

Inhibition of Growth and Biofilm Formation of some Multidrug-Resistant Bacteria by *Streptomyces* Secondary Metabolites

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Abstract

Objective: The present study aimed to investigate the antimicrobial properties of actinobacteria isolated from the rhizosphere soil of palm trees, specifically targeting the inhibition of growth and biofilm formation by specific bacterial pathogens.

Methods: About 15 actinobacteria were isolated from Jeddah and Al-Madina Al-Monawara cities using starch nitrate medium with 5 mg/ml nystatin to prevent fungal growth.

Results: Out of 15 isolates, three showed inhibitory effects against *E. coli*, *Staphylococcus aureus*, MRSA, *S. saprophyticus*, *Salmonella typhi*, and *Klebsiella pneumoniae*, using cross-streak and agar well diffusion techniques. The inhibition zones ranged from 12.5 to 25 mm against MRSA, *S. typhi*, *K. pneumoniae*, and *S. saprophyticus*. These three active actinomycetes were identified based on morphological, cultural, physiological, and biochemical characteristics. The 16S rRNA gene sequences revealed that the isolates belong to the genus *Streptomyces* and were molecularly identified as *Streptomyces yangpuensis*, *S. vinaceusdrappus*, and *S. labedae* with accession numbers PV639342, PV639359, and PV639361. They demonstrated excellent antibacterial activity, and the organic extract of *S. vinaceusdrappus* SJ5 was the most effective against almost all tested pathogens. The minimum inhibitory concentration (MIC) ranged from 6.25 to 25 mg/mL and exhibited significant inhibitory effects on bacterial biofilm formation. GC-MS analysis of *S. vinaceusdrappus* extract identified derivatives with known antibiofilm activity, such as 9,12,15-Octadecatrienoic acid methyl ester, Pentaacetate, and hexadecanoic acid methyl ester from the culture supernatant.

Conclusion: Actinomycetes of the genus *Streptomyces* from date palm soil exhibit inhibitory effects on certain resistant bacterial pathogens.

Keywords: Actinobacteria, anti-bacterial agents, biofilms/drug effects, natural products/pharmacology, bacterial infections/microbiology

Introduction

Public health is increasingly at risk from illnesses brought on by pathogens that are resistant to several drugs.¹ The overuse and careless antibiotic consumption are the primary causes of the rise in resistance in these strains.² In addition to medical facilities and healthcare settings, multidrug-resistant bacteria issues are prevalent in other domains such as the environment and food.³ In addition to increasing bacterial growth and persistent infections, multidrug-resistant bacteria's ability to form biofilms shields them from antimicrobials and enhances their resistance to environmental factors and host defenses. Pathogens enhance these traits in a partnership relationship through a signaling mechanism called quorum sensing.^{4,5} The growth of bacterial biofilms in the food processing sector can provide a habitat for harmful microbes and cause a lot of spoilage, which can contaminate food and reduce shelf life.⁶ Biofilm is considered an essential indication of infection and is responsible for antibiotic and disinfectant resistance.⁷ Consequently, infections become more challenging to control, and bacteria become more virulent, which promotes the hunt for novel approaches to treating drug-resistant diseases. Many natural substances are used to control infections and prevent biofilm formation.⁸ The beneficial biological activities of microbial secondary metabolites are attracting a lot of interest, especially when it comes to their positive effects on human health. Soil bacteria are among the microorganisms that exhibit novel biological activity and produce distinctive and innovative secondary metabolites. The organisms that live in soil,

an ecological niche that is widely used, produce secondary metabolites and physiologically active natural substances such as therapeutically required antibiotics. A varied category of filamentous prokaryotes, actinomycetes are typically Gram-positive. Among actinomycetes, *Streptomyces* is still a major source of new bioactive compounds and has long been acknowledged as a rich source of beneficial secondary and continues to be a major source of novel bioactive molecules among actinomycetes.^{9,10} The genus *Streptomyces* generates 75–80% of all commercially and medicinally used antibiotics, and it generates half of the total amount of soil actinomycetes. Peptides, alkaloids, sterols, fatty acids, terpenes, and derivatives of amino acids are examples of secondary metabolites produced by actinobacteria.¹¹

According to earlier research, these secondary metabolites have unique biological properties and may be employed in the creation of antibiotics.¹² This study aims to investigate the antibacterial activity and biofilm inhibition of soil Actinomycetes, which are rich in bioactive compounds that inhibit bacteria, resistant to several drugs.

Methodology

Collection of Soil Samples and Isolation of Actinobacteria

Soil samples were collected from different sites in Jeddah city and Almadinah al-Monawara, Saudi Arabia. The rhizosphere palm tree soil was collected from a 15 cm depth below the soil

surface and transferred to the lab in sterilized polythene bags. These soil samples were air-dried for 3 days at 45°C, crushed, and these samples were used for the isolation of Actinomycetes by using serial dilution method and the spread plate technique.¹³ These dilutions were spread on starch nitrate agar containing nystatin (30 mg/ml) and incubated at 28°C for 6–7 days.^{14,15}

Morphological, Physiological, and Biochemical Observation

The potential isolate was observed for aerial spore, mycelia color, spore chain morphology, and other microscopic characters. The taxonomic identification of actinomycetes was based on Bergey's Manual.^{16,17} The different biochemical tests, like hydrolysis of starch, casein, lipid, and cellulose, were performed.

