Original Research Report

ANTIBIOTIC-PRODUCING *Streptomyces sp.* ISOLATED FROM THE SOIL OF A MANGROVE ECOSYSTEM

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ABSTRACT

A mangrove ecosystem in Surabaya, Indonesia, has a high salinity, pH, potassium, phosphorus, and nitrate contents. This ecosystem comprises a mixture of sand, dust, mud, and clay, which has the potential to be a conducive environment for the isolation of *Streptomyces*. The importance of *Streptomyces* in biotechnology lies in its ability to produce bioactive secondary metabolites, which represent a valuable reservoir of antibiotics. This study aimed to assess the antibiotic activity exhibited by *Streptomyces* sp. isolated from the soil of a mangrove ecosystem in Wonorejo, Surabaya, Indonesia. The analysis focused on the potential of *Streptomyces* sp. to produce antibiotics that work against Gram-positive bacteria (i.e., *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*) as well as Gram-negative bacteria (i.e., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium*). The antibacterial activity test was conducted using the modified agar diffusion method. Observations were performed to identify any clear zone formation around the *Streptomyces* sp. agar colonies with a diameter of 0.8 cm and a height of 3 mm. The clear zone diameter was measured every 24 hours during the 10-day incubation period to assess the diversity of antibacterial activity. The antibacterial profile of *Streptomyces* sp. exhibited varying levels of activity against different bacterial strains in the tests conducted. The inhibition zone diameters demonstrated the highest levels of activity in *Bacillus subtilis* (15.9 mm) on day 7, *Staphylococcus aureus* (27.6 mm) on day 2, *Pseudomonas aeruginosa* (24.3 mm) on day 7, *Escherichia coli* (29.2 mm) on day 5, and *Salmonella Typhimurium* (27.5 mm) on day 7. The results indicated that *Streptomyces* sp. had inhibitory effects against Gram-positive bacteria as well as Gram-negative bacteria. In conclusion, *Streptomyces* sp. found in the soil of mangrove ecosystems has the ability to produce antibiotics.

Keywords: *Streptomyces* sp.; mangrove; antibiotics; biodiversity; good health and well-being

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Highlights:
1. The unexplored soil of mangrove ecosystems in Surabaya, Indonesia, has the potential to be home to biodiversity, including *Streptomyces* sp. that can produce antibiotics.
2. *Streptomyces* sp. has antibacterial properties against Gram-positive and Gram-negative bacteria, and the duration of incubation plays a critical role in regulating the antibacterial activity.

INTRODUCTION

Mangrove forests can absorb contaminants originating from upstream areas through rivers and surrounding dry lands through rainwater. These contaminants contain various compounds, such as ammonia, nitrate, and nitrite, which serve as nutrients for decomposing bacteria (Chrisyariati et al. 2014, Alongi 2020). The structure and composition of a mangrove ecosystem have a direct correlation with its nutrient profile. The decrease in nutrient content of the ecosystem is expected to have a negative impact on mangrove growth. Nitrogen and phosphorus are the basic inorganic nutrients that are essential for the process of primary production. The ideal nutrient profiles of mangrove vegetation

are 0.211–0.252 mg/L for nitrate, 0.135–0.277 mg/L for nitrite, 0.51–0.74 mg/L for ammonia, and 0.109–0.140 mg/L for phosphate (Frederika et al. 2021).

Mangrove ecosystems are categorized based on their biophysical types. The categorization includes numerous types of mangroves, i.e., deltaic, estuarine, lagoonal, and open-coast mangroves (Worthington et al. 2020). A study conducted by Constance et al. (2022) examined the lagoonal mangrove ecosystem located in Aldabra Atoll, Seychelles. The findings of the study estimated an average aboveground biomass (AGB) of 82±13 Mg ha^{-1}. In another study, Palla et al. (2018) conducted research that examined tropical mangrove forests. The researchers found a microbial composition consisting of 91% bacteria and fungi, 7% algae, and 2% protozoa.

