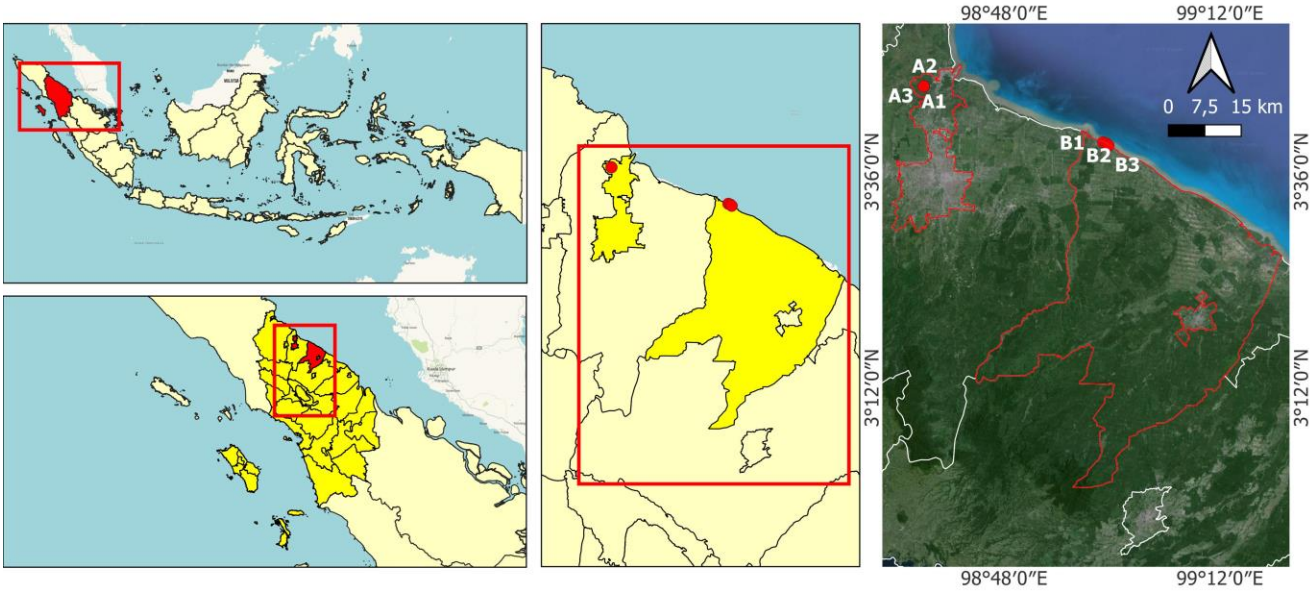


Figure 1. Location of water and sandy sediment samples for isolation actinomycetes from A. Sicanang Mangrove and; B. Cermin Beach in North Sumatra Province, Indonesia. The alphabet of A1-3 and B1-3 indicated the sampling point from each location

Actinomycetes isolation

Sandy sediment samples from the two locations were combined in each container and air-dried. A total of 10 g of sandy sediment was suspended in 90 mL of sterile saltwater and serially diluted to 0.001. An aliquot (0.1 mL) of the dilution was spread on plates containing Starch Casein Agar (SCA) and Humic Acid Vitamin Agar (HVA) supplemented with 50 µg/mL nalidixic acid and 50 µg/mL cycloheximide. Actinomycetes were directly isolated from water samples by inoculating 0.1 mL seawater into the isolation medium. The culture medium was incubated at room temperature for 10-15 days until actinomycetes colonies were visible on the isolation medium. The colonies were then sub-cultured several times on yeast extract malt agar (ISP-2) until pure cultures were obtained (Saleem et al. 2023).

Morphological characterization of actinomycetes

The colonies, such as shape, color, margin, elevation, surface color, and melanin formation, of the actinomycetes isolates grown on International *Streptomyces* Project (ISP)-2, ISP-3, ISP-4, and ISP-7 media were characterized using macroscopic and microscopic methods. Actinomycetes morphology was observed under a microscope using the slide culture method. The morphological characteristics of actinomycetes in cultures incubated for 10 days were observed at 1000× magnification (Sharma et al. 2014).

Primary selection of antibiotic-producing actinomycetes

The antibiotic-producing ability of actinomycetes was assessed through antagonist tests against the test bacteria, namely MRSA and *S. aureus* (ATCC 25923), using the agar plug diffusion method. The test bacteria were suspended in a sterile physiological solution at a cell density equivalent to 0.5 McFarland standard. The bacterial suspension was spread with a sterile cotton bud on a Müller-Hilton agar (MHA) medium. Actinomycetes isolates grown on ISP-2 medium for 10 days were extracted with a cork borer with a diameter of approximately 9 mm, and the extracted agar plug was placed on an MHA containing the test bacteria. Agar plugs of each actinomycete isolate were placed on three MHA plates. The cultures were incubated at 37°C for 24 h. The diameter of the inhibition zone, represented as a clear zone around the agar plug, was measured using a caliper, and the activity index value was determined (Balouri et al. 2016). All values from the primary selection used three replications. The inhibition index of actinomycetes was calculated as described by Apsari et al. (2019) using the following formula:

$$Inhibition\ index = \frac{Inhibition\ zone\ diameter - Colony\ diameter}{Colony\ diameter}$$

Secondary selection of antibiotic-producing actinomycetes

Preparation of methanol and ethyl acetate extracts

The antibiotic-producing ability of actinomycetes exhibiting high inhibitory activity from the primary selection was assessed. Actinomycetes cultures grown on ISP-2 agar medium for 10 days were utilized for this purpose. Four agar plates were cut into pieces, transferred to an Erlenmeyer flask, and then wrapped in aluminum foil. Methanol (MeOH) and ethyl acetate (EtOAc) (pro analysis grade) solution were then added into corresponding Erlenmeyer flasks containing the actinomycetes culture until submerged. Subsequently, the flasks were incubated for 72 h in a shaker incubator at 28°C. The solvents were then separated from the agar culture pieces by filtering through a filter paper. Concentrated MeOH and EtOAc crude extracts were obtained using a rotary evaporator. The rotary evaporator present in the USU FMIPA Organic Chemistry Laboratory was used for this purpose.