Molecular Identification and Phylogenetic Analysis

Finally, the strain was identified by 16S RNA sequencing. Gene Universal primers, 27F (5-AGAGTTTGATCCTGGCTCAG-3) and 1525R (5-AAAGGAGGTGATCCAGCC-3) were used for the PCR amplification of 16S rDNA of three isolates. Sequencing of the PCR amplicons of the isolates was done by Macrogen, Inc (Seoul, Korea). The 16S rRNA sequence data were compared with available sequence data in the GenBank at the National Center of Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program. The isolates were identified based on the sequence similarity to their closest homolog.¹⁸

Antimicrobial Activity and Screening of Actinobacteria

Primary and secondary screening are the two stages of the isolation screening process. In primary screening, the antibacterial activity of pure isolates was determined by the cross-streak method.¹⁹ Following secondary metabolite extraction, the isolate was subjected to secondary screening using the agar well diffusion technique.

Primary Screening

All the tested bacterial pathogens were obtained from KAUH, Saudi Arabia. The antibiotic sensitivity of isolated actinobacteria was assayed by the cross-streak method. After inoculating the actinobacteria at the center of the plates, the test organisms *Staphylococcus aureus* (MRSA) (ATCC 25923), *E. coli* (ATCC 25922), *Salmonella typhi* (ATCC 13311), *Klebsiella pneumoniae*, and *S. saprophyticus* were streaked perpendicular to the isolate. The plates were incubated at 37°C for 24 hrs. Three replicates were included in all experiments, and the mean value was calculated and compared. Based on the presence and absence of a zone of inhibition, the actinobacteria were selected that produce an antimicrobial compound. After that, the agar well diffusion test was used to determine the extracted filtrate's antibacterial activity against the chosen microorganisms.^{20,21}

Extraction of Bioactive Compound

The most active actinomycete isolates, named SM2.1, SJ5, and SJ7 were grown in starch nitrate broth medium at 120 rpm and 28°C for eight days. After growth, the culture broth was

centrifuged at 5,000 rpm for 20 minutes at 4°C. The active materials were extracted using a separating funnel and ethyl acetate, and the top layer was collected, dried, and the antibacterial activity was measured using different bacterial pathogens.^{22,23}

Secondary Screening using Agar Well Diffusion Method

The filtrate obtained from the isolated actinomycetes was used to test antimicrobial activity. The overnight cultures like *S. typhi* (ATCC 13311), *S. aureus* (MRSA) (ATCC 33591), *K. pneumoniae*, and *S. saprophyticus* were used as test organisms. The test organisms were spread uniformly on an agar plate, and wells were bored in the agar surface. Three wells of 6 mm diameter were made on the agar plate with the help of sterile cork borers, and 100 µl of crude extract was poured into the wells. Then the wells were filled with the culture filtrate. Then, plates were incubated at 37°C for 24 to 48 hrs.²⁴

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentrations (MICs) of the crude extracts against four bacterial strains were determined. Bacterial cultures were grown overnight in nutrient broth medium. The crude extracts were dissolved in DMSO to a concentration of 100 mg/ml, and 100 µl was added to the first well of a sterile 96-well microtitre plate containing 100 µl of Muller-Hinton broth. One hundred microliters of appropriately adjusted bacterial cultures were added to each well. The bacteria were subjected to extract concentrations of 50, 25, 12.5, 6.5, 3.25, and 1.625 mg/ml. The plates were incubated at 37°C for 24 h. The MIC value was recorded as the lowest concentration of the crude compounds that inhibited the visible growth of the test organisms.²⁵

Antibiofilm Activity using Spectrophotometric Assay

Prevention of biofilm formation was detected by the method described by Plyuta et al.²⁶ Fresh culture of strong biofilm producer selected bacteria was used to prepare bacterial suspension in TSBG broth and adjusted to 0.5 Mac Farland as described before. Individual wells of sterile 96-well flat-bottom tissue culture plates were filled with 100 µL of the diluted cultures and 100 µL of the extract, then the plates were incubated at 37°C for 24 hrs. After incubation, the wells' liquid contents were aspirated, and the formed biofilm was washed and stained with 0.1% CV. The biofilm inhibition was calculated after comparing to the control (without bacterial extract).

Assessment of Antibiofilm Activity of Bacterial Extract using Light Microscopy

Light microscopic assessment of the antibiofilm activity for the selected tested materials against bacterial isolates was accomplished by following the method described by Musthafa et al.²⁷ with some modifications. Overnight grown colonies of the tested bacterial isolate (MRSA) were added to a freshly prepared TSB with 1% glucose and incubated at 37°C for 24 hrs. A total of 500 µL of inoculated broth of MRSA was treated with 500 µL of filter-sterilized SJ5 extract. About 50 µL of the content was gently removed and spread on a clean glass

slide in a sterile petri dish which was incubated for 24 hrs at 37°C. The slide was washed, and the formed biofilm was stained with 0.1% crystal violet and examined using a Light microscope and compared to a control without actinomycete extract.

