



Antimicrobial Potential of Rhizosphere-Associated Actinomycetes Isolated from *Salvia rosmarinus* and *Mentha spicata*

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ABSTRACT

Background and Objective: Rhizosphere-associated actinomycetes are promising sources of microorganisms with antimicrobial potential. This study aimed to isolate actinomycete from the rhizospheres of rosemary (*Salvia rosmarinus*) and spearmint (*Mentha spicata*) to and evaluate their antagonistic activity against bacterial and fungal pathogens.

Materials and Methods: Rhizosphere soil samples were collected from rosemary and spearmint, and actinomycetes were isolated using culture-based methods involving sample pretreatment, serial dilution, and cultivation on selective media. All isolates were screened for antagonistic activity using the cross-streak method on International *Streptomyces* Project medium 2 (ISP2) and tryptic soy agar (TSA). The target microorganisms included *Escherichia coli*, *Pseudomonas aeruginosa*, methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecalis*, *Listeria monocytogenes*, and *Candida albicans*. Nine isolates were selected for further investigation. The isolates were cultivated in tryptic soy broth, and cell-free culture supernatants (CFS) were prepared by centrifugation and membrane filtration. The antimicrobial activity of CFS was evaluated by agar-well diffusion method. Selected isolates were subjected to 16S rRNA gene sequencing for molecular identification.

Results and Conclusion: Among 36 isolates screened by the cross-streak method, 15 exhibited antimicrobial activity, with inhibition profiles varying according to the isolate, test organism, and culture medium. RR1 showed the broadest antagonistic activity in this assay. In the agar-well diffusion assay, the CFS of RR1 inhibited all seven test organisms, with inhibition zones of 12–19 mm, whereas MR4 produced 20-mm inhibition zones against both MSSA and MRSA but showed no detectable inhibition of the other test organisms. The activity profiles differed between the two assays for some isolates. Based on 16S rRNA gene sequence comparisons, RR1, RR13, and RR29 showed 100% sequence identity with *Streptomyces amrissarensis*, while MR4 showed 100% identity with *Streptomyces antibioticus*. These results identify RR1 as a promising candidate for further investigation of broad-spectrum antimicrobial activity and MR4 for studies of anti-staphylococcal activity. However, further characterization, including minimum inhibitory concentration determination and chemical analysis of active compounds, is required to establish the potency and nature of their antimicrobial activity.

What is “already known”:	<ul style="list-style-type: none"> • Rhizosphere-associated <i>Streptomyces</i> spp. represent promising sources of antimicrobial compounds. • Antimicrobial activity depends on isolates and target microorganisms, highlighting the importance of screening strains against a diverse panel of pathogens.
What this article adds:	<ul style="list-style-type: none"> • Antimicrobial activity of actinomycetes isolated from the rhizospheres of rosemary (<i>Salvia rosmarinus</i>) and spearmint (<i>Mentha spicata</i>). • Molecular identification revealed selected isolates were related to <i>Streptomyces amrirsarensis</i> and <i>Streptomyces antibioticus</i>. • Isolates show distinct antimicrobial profiles: broad inhibitory activity by RR1 & high activity of MR4 against methicillin-susceptible and methicillin-resistant <i>S. aureus</i>.

1. INTRODUCTION

Antimicrobial resistance is a major global health concern that increasingly compromises the treatment of bacterial infections and underscores the need to discover new antimicrobial agents. The World Health Organization has also identified multiple drug-resistant bacterial pathogens as priorities for research and development of new treatment options [1]. In this regard, natural products remain important sources of bioactive compounds, and actinomycetes are particularly valuable because of their extensive capacity to produce structurally diverse secondary metabolites [2,3]. Although *Streptomyces* has been extensively investigated, recent research continues to identify new compounds and biosynthetic potential within this genus, supporting further exploration of strains from under investigated environments [4-6].

The rhizosphere represents a dynamic ecological interface where plant roots shape microbial community composition and activity through the release of root exudates and other rhizodeposits. These compounds serve as sources of nutrients and chemical signals that influence microbial recruitment, colonization, and interactions within the surrounding soil [7]. Such interactions contribute to the ecological selection and functional diversity of rhizosphere microorganisms, including actinobacteria, many of which are recognized for their capacity to produce diverse bioactive secondary metabolites [2,3,8]. Research on medicinal-plant-associated microbial communities has further highlighted the diversity of rhizosphere actinobacteria and their potential antimicrobial and other biologically relevant activities [9,10].

Actinomycetes, particularly members of the genus *Streptomyces*, are recognized as important producers of bioactive secondary metabolites with diverse antimicrobial activities. Previous studies have demonstrated that compounds obtained from actinomycetes can inhibit clinically important bacterial pathogens, including antimicrobial-resistant strains, highlighting their potential as sources of new antimicrobial leads [4-6].