Previous research has documented the presence of several bacterial strains, such as *Streptomyces*, within mangrove ecosystems. *Streptomyces* is a Gram-positive, rod-shaped, filamentous bacterium characterized by its hyphae diameter of 0.5–1.0 mm. It has an aerobic microbial life and a diaminopimelic cell wall. In contrast to the rapid growth observed in other bacterial colonies, *Streptomyces* colonies present with a slow emergence and strong adhesion to agar media. These colonies have distinct clusters or granules in liquid culture media, with their characteristics highlighted by their opaque appearance (Chater 2016, Al-Dhabi et al. 2018, 2019). The growth of *Streptomyces* begins with the germination of the spores and the development of hyphae. Afterwards, the primary and secondary mycelia start to form, leading to the development of unigenomic spores at the end of the cycle. The secondary mycelium is responsible for the expression of genes or proteins that are involved in secondary metabolism, both in solid and liquid cultures (Manteca & Yagüe 2018).

Streptomyces, a microorganism that was formerly misidentified as a fungus, infrequently induces pathogenic conditions. The microorganism is characterized by its filamentous structure and sporulation capability. It has a notable guanine + cytosine (G+C) content of 73%, which is higher than many other organisms. *Streptomyces* can thrive within a pH range of 3–9 and a temperature of 15–45°C. The optimal growth process for *Streptomyces* is feasible at normal room temperature. However, the growth of *Streptomyces* is strongly influenced by pH levels. The growth rate decreased significantly at pH levels of 3 and 9. The ideal pH level for achieving maximum growth of *Streptomyces* sp. is 7 (Baskaran & Muthukumarasamy 2017).

Streptomyces remains one of the main natural sources for antibiotics and several bioactive compounds. It plays an important role as the primary producer of approximately two-thirds of antibiotics in the fields of medicine and agriculture. However, the secondary metabolic pathways provided by *Streptomyces* have not been explored enough in recent laboratory culture research (Manteca & Yagüe 2018, Karthik & Kalyani 2023). When conducting research on *Streptomyces*, nitrogen concentration serves as an indicator of its presence within mangrove ecosystems. One of the characteristics of *Streptomyces* is its ability to enzymatically convert nitrate compounds into nitrite. Therefore, differences in mangrove soil texture can potentially influence the nitrogen concentration, which in turn acts as a reliable marker for the existence of various kinds of *Streptomyces* bacteria (Madigan et al. 2019).

Water pollution poses harmful effects on human individuals and populations, as well as on mangrove ecosystems. However, some organisms can flourish even in the presence of polluted waste, thereby contributing to the overall biodiversity of these ecosystems. The *Streptopmyces* bacteria that survive in contaminated mangrove ecosystems are considered distinct isolates (Al-Ansari et al. 2019). Instances of water pollution frequently arise in Surabaya, Indonesia, particularly along the rivers and mangrove forests, with a notable concentration downstream of ponds in the estuaries and coastal areas. The area with the widest and most diverse mangrove ecosystem along the Surabaya shoreline is located on the east coast of the city (Syamsu et al. 2018, Sukojo & Arindi 2019). Hence, this study aimed to assess the antibiotic profile of *Streptomyces* sp. isolates obtained from the mangrove ecosystem soil in Wonorejo, Surabaya, Indonesia. The assessment focused on the activity of these isolates against both Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

The technique used was a modified agar diffusion method. Agar molds were made every 24 hours for ten days using *Streptomyces* sp. which was 0.8 cm in diameter and 3 mm high. To test potential, the test object was then inserted into the test medium. Agar mold containing *Streptomyces* sp. attached to culture media that has previously been inoculated with test bacteria to carry out antibacterial activity tests. The media used in this study was ISP-4 (International *Streptomyces* Project medium 4).

Microscopic identification of *Streptomyces* sp.