Inhibitory activity of MeOH and EtOAc extracts

MeOH and EtOAc extracts of potential isolates were used as test materials to inhibit the growth of MRSA and *S. aureus* (ATCC 25923). The antibiotic activities of the extracts were tested using the disk diffusion method. MRSA and *S. aureus* suspensions were prepared to achieve a density equivalent to 0.5 McFarland (1-2×10^8 CFU/mL). The crude MeOH and EtOAc extracts were diluted with 10% dimethyl sulfoxide (DMSO) to obtain concentrations of 75%, 50%, and 25%. A sterile cotton bud spread MRSA and S. aureus suspensions on an MHA medium. Next, 0.1 mL of MeOH and EtOAc extract was applied to a 6-mm diameter sterile disk (Oxoid) and placed on top of the MHA medium containing the test bacterial culture. The antibiotic chloramphenicol (10%) was used as a positive control, while 10% DMSO was used as a negative control. The bacterial cultures were incubated for 24 h at 37°C. Observations were made by examining the clear zones forming around the paper disk and measuring them using a caliper. The percentage of antibacterial activity index of the extract was determined by calculating the ratio of the diameter of the extract inhibition zone (clear zone) to that of the antibiotic inhibition zone (control) (Khalaf et al. 2019), using the formula shown below. The experiment was repeated thrice. Data were analyzed using ANOVA to obtain significantly different means.

$$\%\ Antibacterial\ activity\ index = \frac{Inhibition\ zone\ of\ extract}{Inhibition\ zone\ of positive\ control} \times 100$$

Molecular identification of potential bacteria

The isolates exhibiting the highest inhibitory activity were identified at the molecular level; initially, the actinomycetes DNA was isolated using the Wizard Genome Purification Kit. The isolated DNA was amplified using a polymerase chain reaction (PCR) machine with 16S rRNA gene primers 27F (5′-AGAGTTTGATCATGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) (Sharma and Manhas 2019). A total of 2 µL template DNA was mixed with 12.5 µL of 2× Go Taq Green Master Mix, 1 µL of each primer, and 8.5 µL of nuclease-free water to obtain a 25 µL reaction mix. The PCR machine was programmed with a pre-denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56.5°C for 1 min, elongation at 72°C for 1 min, and a final post-PCR elongation step at 72°C for 10 min (Salim et al. 2017). The PCR products were visualized using 1% agarose gel electrophoresis (1 g of agarose in 100 mL 1× TAE) stained with ethidium bromide. PCR-amplified

DNA samples were loaded into the gel wells alongside a 1-kb DNA marker, and the electrophoresis was conducted at 80 volts and 400 mA for 60 min. The amplified PCR product was visualized under a UV transilluminator. Finally, the amplified DNA was sent to Macrogen Inc. for sequencing.

Bioinformatics analysis

The DNA base sequence obtained from Macrogen Inc. was stored in FASTA format and searched for genetic similarity with reference data in the GenBank database using the Basic Local Alignment Search Tool nucleotide (BLASTn) program on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, a reference sequence database with the highest level of similarity was selected, and the alignment was performed using ClustalW in the MEGA 11 software. Finally, a phylogenetic tree was constructed using the neighbor-joining phylogeny program with the Kimura-2-parameter method in MEGA 11 and bootstrap analysis was set to 1,000 iterations.

RESULTS AND DISCUSSION

Isolation of actinomycetes from North Sumatra

Moreover, 30 actinomycetes isolates were obtained from the Cermin Beach and Sicanang Mangrove forest area using SCA and HVA media. From Sicanang Mangrove were collected 14 isolates from sandy sediment and 1 isolate from water, while at Cermin Beach were 12 isolates from sandy sediment and 4 isolates from water. The higher number of actinomycetes isolates obtained from Cermin beach sediment compared to seawater samples, despite similar environmental conditions such as temperature of 30°C, pH of 7.5, and salinity of 30 ppm, may be attributed to the dynamic nature of surface seawater influenced by wave currents, climate, and tides, resulting in a lower actinomycetes abundance. According to Jagannathan et al. (2021), actinomycetes found in marine habitats, ranging from the surface to seabed environments, are influenced by environmental conditions such as depth, mineral content, and symbiosis with marine micro-macrofauna. In mangrove forest environments, geographical factors such as location and organic content in root areas influence actinomycetes diversity (Shrestha et al. 2021).

Actinomycetes colonies on an isolation medium can be distinguished from bacterial colonies by their rough surface shape, contrasting with their shiny and transparent appearance. Actinomycetes colonies that grew on the isolation medium appeared as small white and yellow spots. Nutrients present in HVA medium, such as humic acid, are used by actinomycetes as carbon and nitrogen sources to accelerate sporulation. Actinomycetes isolates were also grown on SCA medium. Although actinomycetes isolates were grown using the same method, some isolates on SCA medium had varied colony colors. Actinomycetes colonies on SCA medium are light colored with powdery spores that do not spread like molds. In addition to selective media, antibiotics such as cycloheximide and nalidixic acid were incorporated to inhibit the growth of fungi and Gram-negative bacteria. Furthermore, pre-treatment of samples with higher temperatures in an oven at 40°C, 65°C, or 110°C (Fang et al. 2017) or the addition of a germicide solution of 1.5% phenol (Putri and Sumerta 2020) was needed to induce slow-growing actinomycetes, reduce contamination and obtain actinomycetes in the sample.

Characterization of actinomycetes

The colonies of actinomycetes isolates obtained from the two locations showed morphological variations in shape, color, margins, elevation, and colony surface, as shown in Table 1. Most actinomycetes isolate formed irregular colonies that adhered to the medium, emitted an earthy aroma, and produced conidia during an incubation period of 10 days. Actinomycetes colonies grew by adhering to the medium, developing wrinkled and rough surfaces, as shown in Figure 2. These findings are consistent with those of Rathore et al. (2019), who found that most actinomycetes colonies have irregular shapes, raised elevations, and wrinkled surfaces. Almost all isolates of actinomycetes show slow growth. Colonies of actinomycetes in agar medium are easy to distinguish from bacteria or fungi. The texture and color of the colony varied from white, white-beige, and yellow to grey (Figure 2).