Identification of Potential Bioactive Compounds by GC-MS

Identification of the chemical compounds present in the crude extract was carried out by GC-MS. The oven temperature was programmed from 50 to 300°C at specific rates. The components of the extracts were identified based on the comparison of their GC relative retention time and mass spectra with those from the Wiley and NISTT (National Institute of Standards and Technology) libraries.

Results

Isolation, Morphological, and Molecular Characterization of Actinobacterial Isolates

In the current study, 15 actinobacteria were isolated from soil samples from Jeddah and Al-Madinah al-Monawara, Saudi Arabia. Actinobacteria were distributed differently depending on the soil's texture and cultivation level; rhizosphere soil had the highest concentration of them. After that, the isolates were inoculated onto SNA medium to purify them. The pure strains were categorized according to their morphology, which includes spore color, aerial and substrate mycelium, and colony morphology and color, which ranged from light grey, pale pink, beige, and yellowish (Table 1, Figure 1), in addition to

Table 1. Isolation of different actinomycetes from soil and their morphological characters

Site of isolation	Isolate code	Growth	Gram stain	Color of aerial mycelium	Color of substrate mycelium	Soluble pigment	Antagonistic activity
							<i>E. coli</i>
Jeddah city	SM1	Heavy	+	Pale Green	Brown	+	–
	SM2.1	Heavy	+	Beige	Pale yellow	–	+
	SM3	Heavy	+	Pale yellow	Yellow	–	–
	SM4	Heavy	+	Pale pink	Brown	+	+
	SM5	Heavy	+	Grey	Beige	–	–
	SM6	Heavy	+	Pale green	Beige	–	–
Al-Madinah al-Munawarah city	SJ1	Heavy	+	Grey	Brown	–	–
	SJ2	Heavy	+	Grey	Beige	–	–
	SJ3	Heavy	+	Green	Beige	–	–
	SJ4	Heavy	+	Pale yellow	Beige	+	+
	SJ5	Heavy	+	Pale grey	Brown	+	+
	SJ6	Heavy	+	Grey	Cream	–	–
	SJ7	Heavy	+	Grey	Green	+	+
	SJ8	Heavy	+	Beige	Beige	–	+
	SJ9	Heavy	+	Pale yellow	Golden yellow	+	+

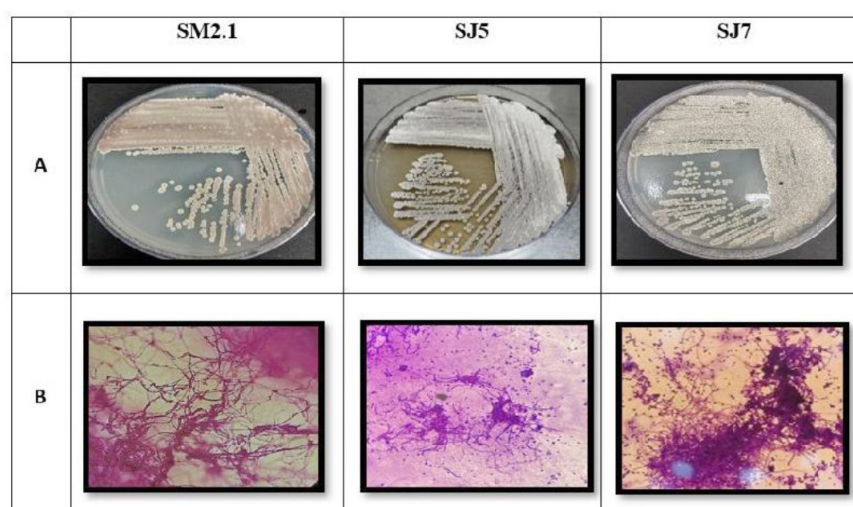


Fig. 1 (A): Morphological characterization of actinobacterial isolates on starch casein agar plates. (B): Gram stain of the selected actinomycete isolates examined under light microscope.

the microscopic appearance of colonies after simple staining. All isolates were Gram-positive filamentous bacteria with high growth on starch nitrate medium. The molecular identification of isolates by amplification of the 16S-rRNA gene was done for isolates SM2.1, SJ5, and SJ7, the most active isolates against some bacterial pathogens, using universal primers 27F' and 1492R'. In 16S-rRNA sequencing, alignment of the nucleotide sequences of strain SM2.1, SJ5, and SJ7 exhibited a similarity of 99.99%, 100% and 100 % with closely related *Streptomyces yangpuensis*, *Streptomyces vinaceusdrappus*, and *Streptomyces labedae*, respectively (Table 2). The phylogenetic relatedness of strains with their closely related species obtained by the neighbor-joining method is shown (Figure 2).