The *Streptomyces* sp. bacteria were grown on International *Streptomyces* Project 4 (ISP-4) medium, with catalog number 277210, produced by Difco Laboratories (Detroit, MI, USA). The

incubation period was carried out for four days at a temperature of 28°C. Microscopic observation was conducted by transferring a loopful of *Streptomyces* sp. using a sterile inoculation loop into an object glass filled with sterile water, then examining it under an Olympus U-5RE-2 microscope (Munday Scientific, Sanford, NC, USA) with 400X magnification by adding immersion oil (Girkin 2019).

Preparation of *Streptomyces* sp. cell suspension

The preparation of *Streptomyces* sp. cell suspension started by adding 10 mL of sterile phosphate buffer solution (product number P3228, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) with a pH of 7 to ISP-4 agar slants (product number BD 277210, Becton Drive, Franklin Lakes, NJ, USA) containing *Streptomyces* sp. obtained from the soil of a mangrove ecosystem in Wonorejo, Surabaya, Indonesia (-7.305096, 112.843754), which had been incubated at 30°C for 24 hours. Subsequently, the agar slants were shaken until all colonies on the agar surface were released and suspended in a phosphate buffer with a pH level of 7. The inoculum was then measured at a transmittance of 25% with a wavelength of 580 nm using a Spectronic 20 (Thermo Fisher Scientific, Waltham, MA, USA). Afterwards, 5 mL of cell suspension was mixed with 15 mL of ISP-4 agar media. The agar media had been thawed at a temperature of 45°C and subsequently incubated at 28°C. The agar print, consisting of *Streptomyces* sp. with a diameter of 0.8 cm and a height of 3 mm, was taken every 24 hours for 10 days. The specimens were then placed on the test medium for potency testing (Rütten et al. 2022).

Antibacterial bioassay of *Streptomyces* sp.

The *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium* strains used in this study were sourced from the Microbiology Laboratory of the Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The bacteria were cultured in 10 mL of nutritional agar slant media and incubated at a temperature of 30°C for 24 hours. Petri dishes were prepared by adding 15 mL of nutrient agar medium (Oxoid CM0003B, Oxoid Ltd., Basingstoke, UK) as a foundational layer. A volume of 10 µL of *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium* was added to each petri dish as a seed coating. The bacteria had a transmittance (T) of 25% measured using a spectrophotometer (Spectronic 20, Thermo Fisher Scientific, Waltham, MA, USA). The antibacterial activity test was carried out by attaching an agar print containing *Streptomyces* sp. onto the culture medium that had been previously inoculated with the test bacteria. Afterwards, the specimens were incubated at a temperature of 28°C for 24 hours. The measurement of the inhibition zone around the cultured colonies was conducted using a caliper. A volume of 10 µL of streptomycin sulfate solution with a concentration of 250 ppm was added to a well with dimensions of 0.8 cm in diameter and 3 mm in height. This study used positive and negative control groups. The positive control group contained antibiotic streptomycin sulfate on the test bacteria, while the negative control group contained sterile phosphate buffer. The positive control showed the presence of an inhibition zone around the cultured petri dish, while the negative control showed no inhibition zone at all. The presence of an inhibition zone surrounding the culture colonies on the petri dish was indicative of a positive result, suggesting that the *Streptomyces* sp. isolates could produce antibiotics by inhibiting bacterial growth. The antibacterial activity test of *Streptomyces* sp. was conducted in three replications (Al-Ansari et al. 2019).

RESULTS

After being incubated at a temperature of 28°C for two days, *Streptomyces* sp. isolates obtained from the soil of the mangrove ecosystem in Surabaya, Indonesia, exhibited distinct olfactory characteristics reminiscent of soil, along with the presence of small colonies. The appearance of the colonies' surface was initially observed on day 2, with a relatively smooth texture. The surface of the colonies had formed completely by day 4. The granularity of mycelium was also revealed during the observation. The colonies of *Streptomyces* sp. that were 14 days old showed several distinct characteristics. They were white, dry, and turbid, with no exudate present. The colonies had a circular shape and a convex surface that was not translucent. The growth of the colonies was slow, and they had rather thick spores, as seen in Figure 1.