Table 1. Colony characteristics of actinomycetes isolates obtained from Cermin beach and Sicanang mangrove forest when cultured on ISP-2 medium for 10 days

Isolate code	Shape	Color	Margin	Elevation	Surface
SPC 1	Irregular	White	Undulated	Umbonated	Wrinkled
SPC 2	Irregular	White	Lobate	Raised	Rough
SPC 3	Irregular	White	Undulated	Umbonated	Wrinkled
SPC 4	Irregular	Yellow	Lobate	Umbonated	Wrinkled
SPC 5	Irregular	White	Undulated	Umbonated	Wrinkled
SPC 6	Irregular	White	Undulated	Umbonated	Wrinkled
SPC 7	Irregular	White beige	Lobate	Raised	Wrinkled
SPC 8	Irregular	White	Undulated	Raised	Rough
SPC 9	Irregular	Yellow	Undulated	Umbonated	Wrinkled
SPC 10	Irregular	White	Undulated	Umbonated	Wrinkled
SPC 11	Irregular	White beige	Curled	Raised	Wrinkled
SPC 12	Circular	White	Entire	Raised	Rough
APC 1	Irregular	White beige	Curled	Umbonated	Wrinkled
APC 2	Irregular	White beige	Lobate	Umbonated	Wrinkled
APC 3	Irregular	White	Undulated	Raised	Rough
SMC 1	Irregular	White	Undulate	Umbonate	Wrinkled
SMC 2	Irregular	White	Undulate	Umbonate	Wrinkled
SMC 3	Irregular	White	Lobate	Umbonate	Wrinkled
SMC 4	Irregular	Yellow	Undulate	Umbonate	Wrinkled
SMC 5	Irregular	Gray	Undulate	Raised	Rough
SMC 6	Irregular	White beige	Undulate	Umbonate	Wrinkled
SMC 7	Circular	White	Lobate	Convex	Wrinkled
SMC 8	Irregular	White beige	Undulate	Umbonate	Wrinkled
SMC 9	Circular	Gray	Entire	Convex	Rough
SMC 10	Irregular	White	Undulate	Umbonate	Wrinkled
SMC 11	Irregular	White beige	Entire	Raised	Wrinkled
SMC 12	Circular	Yellow	Undulate	Umbonate	Wrinkled
SMC 13	Irregular	White	Undulate	Umbonate	Wrinkled
SMC 14	Irregular	White beige	Undulate	Umbonate	Wrinkled
AMC 1	Irregular	White	Undulate	Umbonate	Rough

Note: SPC: Cermin beach sediment; APC: Cermin beach water; SMC: Sicanang mangrove sediment; AMC: Sicanang mangrove water

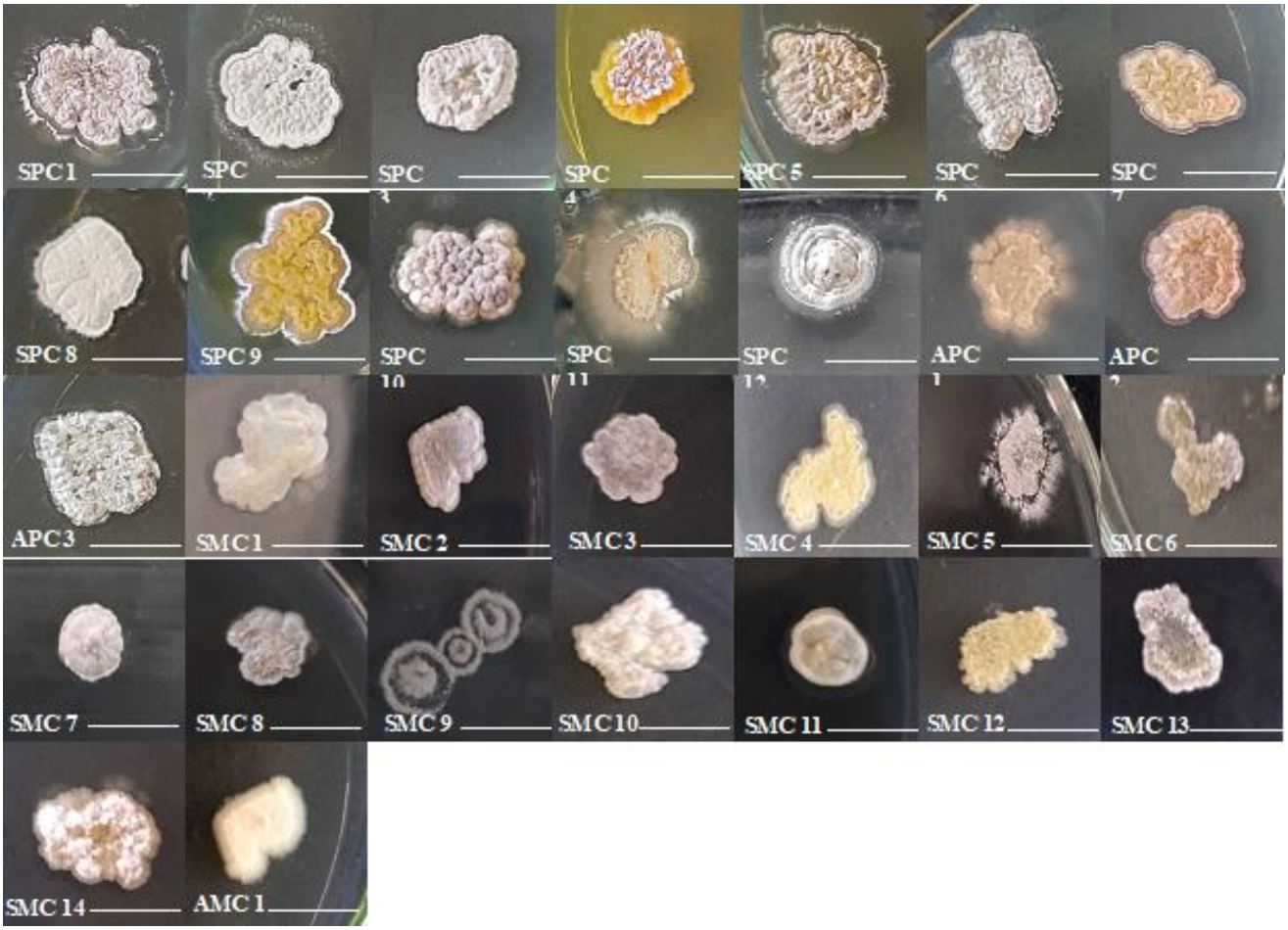


Figure 2. Macroscopic characteristics of actinomycetes colonies on ISP-2 medium after 10 days of incubation. Scale bar: 1 cm

Morphological differentiation of actinomycetes colonies is based on their growth characteristics on ISP medium. Actinomycetes isolates were cultured on the new ISP medium to maintain the isolates in their exponential phase and support their development. Differences in media can also influence physiological characteristics, such as pigment formation in actinomycetes isolates (Table 2).

Most actinomycetes isolates exhibited predominantly white aerial and substrate mycelia, with some displaying yellow and gray hues. The variety of colors of isolates in ISP medium plays an important role in characterizing the isolates. Actinomycetes, especially the *Streptomyces* genus, exhibit high morphological variability and color the medium. The variety of colors depends on the age and type of medium (Perez-Corral et al. 2022). Pigments produced by actinomycetes may dissolve in the media, resulting in visible colony colors; however, some actinomycetes produce pigments that remain in the mycelium. The change in color of substrate mycelia from bright to dark on the ISP-7 medium indicates the ability of actinomycetes isolates to produce melanin pigments. ISP-7 medium contains tyrosine, the main nitrogen source responsible for melanin. The mycelial substrate color of actinomycetes indicates melanin production in ISP-7 mediums, such as brown and black. Fifteen isolates produced a black color on the mycelial substrate. In comparison, three isolates produced a brown color, according to Sheefaa et al. (2022); brown, greenish-black, and black colonies indicate the presence of melanin. In contrast, the absence of such colors indicates no melanin pigment formation.