The biochemical results of bacterial isolates were summarized in Table 3 and Figure 3. Antibiotic susceptibility pattern of the tested bacterial isolates to some antibiotics was summarized in Figure 2A. The isolate SJ5 was sensitive

to the antibiotics Gentamicin (GM) and Neomycin (NE), while it was resistant to Bacitracin (BA), Chloramphenicol (C), Penicillin G (PG), and Polymyxin B (PB). The results showed the effect of different temperatures, initial medium pH values, and NaCl concentrations on the growth of the three selected actinobacteria. Minimum growth of the three tested isolates was obtained at 15°C, while maximum growth was recorded between 25 and 40°C. Unexpectedly, isolates SJ5 and SJ7 demonstrated robust growth at 50°C, suggesting potential applications in high-temperature environments. All isolates showed excellent growth at pH 7–9, while isolates

Table 2. Molecular identification (based on 16S rRNA amplification) of the three actinobacterial strains isolated from soil

Code of isolate	Organism	GenBank accession no.	Similarity %	Query cover
SM2.1	<i>Streptomyces yangpuensis</i>	PV639342	99.90%	100%
SJ5	<i>Streptomyces vinaceusdrappus</i>	PV639361	100%	100%
SJ7	<i>Streptomyces labedae</i>	PV639359	100%	99.29%

Table 3. Biochemical characteristics of the three actinobacterial isolates

Character	Isolated actinomycetes		
	SM2.1	SJ5	SJ7
Temperature (°C)	20–40	15–50	50–20
pH	5–9	5–9	9–6
NaCl concentration (%)	0.5–10	0.5–5.0	0.5–10
Enzyme production			
Protease	+	+	+
Cellulase	–	+	+
Amylase	+	+	+
Catalase	–	+	+

+: Production; -: No production.

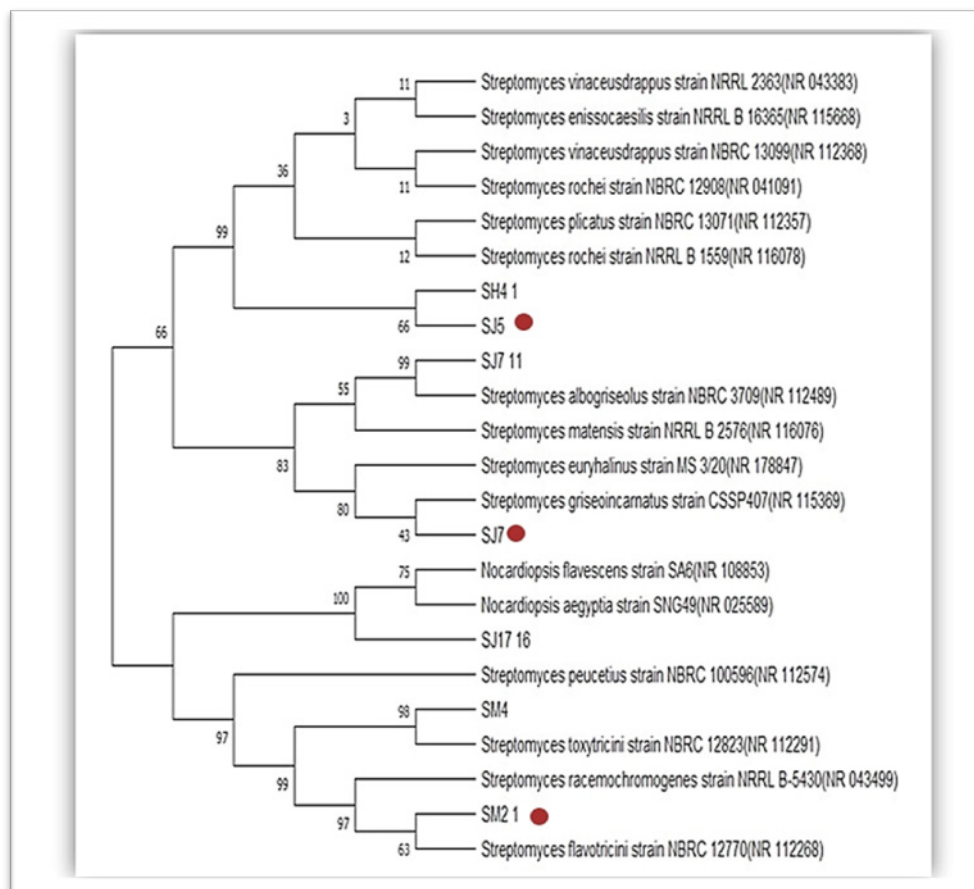


Fig. 2 Phylogenetic tree indicating the taxonomic position of the three isolates of genus *Streptomyces*.