The results of the antimicrobial activity test indicated that the *Streptomyces* sp. isolates exhibited effects against both Gram-positive bacteria (i.e., *Bacillus subtilis* and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (i.e., *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella Typhimurium*). This activity was evidenced by the formation of inhibition zones. The observed activity against the tested bacteria varied in terms of the initial detection time, the diameter of the inhibition zones, the duration of inhibitory activity, and the time required to reach the maximum production of antibiotics.

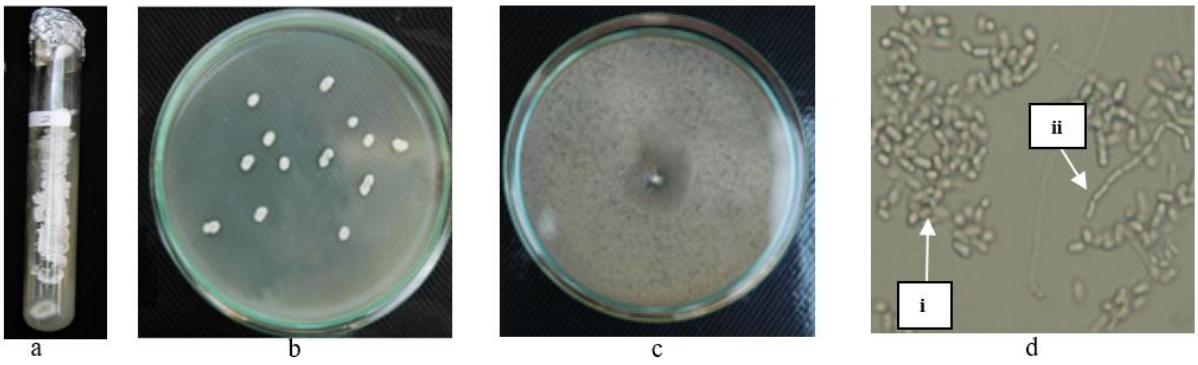

Figure 1. The characteristics of *Streptomyces* sp. through macroscopic and microscopic observations.
Notes: (a, b) Characteristics of *Streptomyces* sp. shown in the macroscopic observation. (c) Screening of the antibacterial activity of *Streptomyces* sp. against *Bacillus subtilis* using diffusion method. (d) Microscopic observation showing the characteristics of *Streptomyces* sp., specifically spore (i) and hyphae (ii).

The *Streptomyces* sp. isolates showed antibacterial activity against *Bacillus subtilis* starting from day 2 until day 7. The peak of the activity was seen on day 7, as evidenced by the largest inhibition zone diameter of 15.9 mm. Between days 8 and 10, a decline in activity was observed, as indicated by the reduced diameter of the inhibition zone. On day 10, the inhibition zone reached its smallest diameter of 11 mm.

In the test against *Staphylococcus aureus* ATCC 25923, the *Streptomyces* sp. isolates showed antibacterial activity, which started on day 2 and reached its peak on the same day. This was evidenced by the observation of the largest inhibition zone diameter at 27.6 mm. There was an apparent decrease in activity from day 3 to day 10, as demonstrated by the reduction in the diameter of the inhibition zone.

The *Streptomyces* sp. isolates demonstrated antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853, with the effect becoming evident on day 2 and reaching its peak on day 7. The presence of the largest inhibition zone diameter at 24.3 mm was indicative of antibacterial activity. On day 8, a decrease in activity was observed, with the inhibition zone diameter reducing to 13.6 mm. Later, on day 9, the activity began to stop.