Primary screening of North Sumatra actinomycetes isolates against MRSA and *S. aureus*

An antagonist test conducted on actinomycetes isolates from North Sumatra revealed that 13 of 30 isolates inhibited the growth of MRSA and *S. aureus* (ATCC 25923) (Table 3). Agar plug culture actinomycetes on ISP 2 medium may have bioactive substances produced by these actinomycetes isolates. This was evidenced by forming a clear zone around the isolate on MHA medium.

Thirteen actinomycetes isolates collected from Cermin Beach and Sicanang Mangrove forest areas showed inhibitory effects on MRSA and *S. aureus* (ATCC 25923), with the highest activity index, recorded as 1.8 against MRSA and 2.0 against *S. aureus* (ATCC 25923) by SPC 3, 4, and 9. According to Ouchari et al. (2019), inhibition zone diameter can be categorized as follows: >20 mm for very strong, 10-20 mm for strong, 5-10 mm for medium, and <5 mm for low. Most inhibition zones observed in the antagonist test fell within the strong category. Actinomycetes isolates from water did not exhibit inhibition zones against MRSA.

Table 2. Color characteristics of mycelia of North Sumatra actinomycetes isolate cultured on different ISP media for 10 days

Isolate code	Mycelia color on ISP medium					
	ISP-3		ISP-4		ISP-7	
	Aerial	Substrate	Aerial	Substrate	Aerial	Substrate
SPC 1	White	White	White	White	Black	Black
SPC 2	White	White	White	White	White	White
SPC 3	White	White	White	White	Black	Black
SPC 4	Yellow	Yellow	White	Yellow	Black	Black
SPC 5	White	White	Gray	Gray	Black	Black
SPC 6	White	Yellow	White	White	Black	Black
SPC 7	White	White	White	White	Brown	Brown
SPC 8	Yellow	Yellow	White	White	White	White
SPC 9	Yellow	Yellow	White	Yellow	Black	Black
SPC 10	White	White	White	White	Black	Black
SPC 11	White	White	White	White	White	White
SPC 12	White	White	White	White	White	White
APC 1	White	White	White	White	Black	Black
APC 2	White	White	White	White	Brown	Brown
APC 3	Gray	Gray	White	Gray	Brown	Brown
SMC 1	White	White	Gray	Gray	Black	Black
SMC 2	White	White	White	White	Black	Black
SMC 3	White	White	White	White	Gray	Gray
SMC 4	White	White	White	White	White	White
SMC 5	Gray	Gray	Gray	Gray	White	White
SMC 6	Gray	Gray	White	White	Black	Black
SMC 7	Gray	Gray	White	White	Black	Black
SMC 8	Gray	Gray	Gray	Gray	Black	Black
SMC 9	Gray	Gray	White	White	White	White
SMC 10	White	White	White	White	Black	Black
SMC 11	White	White	Yellow	Yellow	Black	Black
SMC 12	Yellow	Yellow	White	White	White	White
SMC 13	Gray	Gray	Gray	Gray	White	White
SMC 14	White	White	White	White	White	White
AMC 1	Gray	Gray	Gray	Gray	White	White

Table 3. The diameter of the clear zone formed by potential actinomycetes isolates against MRSA and *S. aureus* (ATCC 25923)

Actinomycetes isolates	Clear zone diameter (mm)		Activity index	
	MRSA	*S. aureus* (ATCC 25923)	MRSA	*S. aureus* (ATCC 25923)
SPC 1	15.6 ± 0.24	18.5 ± 0.36	0.7	1
SPC 2	0	0	0	0
SPC 3	25.7 ± 0.15	27 ± 0.15	1.8	2
SPC 4	26.2 ± 0.26	27.3 ± 0.20	1.8	2
SPC 5	15.8 ± 0.23	18.9 ± 0.15	0.7	1.1
SPC 6	22.1 ± 0.35	24.9 ± 0.23	1.4	1.7
SPC 7	0	0	0	0
SPC 8	0	0	0	0
SPC 9	26 ± 0.17	28.2 ± 0.32	1.8	2
SPC 10	22 ± 0.12	22.8 ± 0.12	1.4	1.5
SPC 11	0	0	0	0
SPC 12	0	0	0	0
APC 1	0	0	0	0
APC 2	0	0	0	0
APC 3	0	0	0	0
SMC 1	0	0	0	0
SMC 2	3.93 ± 0.82	12.3 ± 1.45	0.10	0.37
SMC 3	0	0	0	0
SMC 4	0	0	0	0
SMC 5	0	0	0	0
SMC 6	12.56 ± 1.12	13.96 ± 1.82	0.40	0.53
SMC 7	0	0	0	0
SMC 8	0	0	0	0
SMC 9	21.3 ± 1.8	23.63 ± 1.52	1.39	1.65
SMC 10	19.76 ± 1.28	21.6 ± 0.33	1.20	1.43
SMC 12	17.7 ± 1.25	19.53 ± 0.83	0.98	1.19
SMC 13	0	0	0	0
SMC 14	11.63 ± 0.82	15.5 ± 0.85	0.33	0.72
AMC 1	0	0	0	0

It does not mean the isolates do not produce secondary metabolites; possibly the metabolite was ineffective for MRSA. Production of secondary metabolites can be improved by optimizing carbon and nitrogen sources in the fermentation medium. Singh et al. (2017) highlighted the importance of carbon and nitrogen sources in medium metabolite production. Vijayakumar et al. (2012) reported that S*treptomyces afghaniensis* VPTS3-1 from India, when cultured in eight media with different carbon and nitrogen compositions, produced different zone inhibition against tested microorganisms. The suitable carbon and nitrogen sources were starch KNO_3 in a starch-casein medium. From primary selection, ten actinomycete isolates with a higher index of antibacterial activity against MRSA were chosen for further steps.