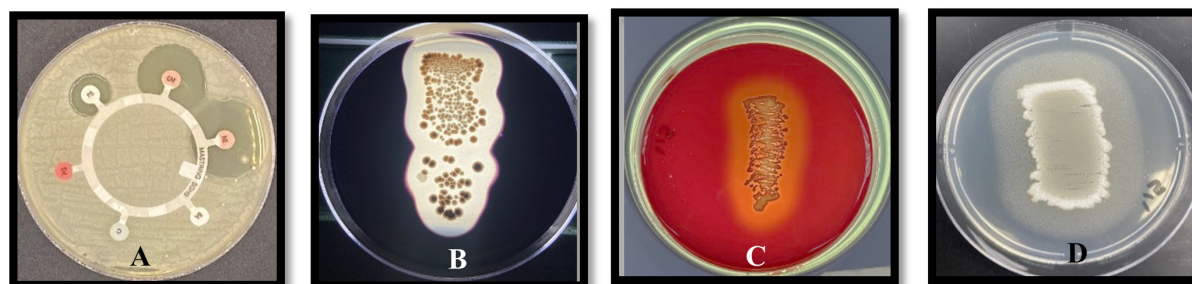


Fig. 3 Biochemical characteristics of bacterial isolate SJ5. (A): Antibiotic susceptibility pattern to some antibiotics, enzyme production by the isolate SJ5, (B): Amylase activity, (C): Cellulase activity, (D): Protease activity.

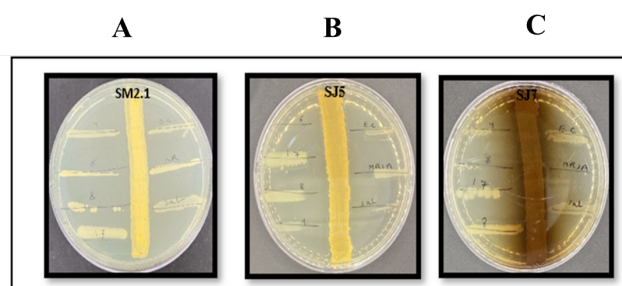


Fig. 4 Primary screening by cross streak method (A): SM2.1 extract, (B): SJ5 extract (c): SJ7 extract.

Extract	Tested bacteria			
	MRSA	<i>S.typhi</i>	<i>K.pneumoniae</i>	<i>S.saprophyticus</i>
SM2.1				
SJ5				
SJ7				

Fig. 5 Antimicrobial activity of actinobacterial SM2.1, SJ5, and SJ7 extracts against pathogenic test organisms.

SM2.1 and SJ5 showed good growth at pH 5. Furthermore, the results showed that isolates SM2.1, SJ5, and SJ7 exhibited good amylase activity, and all isolates were positive for protease. Additionally, two isolates, SJ5 and SJ7, were positive for Cellulase and Catalase activity.

Antibacterial Activity of the Three Selected Actinobacterial Isolates

Out of 15 pure actinomycete isolates, 3 isolates were selected, identified, and then subjected to primary screening. They

showed good antimicrobial activity against the test bacterial pathogens during primary screening by the perpendicular streaking method (Figure 4).

As shown in Figure 5, it was noticed that the three isolates had good antibacterial activity against all the tested bacteria. The filtrate from isolate SM2.1 inhibited the growth of MRSA with an inhibition zone of $(25 \pm 1.5 \text{ mm})$ and showed inhibition zone against *K. pneumoniae* and *S. saprophyticus* $(15.0 \pm 0.1 \text{ mm})$. The crude extract recorded a higher inhibition zone against MRSA with an inhibition zone of $24 \pm 0.12 \text{ mm}$.

The filtrate from isolate SJ5 showed the highest antagonistic activity against *S. typhi* (23.0 ± 0.5 mm) as recorded in Figure 5, but MRSA showed an inhibition zone of 22 ± 0.2 mm. The lowest inhibition zone was against *K. pneumoniae* with an inhibition zone of 17 ± 0.2 mm. The crude extract recorded a higher inhibition zone against *K. pneumoniae* with inhibition zones of (26 ± 1.5 mm) and showed a similar inhibition zone was recorded against MRSA and *S. typhi* with inhibition zones of (25 ± 1.5 mm).

However, the filtrate from isolate SJ7 was active against some pathogens with an inhibition zone of (20 ± 0.5 mm) against *S. saprophyticus* (Figure 5). The crude extract showed a higher inhibition zone against MRSA with an inhibition zone of (16 ± 0.5 mm). Figure 6 summarizes the antimicrobial activity of the filtrate and the extracts of the three *Streptomyces* isolates detected using the agar well diffusion method against some selected bacterial pathogens.

In the present research study, Table 4 showed the Minimum Inhibitory Concentrations (MICs) for the three selected extracts against the tested bacterial isolates. The inhibition of growth was determined at OD 600 nm. In

control samples, the growth was high and ranged from 0.5–0.7, while the addition of SJ5 extract decreased the growth, and this was clear in the case of using the extract against the selected bacterial pathogens. The MIC was recorded for the three extracts against the four bacterial pathogens. They ranged from 12.5–50 mg/ml. The highest effect was by SJ5 and SJ7 extract against *S. saprophyticus* and MRSA (6.25 mg/ml), while the lowest effect was by using isolate SM2.1 and isolate SJ7 (50 mg/ml) against MRSA, *S. typhi*, and *K. pneumoniae*.