The *Streptomyces* sp. isolates showed antibacterial activity against *Escherichia coli* ATCC 25922, starting on day 1 and attaining maximum activity on day 5. The largest diameter of the inhibition zone measured was 29.2 mm. However, there was a decline in activity on day 6, as suggested by the decreasing diameter of the inhibition zone. On day 6, an inhibition zone with a diameter of 24.5 mm was observed. The inhibition zone diameter shrank to 21.8 mm on day 7 and 20.7 mm on day 8. By day 9, the inhibitory activity started to stop.

Lastly, the *Streptomyces* sp. isolates demonstrated antibacterial activity against *Salmonella typhimurium*, which was initially observed on day 2. The activity reached its peak on day 7, as indicated by the largest inhibition zone diameter of 27.5 mm. Nevertheless, there was a decrease in activity on day 8, as shown by the diminishing diameter of the inhibition zone. The activity came to a halt on day 10.

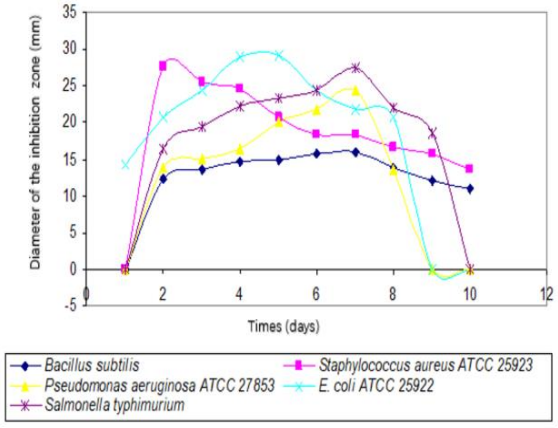

Figure 2. Activity profile of *Streptomyces* sp. according to the inhibition zones.

The antibacterial activity of *Streptomyces* sp. was expressed using inhibition zones, which were outlined throughout this section. Table 1 presents a more comprehensive set of data regarding the inhibition zones. Meanwhile, Figure 2 displays the activity profile of *Streptomyces* sp. in terms of its ability to inhibit the growth of the test bacteria.

Table 1. Inhibition zones from the antibacterial activity test of *Streptomyces* sp. using the modified agar diffusion method.

Groups	Repetition	Diameter of the inhibition zone around the test bacteria (mm)				
		B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*S. Typhimurium*
Positive c.	1st	21.5	14.3	16.3	12.5	11.8
	2nd	20.9	13.5	16.5	11.9	12.9
	3rd	21.8	14.2	15.8	11.6	12.8
	Mean	21.4	14	16.2	12	12.5
Negative c.	1st	0	0	0	0	0
	2nd	0	0	0	0	0
	3rd	0	0	0	0	0
	Mean	0	0	0	0	0
Day 1	1st	0	0	0	13.4	0
	2nd	0	0	0	14.4	0
	3rd	0	0	0	15.1	0
	Mean	0	0	0	14.3	0
Day 2	1st	12.3	27.1	0	21	16.7
	2nd	10.9	27.1	0	20.5	18
	3rd	13.4	28.6	0	20.9	14.8
	Mean	12.2	27.6	0	20.8	16.5
Day 3	1st	13	25.8	13	24.1	19.1
	2nd	13.5	26	13.6	25.2	19
	3rd	14.6	24.7	14.8	23.9	20.1
	Mean	13.7	25.5	13.8	24.4	19.4
Day 4	1st	15.3	25.3	16	28.6	22.3
	2nd	13.9	26	14.7	27.9	22.7
	3rd	14.9	22.8	14.9	30.2	21.9
	Mean	14.7	24.7	15.2	28.9	22.3
Day 5	1st	13.8	19.8	15.9	29.1	21.8
	2nd	15.8	21.1	16.4	20.1	23.8
	3rd	15.1	21.5	16.6	28.4	24.3
	Mean	14.9	20.8	16.3	29.2	23.3
Day 6	1st	16.4	18.5	20.5	23.9	23.9
	2nd	15.8	18	20.1	24.5	25.5
	3rd	15.2	18.4	19.4	25.1	24.1
	Mean	15.8	18.3	20	24.5	24.5
Day 7	1st	14.9	17.6	20.4	23.3	27
	2nd	15.5	19.1	22	21.8	28.3
	3rd	17.3	18.2	23.3	20.3	27.2
	Mean	15.9	18.3	21.9	21.8	27.5
Day 8	1st	13.5	17.7	24	18.8	21.4
	2nd	15	16.1	25.2	22.3	21.6
	3rd	13.2	16.3	23.7	21	23
	Mean	13.9	16.7	24.3	20.7	22
Day 9	1st	10.8	16.5	14.3	0	19.1
	2nd	12.5	14.9	15.5	0	18.3
	3rd	13	16	11	0	18.4
	Mean	12.1	15.8	13.6	0	18.6
Day 10	1st	10.1	14.1	0	0	0
	2nd	11.1	13	0	0	0
	3rd	11.8	13.4	0	0	0
	Mean	11	13.5	0	0	0