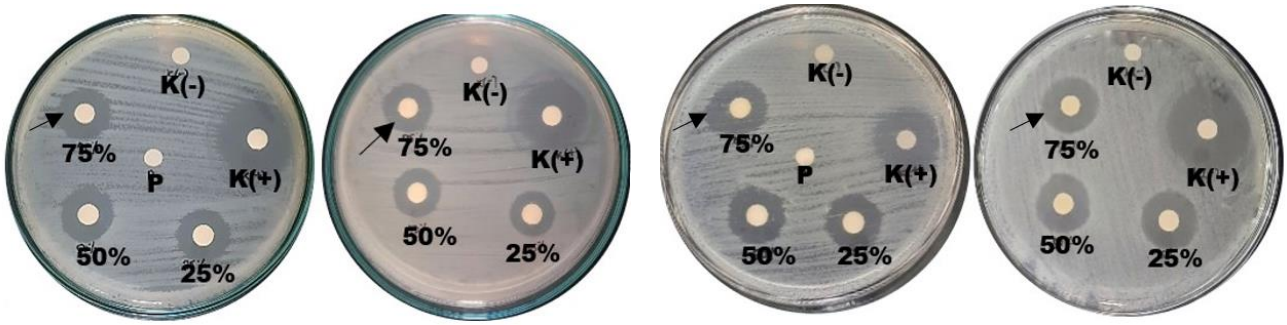
Antibacterial activity of MeOH and EtOAc actinomycetes extracts against MRSA

The antibacterial test results of the crude actinomycetes isolate extracts against MRSA and *S. aureus* (ATCC 25923) showed that the MeOH and EtOAc extracts inhibited the growth of the test bacteria based on the antibacterial index (Figure 3). The difference in the ability of the isolates to produce clear zones possibly depends on the secondary metabolites present in the actinomycetes isolates. Statistically, the type of actinomycetes isolates and the solvent extract was significantly affected by antibacterial activity value.

Table 4. The antibacterial activity index*) (%) of actinomycetes culture extracts against MRSA and *S. aureus* (ATCC 25923)

Bacterial pathogen	Isolate code	Activity index (%) of MeOH extracts			Activity index (%) of EtOAc extracts		
		75%	50%	25%	75%	50%	25%
MRSA	SPC 1	$56 \pm 0.40^{a,2}$	0	0	$55 \pm 1.18^{a,3}$	$50 \pm 0.95^{b,4}$	$49 \pm 0.66^{b,4}$
	SPC 3	$44 \pm 0.66^{c,4}$	$41 \pm 0.84^{c,3}$	$33 \pm 0.79^{d,4}$	$62 \pm 0.30^{a,2}$	$53 \pm 0.79^{b,4}$	$50 \pm 0.79^{b,3}$
	SPC 4	$85 \pm 0.30^{a,1}$	$72 \pm 0.95^{c,1}$	$68 \pm 1.39^{d,2}$	$83 \pm 0.95^{a,1}$	$78 \pm 1.15^{b,2}$	$74 \pm 0.30^{c,1}$
	SPC 5	$49 \pm 0.66^{a,3}$	$41 \pm 0.53^{b,3}$	$34 \pm 0.91^{c,4}$	$49 \pm 0.53^{a,4}$	$43 \pm 0.80^{b,5}$	$34 \pm 0.76^{c,6}$
	SPC 6	$50 \pm 0.15^{d,3}$	$45 \pm 0.76^{e,2}$	$41 \pm 0.30^{f,3}$	$83 \pm 0.46^{a,1}$	$67 \pm 0.95^{b,3}$	$62 \pm 0.66^{c,2}$
	SPC 9	$85 \pm 0.26^{a,1}$	$74 \pm 0.76^{b,1}$	$71 \pm 1.46^{b,1}$	$86 \pm 0.66^{a,1}$	$76 \pm 0.92^{b,2}$	$74 \pm 0.92^{b,1}$
	SPC 10	$47 \pm 0.66^{a,3}$	$36 \pm 0.69^{c,4}$	$35 \pm 0.52^{c,4}$	$48 \pm 0.79^{a,4}$	$43 \pm 0.30^{b,5}$	$33 \pm 0.45^{c,6}$
	SMC 9	$82 \pm 0.53^{b,1}$	$74 \pm 0.79^{c,1}$	$65 \pm 1.09^{d,2}$	$86 \pm 0.80^{a,1}$	$81 \pm 0.54^{b,1}$	$76 \pm 0.40^{c,1}$
	SMC 10	$48 \pm 0.92^{a,3}$	$36 \pm 0.79^{c,4}$	$32 \pm 0.26^{d,4}$	$48 \pm 0.26^{a,4}$	$43 \pm 0.92^{b,5}$	$38 \pm 0.53^{c,5}$
	SMC 12	$47 \pm 1{,}18^{c,3}$	$44 \pm 0{,}84^{d,2}$	$42 \pm 0.40^{d,3}$	$63 \pm 0.40^{a,2}$	$50 \pm 0.26^{b,4}$	$46 \pm 0.45^{c,4}$
S. aureus	SPC 1	$65 \pm 0.66^{b,3}$	0	0	$67 \pm 0.95^{a,5}$	$63 \pm 0.92^{b,5}$	$57 \pm 0.45^{c,5}$
ATCC 25923	SPC 3	$59 \pm 0.66^{b,5}$	$55 \pm 0.30^{c,3}$	0	$65 \pm 1.05^{a,5}$	$53 \pm 0.91^{c,6}$	$48 \pm 0.66^{d,6}$
	SPC 4	$83 \pm 0.66^{b,2}$	$74 \pm 1.05^{c,2}$	$65 \pm 1.21^{d,2}$	$85 \pm 0.15^{a,3}$	$81 \pm 0.76^{b,3}$	$76 \pm 0.91^{c,3}$
	SPC 5	$62 \pm 0.79^{a,4}$	$58 \pm 0.76^{b,3}$	0	$40 \pm 0.79^{c,6}$	$38 \pm 0.79^{c,7}$	$34 \pm 0.54^{d,7}$
	SPC 6	$65 \pm 0.69^{a,3}$	$57 \pm 0.95^{b,3}$	$46 \pm 0.92^{c,3}$	$65 \pm 1.64^{a,5}$	$57 \pm 0.26^{b,6}$	$46 \pm 0.40^{c,6}$
	SPC 9	$79 \pm 2.23^{b,2}$	$70 \pm 0.91^{c,2}$	$63 \pm 1.31^{c,2}$	$87 \pm 1.05^{a,3}$	$83 \pm 0.80^{b,2}$	$79 \pm 0.95^{b,2}$
	SPC 10	$56 \pm 0.26^{b,5}$	$50 \pm 1.46^{b,4}$	$41 \pm 0.30^{c,4}$	$71 \pm 1.39^{a,4}$	$68 \pm 1.20^{a,4}$	$67 \pm 1.57^{a,4}$
	SMC 9	$84 \pm 0.45^{b,1}$	$79 \pm 0.40^{c,1}$	$74 \pm 0.79^{c,1}$	$94 \pm 0.15^{a,1}$	$92 \pm 0.53^{a,1}$	$82 \pm 0.66^{b,1}$
	SMC 10	$47 \pm 0.53^{d,6}$	$43 \pm 0.40^{e,5}$	$38 \pm 0.61^{f,4}$	$85 \pm 0.26^{a,3}$	$78 \pm 0.40^{b,3}$	$73 \pm 0.66^{c,3}$
	SMC 12	$63 \pm 0.69^{d,4}$	$50 \pm 0.45^{e,4}$	$46 \pm 0.78^{f,3}$	$90 \pm 1.14^{a,2}$	$84 \pm 0.26^{b,2}$	$76 \pm 1.82^{c,3}$