Figure 7 detects the biofilm inhibitory activity of the selected extract of the actinobacterial isolate SJ5. The results of antibiofilm of the tested SJ5 extract after 24 hrs showed that a clear inhibition of the formed biofilms as compared to the control on using the selected bacteria. The lowest percentage was obtained by using the SJ5 extract against MRSA after 24 hrs. Light microscopic assessment of the tested bacterial biofilms of MRSA before and after treatment by SJ5 extract is shown in Figure 8. In the case of control, the bacterial cells covered the slide and formed a strong biofilm, which is very compacted, while in the treated case, the biofilm is thin. In the present results, it was observed that the SJ5 extract showed the highest inhibitory effect on the biofilms for MRSA bacteria.

Composition of the Extract Detected by GC-MS

The GC-MS analysis results indicated the detection of several organic compounds in *S. vinaceusdrappus* SJ5 extract. The summary of the compounds detected is presented in Table 5, while the spectra of the compounds are presented in Figure 9. The most potent compound, which showed antibacterial activity, might be 9, 12, 15 Octadecatrienoic acid, with a Retention time = 28.81 min. Figure 10 summarizes

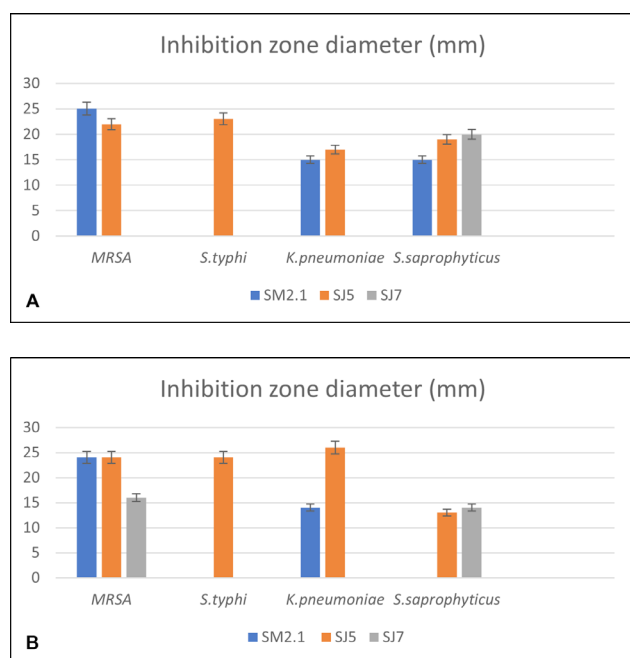


Fig. 6 Antimicrobial activity of three *Streptomyces* isolates by well diffusion method against selected pathogens, A: Actinobacteria filtrate B: Actinobacteria ethyl acetate extract.

Table 4. Minimum inhibitory concentration (MIC, mg/ml) of actinobacterial extract against some pathogenic bacteria

Extract	Minimum inhibition concentration (mg/ml)			
	Test pathogens			
	MRSA ATCC 33591	<i>S. typhi</i> ATCC 13311	<i>K. pneumoniae</i>	<i>S. saprophyticus</i>
SJ5 (control)	25	6.25	25	6.25
SM2.1	50*	ND	50*	12.5*
SJ7	6.2*	ND	ND	50*

ND: not detected. *: Significant results compared to control at $P \leq 0.05$.

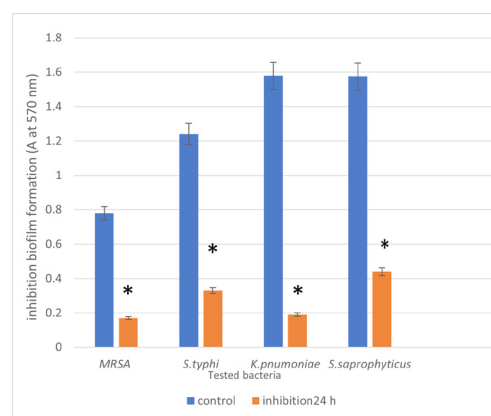


Fig. 7 Biofilm inhibition after 24 hours by SJ5 extract against some selected bacterial pathogens. *: significant results at $P \leq 0.05$.

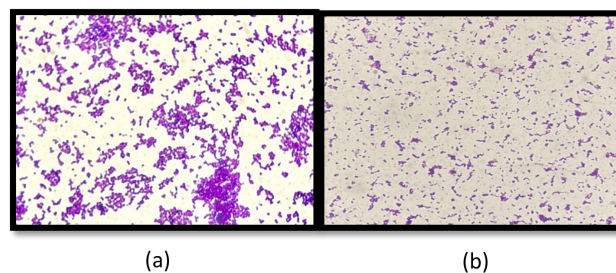


Fig. 8 Antibiofilm activity of crude extract of isolate SJ5 (a) Control MRSA cells (x 1000); (b) Treated MRSA cells by SJ5 extract (x 1000).