Rosemary (*Salvia rosmarinus*) and spearmint (*Mentha spicata*) are aromatic plants of medicinal and biological interest. Investigating actinomycetes associated with these plants may therefore provide an independent route to identifying microbial isolates with promising antimicrobial activity. Accordingly, this study aimed to isolate and screen rhizosphere-associated actinomycetes from rosemary and spearmint against selected bacterial and fungal pathogens. By integrating complementary antimicrobial screening methods with molecular identification, this study sought to identify promising isolates for subsequent investigation of their antimicrobial potential.

2. MATERIALS AND METHODS

2.1. Plant Material and Rhizosphere Sampling

Rhizosphere soil samples were collected from multiple rosemary (*Salvia rosmarinus*) plants (36.593-69350° N, 52.10078201° E) and multiple spearmint (*Mentha spicata*) plants (36.59369350° N, 52.10078-201° E) in Noor, Mazandaran, Iran (2026). For each plant species, a composite rhizosphere soil sample was prepared from the sampled plants. Samples were aseptically collected in sterile containers, protected from direct sunlight during transportation to the laboratory, and processed with minimal delay.



2.2. Isolation of Actinomycetes

Loosely attached soil was removed from the roots, and approximately 2 g of root-associated soil was collected aseptically. Samples were heat-treated at 55°C for 6 min. Subsequently, 1 g of soil was suspended in 10 mL of sterile water and shaken at 100 rpm for 30 min. Serial dilutions were prepared in sterile saline to 10^{-5} , and 100 μ L of each dilution was spread onto International *Streptomyces* Project medium 2 (ISP2) and starch-casein agar (SCA). Four plates were prepared for each medium. The plates were incubated at 28°C for up to four weeks. Colonies displaying actinomycete-like morphology were selected and purified [11].

2.3. Test Microorganisms

The cultures of reference microorganisms were obtained from Microorganisms Bank of Iranian Biological Resource Center (IBRC) and Persian Type Culture Collection (PTCC). Antimicrobial activity was evaluated against *Escherichia coli* (ATCC 25922= IBRC-M 11018), *Pseudomonas aeruginosa* (ATCC 27853= IBRC-M 10832), methicillin-susceptible *Staphylococcus aureus* (MSSA; ATCC 25923= PTCC 1431), methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC 33591= IBRC-M 10690), *Enterococcus faecalis* (ATCC 29212= IBRC-M 10740), *Listeria monocytogenes* (PTCC 1163), and *Candida albicans* (ATCC 10231= IBRC-M 30070).

2.4. Cross-Streak Antagonism Assay

Antagonistic activity was assessed using the cross-streak method on ISP2 agar and tryptic soy agar (TSA) media. Each actinomycete isolate was streaked along the center of an agar plate and incubated at 30°C for 5 days. Fresh cultures of the target microorganisms were then streaked perpendicular to the actinomycete growth without direct contact, followed by incubation for 24 h. Each assay was performed in triplicate. Percentage inhibition was calculated as the length of the growth-inhibition zone along the target streak divided by the total length of the target streak, multiplied by 100 [12]. Control plates containing the target microorganisms without an actinomycete isolate were used to assess normal growth in the absence of the producer isolate.

2.5. Liquid Cultivation and Preparation of Cell-Free Supernatants

A 1 × 1 cm section of fresh actinomycete growth on ISP2 agar was transferred to a 100-mL Erlenmeyer flask containing 20 mL of tryptic soy broth (TSB). Cultures were incubated at 28°C for 72 h with shaking at 150 rpm. Liquid cultivation was performed in two independent replicates. The cultures were centrifuged at $10,000 \times g$ for 10 min at 10°C, and the supernatants were filtered through 0.22- μ m cellulose acetate membranes to obtain cell-free supernatants (CFSs) for antimicrobial testing.

2.6. Agar-Well Diffusion Assay

The antimicrobial activity of cell-free culture supernatants was evaluated using an agar-well diffusion assay adapted from CLSI Mo2 [13], with wells replacing antibiotic disks. Target microbial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard. Mueller–Hinton agar (MHA) was used for bacterial targets, whereas MHA supplemented with 2% glucose was used for *Candida albicans*. Wells 5 mm in diameter were aseptically punched into the agar, and the agar surface was uniformly inoculated with the standardized microbial suspensions using sterile swabs. The bottom of each well was sealed with 30 μ L of sterile molten 1% agar, followed by the addition of 75 μ L of CFS. Sterile uninoculated culture medium served as the negative control, while commercial antibiotic disks served as positive controls. Plates were incubated at 37°C for 24 h, and inhibition-zone diameters were measured. Each CFS obtained from the two independent liquid-culture replicates was tested in triplicate.