Note: Different superscript letters (a, b, c, etc.) across the rows indicate significant differences ($P \leq 0.05$) between rows within the same column. Different numbered superscripts (1, 2, 3, etc.) across the columns indicate significant differences ($P \leq 0.05$) between columns within the same row. Results are based on post-hoc Tukey's HSD test of activity index limited to each pathogen. *)ratio inhibition zone of diameter extract to diameter inhibition of chloramphenicol (22 mm)



MRSA ***S. aureus*** **(ATCC 25923)** **MRSA** ***S. aureus*** **(ATCC 25923)**

A **B**

Figure 3. Antibacterial activity inhibition zone of: A. Methanol (MeOH) SPC 9 Extract and; B. Ethyl acetate (EtOAc) SPC 9 extracts. Note: K (−) DMSO; K (+) Chloramphenicol; P: Penicillin. The arrow shows the clear zone indicating the antibacterial activity of MeOH and EtOAc actinomycetes extracts

The MeOH and EtOAc extracts of the actinomycetes isolates demonstrated varying antibacterial activity indices. Notably, the 75% EtOAc extract of SPC 9 and SMC 9 exhibited the highest activity index at 86% against MRSA; the 75% MeOH extract of SPC 4 and SPC 9 showed a similarly high activity index at 85%. All actinomycetes isolates were soluble in both extracts, but the inhibition zone formed by the EtOAc extract was larger than that formed by the MeOH extract. The results of the antibacterial activity index of the crude extract against MRSA and *S. aureus* (ATCC 25923) are summarized in Table 4.

In addition to the solvent types, the ability of actinomycetes extracts to inhibit MRSA and *S. aureus* (ATCC 25923) also depended on the extract concentration and type of the strain. A high concentration of antibacterial ingredients indicates the presence of more active compounds, leading to increased effectiveness in inhibiting the test bacteria and resulting in a larger antibacterial index activity. The potential isolates with an antibacterial index >80 % against MRSA were SPC 9, SMC 9, and SPC 4. Although all actinomycetes crude extracts in this study exhibited potential in inhibiting MRSA and *S. aureus* (ATCC 25923), their efficacy was not greater than commercial antibiotic chloramphenicol. The active compound of potential extract as an antibacterial activity had not yet been identified and is interesting to be done in further study.

Molecular identification of potential actinomycetes

The PCR amplification results indicated that the potential actinomycetes isolates SPC 9 and SMC 9 had DNA amplicons of approximately 500 bp. Amplicons from isolates SPC 9 were selected for sequencing. The DNA sequences were identified through a BLASTn search based on the 16S rRNA sequence. The BLASTn results for isolates SPC 9 showed similarity levels of 99.71% and 99.28% with *Streptomyces antibioticus* strain cfcc3075, respectively. In contrast, the sequence of isolate SMC 9 had a similarity level of 96.02% with *Streptomyces rochei* strain A1 from the GenBank database on the National Center for Biotechnology Information website. Similarity values between 97-100% indicate the homology of target sequences at the species level.

Based on the BLASTn identification data, the results suggest similarities between potential isolates from *S. antibioticus* and *S. rochei*. To further explore their relationship with actinomycetes sequences of various types, they will be analyzed in a phylogenetic tree using MEGA 11 software (Figure 4).

No studies have specially investigated the antibacterial activity of *S. antibioticus* and *S. rochei* found in North Sumatra Province or Indonesia. These two species of *Streptomyces* were known as potent antibiotic producers. The *S. antibioticus* has long been known as a producer of the antibiotic actinomycin, commonly used as a chemotherapeutic agent. Actinomycin was the first antibiotic isolated by Waksman and Woodruff in 1940 (Singh et al. 2010). Sharma and Manhas (2019) reported that *S. antibioticus* strain M7 from the rhizosphere soil of *Stevia rebaudiana* (Bertoni) Bertoni in India produced antibiotic actinomycin V, X2, and D, which can inhibit several pathogenic bacteria, namely *Bacillus subtilis, Klebsiella pneumoniae* sub sp. *pneumoniae, S. aureus, Staphylococcus epidermidis, Salmonella typhi, Escherichia coli* and MRSA. *Streptomyces antibioticus* strain H12-15, also isolated from marine sediments on the coast of South China, produces two bioactive compounds, neoantimycin A and neoantimycin B, as antifungal and anticancer activity (Hu et al. 2017). In this study, the metabolite compounds from *S. antibioticus* strain SPC 9 as anti-MRSA remain unidentified and require further investigation.

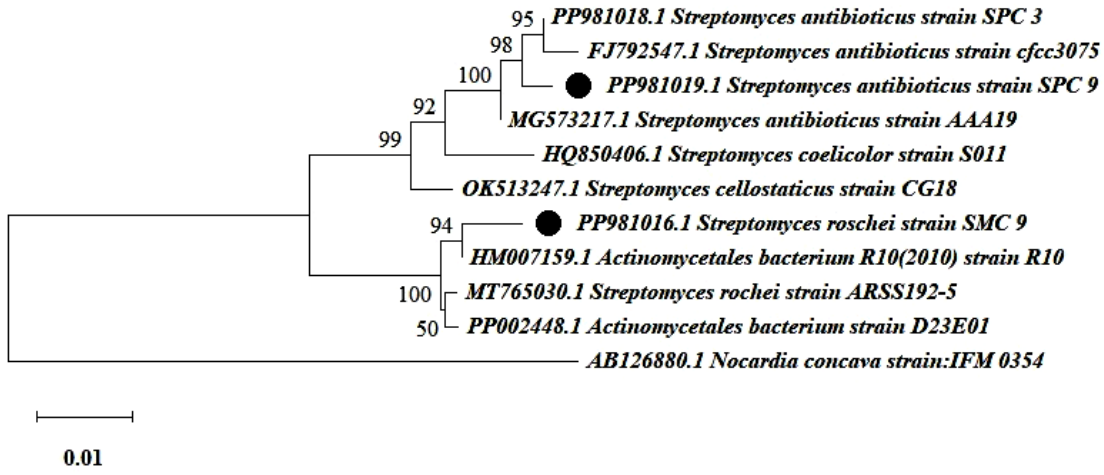


Figure 4. Phylogenetic tree of actinomycetes isolates based on 16S rRNA sequence comparison constructed with MEGA 11 software using the neighbor-joining method with the Kimura-2-parameter and bootstrap 1000