Table 5. Volatile organic compounds detected by GC-MS analysis for ethyl acetate extract of *S. vinaceusdrappus* SJ5

No.	Compound name	Retention time (min) Rt	Molecular Formula	MW
1	Methyl 5,7-hexadecadiynoate	18.04	C ₁₇ H ₂₆ O ₂	262
2	Pentadecanoic acid, 14-Methyl-, Methyl ester	25.76	C ₁₇ H ₃₄ O ₂	270
3	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl) methyl]cyclopropyl] methyl]cyclopropyl] methyl]-, methyl ester	25.87	C ₂₅ H ₄₂ O ₂	374
4	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	28.81	C ₁₈ H ₃₀ O ₂	278
5	Methyl-9,9,10,10-D4-Octadeca Noate	29.40	C ₁₉ H ₃₄ D ₄ O ₂	302
6	1,2-Benzenedicarboxylic acid	35.80	C ₂₄ H ₃₈ O ₄	390
7	Arabinitol, Pentaacetate	39.03	C ₁₅ H ₂₂ O ₁₀	362
8	9,12-Octadecadienoic acid (Z,Z)-, 2,3-Bis[(Trimethylsilyl)Oxy] Propyl ester	39.96	C ₂₇ H ₅₄ O ₄ Si ₂	498

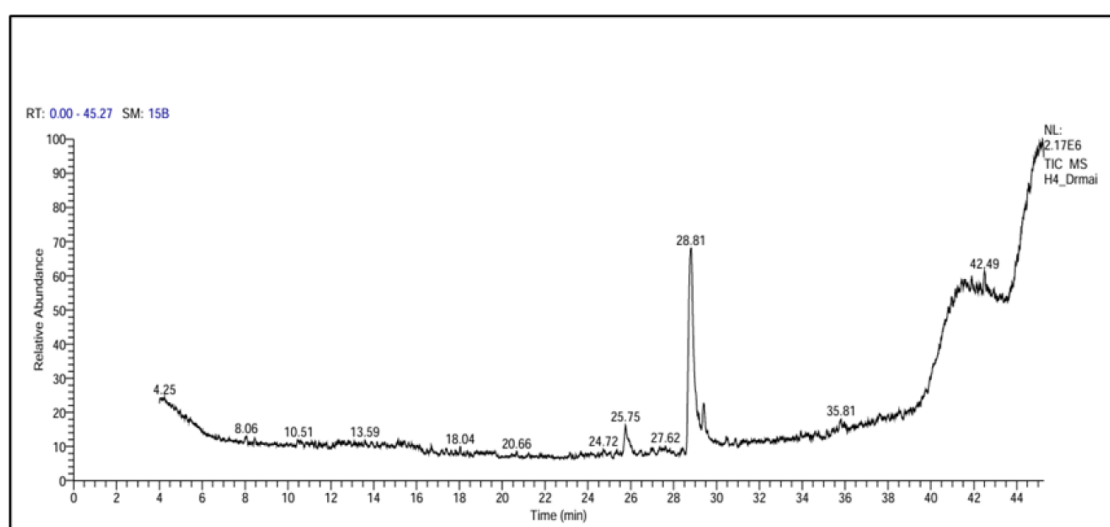
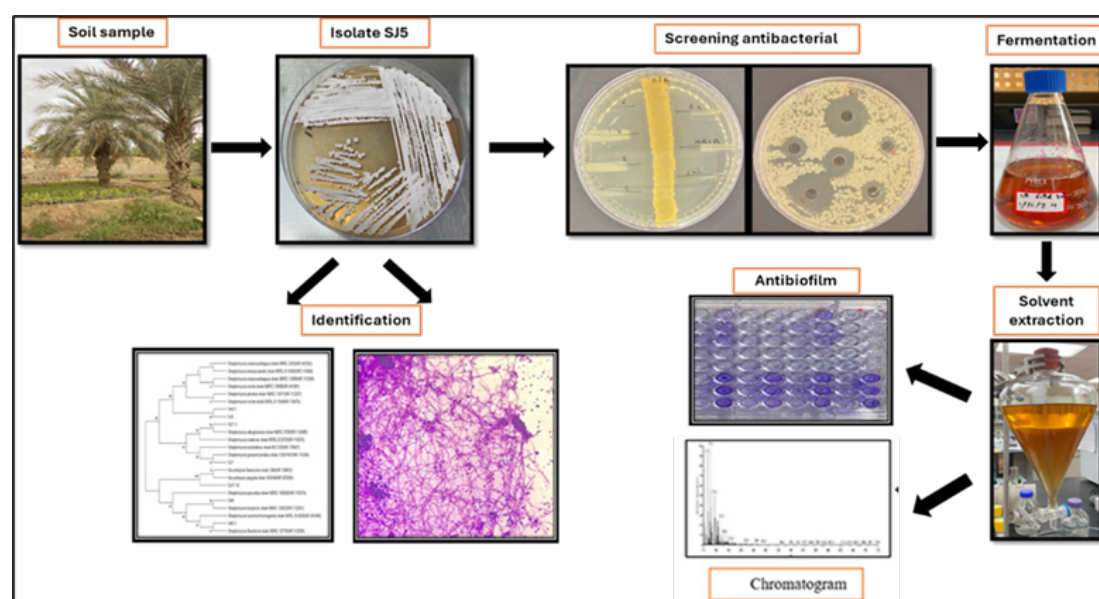
Fig. 9 Compounds detected by GC-MS analysis for ethyl acetate extract of *S. vinaceusdrappus* SJ5.