DISCUSSION

The findings of this study showed that *Streptomyces* sp. was able to produce antibiotics since day 1 of observation, as evidenced by the presence of a clear zone around *Escherichia coli* ATCC 25922. Furthermore, more antibiotics were produced on day 2, resulting in the inhibition of all the test microbes. Antibiotics produced by the *Streptomyces* sp. isolates apparently had varying inhibitory abilities against different test bacteria. The antibiotics exhibited the highest inhibitory power against *Escherichia coli* ATCC 25922 and the lowest inhibitory power against *Bacillus subtilis*. Regrettably, a gradual decline in antibiotic production was seen over time, which was generally notable on day 8. It even became ineffective in inhibiting the growth of *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 on day 9. Nevertheless, the qualitative test of antibacterial activity revealed that the antibiotic-producing *Streptomyces* sp. isolated from the Surabaya mangrove ecosystem has significant potential. The antibiotic potency of *Streptomyces* sp. was evident from its superior inhibitory power compared to the positive control of streptomycin at 250 ppm against the test bacteria. The considerable antibacterial activity of *Streptomyces* sp. isolates against pathogenic bacteria presents a promising opportunity for the development of a highly effective broad-spectrum antibiotic as an alternative treatment for infectious diseases (Balasubramanian et al. 2021). This recommendation aligns with the findings of previous studies on the antimicrobial activity of *Streptomyces* sp., specifically the AA13 and SA32 strains. *Streptomyces* sp. isolates found in the sediments of Lake Oubeira, Algeria, were found to have an inhibitory effect against *Candida albicans* (Adel et al. 2016, Ryandini et al. 2021).

The bacterial strains used for the experiments in this study were from the Gram-positive and Gram-negative classes. The Gram-positive bacteria were *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis*. Meanwhile, the Gram-negative bacteria were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhimurium*. The selection of the test bacteria was determined by taking into account their strong activity, clinical significance, and classification as Gram-positive and Gram-negative bacteria. These criteria allowed this study to assess the potency of broad-spectrum antibiotics. *Bacillus subtilis* is a bacterium with the shape of a rod and the ability to form spores. *Staphylococcus aureus* is a bacterium characterized by its spherical shape and ability to clinically invade the entire body (Tong et al. 2015).

Escherichia coli is a rod-shaped bacterium that can cause numerous diseases, such as urinary tract infections, pneumonia, meningitis, and septicemia. Similarly, both *Pseudomonas aeruginosa* and *Salmonella typhimurium* have a rod shape and can cause infectious diseases in humans. *Salmonella typhimurium*, in particular, is a major causative agent of gastroenteritis. Although many bacteria have shown resistance to certain antibacterials, there are still several effective options, such as the aminoglycoside group of antibiotics that includes amikacin and gentamicin (Percival & Williams 2014, Moradali et al. 2017).