Streptomyces rochei was an antibiotic producer that was isolated from Kukup Beach of Kemadang Village in Yogyakarta, Indonesia, and has the potency to inhibit *S. aureus* ATCC 25923, *B. subtilis* FNCC 0060, *E. coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853 (Ambarwati et al. 2023). Ethanolic extract from culture fermentation of *S. rochei* was potentially MRSA-inhibitory activity (Djinni et al. 2023). Information about chemical metabolites from *S. rochei* as antibacterial activity against MRSA has not been reported yet. Still, for other purposes, *S. rochei* from mangrove sediments in China produced borrelidin as an anticancer (Sun et al. 2018). The potency of *S. rochei* strain SMC 9 to inhibit MRSA from this study is still needed to optimize and characterize the active compound for further study.

Research on antibacterial compound-producing actinomycetes from the Sicanang Mangrove and Cermin Beach areas in North Sumatra province is reported for the first time in this research, particularly focusing on their ability to inhibit MRSA. All isolated actinomycetes can be further investigated for their inhibiting growth activity toward other bacteria or fungi. Although *S. antibioticus* strains SPC 9 and *S. rochei* strain SMC 9 did not demonstrate a greater inhibition zone than the comparator antibiotic (chloramphenicol), the production of secondary metabolites by *S. antibioticus* strains SPC 9 and *S. rochei* strain SMC 9 can still be enhanced through optimization of growth conditions. The metabolites can be further evaluated for therapeutic purposes through in silico, in vitro, or in vivo studies.

In conclusion, a total of 30 actinomycetes isolates were collected from two locations near Cermin Beach and Sicanang Mangrove forests in North Sumatra; 26 isolates were from sediment samples, and 4 were from water samples. After initial screening, 13 actinomycetes isolates were identified as potential inhibitors of MRSA and *S. aureus* (ATCC 25923). In the secondary screening, two actinomycetes culture extracts showed similar inhibition zone sizes, ranging from 7.3 to 19.2 mm, against MRSA and *S. aureus* (ATCC 25923). However, the inhibition zone size was smaller compared to chloramphenicol. Molecular identification revealed that SMC 9 was closely related to *S. rochei*, with a similarity percentage of 96.02%, while SPC 9 had a high similarity (99%) with *S. antibioticus*.

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REFERENCES

Alghamdi BA, Al-Johani I, Al-Shamrani JM, Alshamrani, HM, Al-Otaibi BG, Master KA, Yusofet NY. 2023. Antimicrobial resistance in methicillin-resistant *Staphylococcus aureus*. Saudi J Biol Sci 30 (4): 103604. DOI: 10.1016/j.sjbs.2023.103604.

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.

Apsari PP, Budiarti S, Wahyudi AT. 2019. Actinomycetes of rhizosphere soil producing antibacterial compounds against urinary tract infection bacteria. Biodiversitas 20: 1259-1265 DOI: 10.13057/biodiv/d200504.

Asnani A, Luviriani E, Oedjijono. 2020. Activity of actinomycetes isolated from mangrove Segara Anakan Cilacap toward methicillin-resistant *Staphylococcus aureus* (MRSA). Jurnal Kimia Sains dan Aplikasi 23 (1): 1-7. DOI: 10.14710/jksa.23.1.1-7.

Awad NM, Aboseidah AA, Elshamy AI, Rasmey AHM. 2024. Antimicrobial activities of some actinomycetes isolated from cultivated soil, Egypt. Front Sci Res Technol 8: 40-46.

Ayau P, Bardossy AC, Sánchez-Rosenberg GF, Ortiz R, Moreno D, Hartman P, Rizvi K, Prentiss TC, Perri MB, Mahan M, Huang V, Reyes K, Zervos MJ. 2017. Risk factors for 30-day mortality in patients with methicillin-resistant *Staphylococcus aureus* bloodstream infections. Intl J Infect Dis 61: 3-6. DOI: 10.1016/j.ijid.2017.05.010.

Balouri M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity. J Pharm Anal 6: 71-79. DOI: 10.1016/j.jpha.2015.11.005.

Barka EA, Vatsa P, Sanchez L, Vaillant NG, Jacquard C, Klenk HP, Clement C, Ouhdouch Y, Wezel GP. 2016. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev 80: 1-43. DOI: 10.1128/MMBR.00019-15.

Bhakyashree K, Kannabiran K. 2020. Actinomycetes mediated targeting of drug resistant MRSA pathogens. J King Saud Univ Sci 32 (1): 260-264. DOI: 10.1016/j.jksus.2018.04.034.

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.

Devanshi S, Shah KR, Arora S, Saxena S. 2021. Actinomycetes as an environmental scrubber. In: Abdel-Raouf ME, El-Keshawy MH (eds). Crude Oil-New Technologies and Recent Approaches. Intechopen. DOI: 10.5772/intechopen.99187.

Djinni I, Djoudi W, Boumezoued C, Barchiche H, Souagui S, Kecha M, Mancini I. 2023. Statistical medium optimization for the production of anti-methicillin-resistant *Staphylococcus aureus* metabolites from a coal-mining-soil-derived *Streptomyces rochei* CMB47. Fermentation 9: 381. DOI: 10.3390/fermentation9040381.

Donald L, Pipite A, Subramani R, Owen J, Keyzers RA, Taufa T. 2022. *Streptomyces*: Still the biggest producer of new natural secondary metabolites, a current perspective. Microbiol Res 13 (3): 418-465. DOI: 10.3390/microbiolres13030031.

Fang BZ, Salam N, Han MX, Jiao JY, Cheng J, Wei DQ, Xiao M, Li WJ. 2017. Insights on the effects of heat pre-treatment, pH, and calcium salts on isolation of rare Actinobacteria from Karstic Caves. Front Microbiol 8: 1535. DOI: 10.3389/fmicb.2017.01535.

Fatimah, Suroiyah F, Solikha N, Rahayuningtyas ND, Surtiningsih T, Nurhariyati T, Ni'matuzahroh, Affandi M, Geraldi A, Thontowi A. 2022. Antimicrobial activity of actinomycetes isolated from mangrove oil in Tuban, Indonesia. Biodiversitas 23 (6): 2957-2965. DOI: 10.13057/biodiv/d230622.