Fig. 10 The different steps used for the isolation and identification of actinobacteria, and the extraction and identification of the bioactive secondary metabolites that inhibit the bacterial pathogen growth and biofilm formation.

the different steps used for the isolation and identification of actinobacteria, and the extraction and identification of their bioactive secondary metabolites.

Discussion and Conclusions

Actinomycetes, especially the genus *Streptomyces*, continue to be one of the highest economic and biotechnological benefits, producing most of the antibiotics used in medicine. The rhizosphere of Saudi palm trees is a unique ecological niche because it contains organic materials and nutrients from root exudates, thus it provides an ideal environment for microbial development.²⁸ Common in rhizosphere soils, actinobacteria can produce a wide range of beneficial secondary metabolites and compounds with various characteristics. Furthermore, according to Junaid et al.,²⁹ these bacteria also play an important role in biocontrol against pathogens by producing antimicrobial metabolites.³⁰ The genus *Streptomyces* can be distinguished from other bacterial groups by a broad range of stable phenotypic features such as morphology, physiology, and biochemical traits. Molecular analysis of selected isolates, 16S rRNA gene, confirms their initial identification. The genomic DNA of these isolates was extracted using the method described by Tang et al.³¹ The isolate SJ5 was identified as a new isolate belonging to *Streptomyces vinaceusdrappus* SJ5 and is associated with the rhizosphere soil of the date palm tree. *Streptomyces vinaceusdrappus* is known for producing various bioactive compounds, including the antibiotic amicitin and different secondary metabolites, active against many bacterial pathogens.³² The 16S rDNA gene sequence of isolate SJ7 showed close relation to *Streptomyces labedae*, aligning with results from.³³ According to Marinkovic et al.,³⁴ microdilution and crystal violet tests were used in several studies to evaluate and confirm the antibacterial and antibiofilm properties of various extracts. Our results showed that isolate SJ5 had remarkable activity against harmful bacteria. Isolate SJ5 exhibited the highest activity against methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Salmonella typhi* ATCC 13311, and *Klebsiella pneumoniae*. The lowest concentration of extract still inhibiting growth was 6.25%, observed with isolates SJ5 against MRSA ATCC 33591 and *S. aureus*. Actinomycete secondary metabolites have been targeted because of their ability to produce new compounds for treating pathogens.³⁵

The inhibition was indicated by a clear zone around the well in the diffusion test. These findings agree with previous reports by Li et al.³⁶ Strains such as *S. albogriseolus* and *S. yangpuensi*, which share high sequence homology with our isolates, demonstrated various biological activities, including antibacterial effects, supporting our results.³⁷ Extracts from actinomycetes showed strong antibacterial activity against both Gram-positive and Gram-negative bacteria,³⁸ with inhibitory zones ranging from 15 to 27 mm, consistent with El-Sayed et al.³⁹ This study highlights the potential of Actinomycetes isolated from the rhizosphere soil of the Date palm tree, an important tree with numerous benefits. After initial screening for antibacterial activity, strains SM2.1, SJ5, and SJ7 were active, with SJ5 showing the broadest spectrum in subsequent tests. Sub-MIC doses of *S. vinaceusdrappus* effectively reduced bacterial biofilms by over 50%. Microscopic analyses provided insights into structural changes induced by SJ5 on biofilm formation. GC-MS analysis of the methanol extract from *S. vinaceusdrappus* identified major bioactive compounds, including 9, 12, 15-octadecatrienoic acid methyl ester, Pentaacetate, and hexadecanoic acid methyl ester. These were classified as organic and fatty acids, along with derivatives such as organic alcohols, amino acids, and esters, most of which act as antibacterial and antifungal agents.⁴⁰ Fatty acids disrupt bacterial cell membranes by solubilizing the membrane due to their detergent properties.⁴¹ Fatty acid activity may be either antimicrobial, at high doses, or antibiofilm, at low doses, with potential synergistic effects.⁴² Notably, Pentaacetate and Benzenedicarboxylic acid showed strong antibacterial activity.⁴³ Arabinitol and Pentaacetate also demonstrated significant antibacterial effects,⁴⁴ while hexadecanoic acid methyl ester showed the highest antimicrobial activity against clinical pathogens.⁴⁵ This study confirms that extracts from *Streptomyces* isolates have promising activity against bacteria that form biofilms, showing good antimicrobial potential. Further research aims to purify and characterize these crude extracts, which may lead to the economical production of bioactive compounds for pharmaceutical use, especially *Streptomyces* for treating bacterial infections, cancer, and more diseases.

Conflict of Interest

None. ■

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