The antimicrobial activity in this study was tested using the modified agar diffusion method. The diameter of the apparent inhibition zone was recorded, and all tests were performed in triplicate (Kumar et al. 2014, Palla et al. 2018). This method is a more advantageous alternative for the

assessment of the antimicrobial activity of *Streptomyces* sp. in comparison to the dilution method. This is due to the fact that *Streptomyces* sp., being a bacterium that shares resemblance to a fungus, can grow on both bacterial and fungal media. The dilution method is commonly employed to assess the inhibitory effects of the antibiotic produced by *Streptomyces* sp. against the growth of test bacteria. However, it is worth noting that the continuous cell division in *Streptomyces* sp. may result in turbidity, hence limiting the readability of the results obtained using the dilution method. In addition, the diffusion method is more preferable due to its ease of application and cost-effectiveness. The only drawback of this method is associated with the fluctuations in the growth of *Streptomyces* sp. in ISP-4 culture media. The quantity of *Streptomyces* sp. obtained from the mold may change in every experiment, hence complicating the quantitative measurement of the inhibition zone diameter. Consequently, plenty of replications are required to address this issue.

This study used standard streptomycin sulfate, which is classified as an aminoglycoside derivative, to determine the responsiveness of the test bacteria towards the antibiotic and the suitability of these test bacteria for experimentation (Martineza et al. 2014). The initial step in the antibacterial activity test was the fermentation process of the *Streptomyces* sp. antibiotic. In the process of testing antibacterial activity, nutrient agar was used as a non-selective medium to facilitate the growth of the microbes. The thickness of the media, which contained the test microbes, must remain consistent and homogeneous to avoid fluctuations in the diameter of the resulting inhibition zones. The selected test microbes should have a transmittance level of 25% (Fathoni et al. 2021). This particular transmittance level ensures that the number of bacterial cells is appropriate for the test and fits within the logarithmic growth phase. This condition maximizes the sensitivity of the microbes to antibiotics. The test results of this study revealed variations in the antibacterial activities of *Streptomyces* sp. isolates against the test bacteria in terms of their ability to induce inhibition, the activity duration, and the inhibition zone size. These findings suggested that the secondary metabolites produced by the *Streptomyces* sp. isolates had varying degrees of strength against *Bacillus subtilis*, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Salmonella typhimurium*.

Strength and limitations

This study can contribute to the development of antibiotic products by isolating *Streptomyces* sp. from highly polluted environments. By doing so, this study attempted to obtain robust and specific isolates that can survive in challenging environments, produce antibiotics, and offer sources of biodiversity that have not been widely disclosed. However, only macroscopic and microscopic observations were conducted for the identification of *Streptomyces* sp. and the assessment of its antibacterial activity. Further research at the molecular level is necessary to ascertain the presence of *Streptomyces* sp. in the soil of mangrove ecosystems.

CONCLUSION

Streptomyces sp. derived from mangrove ecosystem soil has inhibitory effects against Gram-positive and Gram-negative bacteria. This species produces secondary metabolites that possess significant potential. These metabolites offer promising opportunities to be developed as alternative drugs for infectious diseases by providing enhanced effectiveness as broad-spectrum antibiotics.

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Conflict of interest

None.

Ethical consideration

This study received an ethical exemption from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, with certificate No. 176/EC/KEPK/FKUA/2023 on 10/7/2023.

Funding disclosure

None.

Author contribution

WR contributed to the conception and design, collection and assembly of the data, analysis and interpretation of the data, drafting of the article, and critical revision of important intellectual content. NMM contributed to the conception and design, analysis and interpretation of the data, drafting of the article, and critical revision of important intellectual content. MP and NW contributed to the administrative, technical, and logistic support as well as the drafting of the article and the critical revision of important intellectual content. A contributed to the critical revision of important

intellectual content. SM contributed to the administrative, technical, and logistic support. WM contributed to the drafting of the article and the critical revision of important intellectual content.

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