Hu C, Zhou SW, Chen F, Zheng XH, 2017. Neoantimycins A and B, two unusual benzamido nine-membered dilactones from marine-derived *Streptomyces antibioticus* H12-15. Molecules 22 (4): 557. DOI: 10.3390/molecules22040557.

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

Jiang J, He X, Cane DE. 2007. Biosynthesis of the earthy odorant geosmin by a bifunctional *Streptomyces coelicolor* enzyme. Nat Chem Biol 3 (11): 711-715. DOI: 10.1038/nchembio.2007.29.

Khalaf OM, Ghareeb MA, Saad AM, Madkour HMF, El-Ziaty AK, Abdel-Aziz MS. 2019. Phenolic constituents, antimicrobial, antioxidant, and anticancer activities of ethyl acetate and n-butanol extracts of *Senna italica*. Acta Chromatograph 31 (2): 138-145. DOI: 10.1556/1326.2018.00412.

Mahjabeen F, Saha U, Mostafa MN, Siddique F, Ahsan E, Fathma S, Tasnim A, Rahman T, Faruq R, Sakibuzzaman M, Dilnaz F, Ashraf A. 2022. An update on treatment options for methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia: A systematic review. Cureus 14 (11): e31486. DOI: 10.7759/cureus.31486.

Mast Y, Stegman E. 2019. Actinomycetes: The antibiotic producers. Antibiotic Basel 8: 5. DOI: 10.3390/antibiotics8030105.

Okwu MU, Olley M, Akpoka AO, Izevbuwa OE. 2019. Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. AIMS Microbiol 5 (2): 117-137. DOI: 10.3934/microbiol.2019.2.117.

Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. 2019. Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga Desert and their taxonomic diversity. Biol Open 8: bio035410. DOI: 10.1242/bio.035410.

Parra J, Wilkinson B, Beaton A, Seipke RF, Hutchings MI, Duncan KR. 2023. Antibiotics from rare actinomycetes, beyond the genus *Streptomyces*. Curr Opinion Microbiol 76: 102385. DOI: 10.1016/j.mib.2023.102385.

Perez-Corral DA, Ornelas-Paz JJ, Olivas-Orozco GI, Acosta-Muniz CH, Salas-Marina MA, Berlanga-Reyes DI, Ruiz-Cisneros MF, Rios-Velasco C. 2022. Molecular, morphological and biochemical characterization of actinomycetes and their antagonistic activity against phytopathogenic fungi. Rev Fitotec Mex 45 (1): 103-115. 10.35196/rfm.2022.1.103

Putri AL, Sumerta IN. 2020. Selective isolation of *Dactylosporangium* and *Micromonaspora* from the soil of karst cave of Simuelue Island and their antibacterial pPotency. Berita Biologi, Jurnal Ilmu-ilmu Hayati 19 (3A): 257-268. DOI: 10.14203/beritabiologi.v19i3.3933.

Rathore DS, Sheikh M, Gohel S, Singh SP. 2019. Isolation strategies, abundance and characteristics of the marine actinomycetes of Kachhighadi, Gujarat, India. J Mar Biol Assoc India 61 (1): 71-78. DOI: 10.6024/jmbai.2019.61.1.2028-11.

Saleem M, Hassan A, Li F, Lu Q, Ponomareva LV, Parkin S, Sun C, Thorson JS, Shaaban KA, Sajid I. 2023. Bioprospecting of desert actinobacteria with special emphases on griseoviridin, mitomycin C and a new bacterial metabolite producing Streptomyces sp. PU-KB10-4. BMC Microbiol 23: 69. DOI: 10.1186/s12866-023-02770-8.

Salim FM, Sharmili SA, Anbumalarmathi J, Umamaheswar K. 2017. Isolation, molecular characterization and identification of antibiotic producing actinomycetes from soil samples. J Appl Pharm Sci 7: 69-75.

Selim MSM, Abdelhamid SA, Mohamed SS. 2021. Secondary metabolites and biodiversity of actinomycetes. J Genet Eng Biotechnol 19 (1): 72. DOI: 10.1186/s43141-021-00156-9.

Sharma M, Dangi P, Choudhary. 2014. Actinomycetes: Source, identification and their application. Intl J Curr Microbiol Appl Sci 3: 801-832.

Sharma M, Manhas RK. 2019. Purification and characterization of actinomycins from *Streptomyces* strain M7 active against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. BMC Microbiol 19: 44. DOI: 10.1186/s12866-019-1405-y.

Sheefaa MI, Sivaperumal P. Antioxidant activities from melanin pigment produced by marine actinobacterium of *Streptomyces* species. 2022. J Adv Pharm Technol Res 13: S84-S87. DOI: 10.4103/japtr.japtr_338_22.

Shrestha B, Nath DK, Maharjan A, Poudel A, Pradhan RN, Aryal S. 2021. Isolation and characterization of potential antibiotic-producing actinomycetes from water and soil sediments of different regions of Nepal. Intl J Microbiol 2021: 5586165. DOI: 10.1155/2021/5586165.

Siddharth S, Aswathanarayan JB, Kuruburu MG, Madhunapantula SRV, Vittal RR. 2021. Diketopiperazine derivative from marine actinomycetes *Nocardiopsis* sp. SCA30 with antimicrobial activity against MRSA. Arch Microbiol 203: 6173-6181. DOI: 10.1007/s00203-021-02582-2.

Singh SB, Genilloud O, Pelaez F. 2010. Terestrial microorganisms-filamentous bacteria. Comprehensive Nat Prod 2: 109-140. DOI: 10.1016/B978-008045382-8.00036-8.

Singh V, Haque S, Niwas R, Srivastava A, Pasupuleti M, Tripathi CKM. 2017. Strategies for fermentation medium optimization: An in-depth review. Front Microbiol 7: 2087. DOI: 10.3389/FMICB.2016.02087.

Sun J, Shao J, Sun C, Song Y, Li Q, Lu L, Ju J. 2018. Borrelidins F-I, cytotoxic and cell migration inhibiting agents from mangrove-derived *Streptomyces rochei* SCSIO ZJ89. Bioorg Med Chem 26: 1488-1494. DOI: 10.1016/j.bmc.2018.01.010.

Vijayakumar R, Panneerselvam K, Muthukumar C, Thajuddin N, Panneerselvam A, Saravanamuthu R. 2012. Optimization of Antimicrobial production by a marine Actinomycete *Streptomyces afghaniensis* VPTS3-1 isolated from Palk Strait, East Coast of India, Indian J Microbiol 52: 230-239 DOI: 10.1007/s12088-011-0138-x.

Zahra R, Zahra S, Hajj RE, Khalil M. 2022. Actinomycetes, promising therapeutic agents: Characteristic and active metabolites. J Biol Today''s World 11: 1-8.