2.7. Molecular Identification

Four isolates exhibiting promising antimicrobial activity were selected for molecular identification based on 16S rRNA gene sequencing. Genomic DNA was extracted using a salting-out method [14]. The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R [15]. PCR amplification was performed with an initial denaturation at 95°C for 5 min, followed by denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s. A final extension was performed at 72°C for 5 min. The amplification products were analyzed by electro-

phoresis on a 1% agarose gel and were electrophoresed at 100 V for 50 min. The amplified 16S rRNA gene products were purified and subjected to Sanger sequencing using primers 27F and 1492R by Pishgam Biotechnology (Iran). Forward and reverse reads were assembled and edited using ChromasPro 2.1.3. The resulting sequences were compared with reference sequences in the NCBI nucleotide database using BLAST to identify the closest sequence matches. Phylogenetic analysis was performed using the neighbor-joining method in MEGA 11. Evolutionary distances were calculated using the Tamura three-parameter model, and branch support was assessed by bootstrap analysis with 1,000 replicates.

3. RESULTS AND DISCUSSION

3.1. Recovery of Rhizosphere Actinomycetes

A total of 36 actinomycete-like isolates were recovered from the rhizospheres of rosemary and spearmint. Rosemary yielded 30 isolates, whereas six isolates were obtained from spearmint. Previous studies have demonstrated that the rhizospheres of medicinal and aromatic plants can serve as sources of antimicrobial actinomycetes [11,16,17]. Elshafie and Camele (2022) isolated rhizospheric actinomycetes from rosemary (*S. rosmarinus*) and other plant species and identified selected isolates as *Streptomyces atratus*, *Streptomyces* sp., and *Arthrobacter humicola* [18]. The selected strains exhibited biological activities that included antifungal potential and plant-growth-promoting effects, highlighting the value of medicinal-plant rhizospheres as sources of functionally diverse actinomycetes. In a related study, Alinejad and Khodakaramian (2024) isolated *Streptomyces* sp. 3Z from the rhizosphere of *Mentha longifolia* and reported antibacterial activity against both Gram-positive and Gram-negative bacteria [19]. More recently, culture-independent profiling of the rhizosphere microbiota of *Mentha spicata* and other *Mentha* taxa detected diverse bacterial communities that included members of the phylum Actinomycetota [20]. Although the latter study did not focus on the

isolation of individual actinomycete strains, it provides complementary evidence for the presence of this bacterial group in *Mentha* rhizospheres. Collectively, these studies provide a relevant ecological context for investigating rhizosphere-derived actinomycetes from rosemary and spearmint and evaluating their antimicrobial potential. The present study extends this line of investigation by examining culturable isolates from the rhizospheres of these two aromatic plants and assessing their activity against a panel of bacterial and fungal test organisms.

3.2. Antagonistic Activity in the Cross-Streak Assay

Of the 36 isolates recovered, 15 exhibited antagonistic activity in the cross-streak assay (Fig 1 and Fig 2). Among the isolates, RR1 exhibited the broadest inhibitory profile, showing activity against all seven targets on both ISP2 and TSA media. Its highest inhibition values (100%) were recorded against MSSA and MRSA on ISP2, whereas inhibition against the same targets on TSA was 83% and 80%, respectively. MR4 also showed strong activity against both *S. aureus* phenotypes, with 100% inhibition of MSSA and MRSA on ISP2 and 83% inhibition of each on TSA. MR4 additionally showed 100% inhibition of *E. faecalis* and 75% inhibition of *L. monocytogenes* on ISP2.

The inhibitory profiles of other isolates varied according to the target microorganism and culture medium. RR12 showed the highest inhibition of *E. coli* (80%) on ISP2, whereas RR29 showed the highest inhibition of *P. aeruginosa* (67%) on ISP2. On TSA, RR29 completely inhibited the growth of both MSSA and MRSA (100% for each). RR13 exhibited activity against all seven targets on TSA, with inhibition values ranging from 20% to 80%; its strongest activity was observed against MSSA (80%) and MRSA (75%). RR26 showed moderate activity against several targets, including MSSA and MRSA on TSA (60% for each) and *L. monocytogenes* (53%). In contrast, some isolates exhibited narrower inhibitory profiles. For example, RR22 showed activity only against *P. aeruginosa* on ISP2 (40%), while RR25 showed activity only against *C. albicans* on ISP2 (36%).





Fig 1. Heatmap of antimicrobial activity of actinomycete isolates against the test microorganisms in ISP2 and TSA media. Colors represent the mean percentage of growth inhibition determined by the cross-streak assay. Darker colors indicate higher inhibition. Values within cells represent mean inhibition percentages. Blank cells indicate no detectable inhibition under the assay conditions. MS *S. aureus*, methicillin-susceptible *S. aureus*; MR *S. aureus*, methicillin-resistant *S. aureus*.

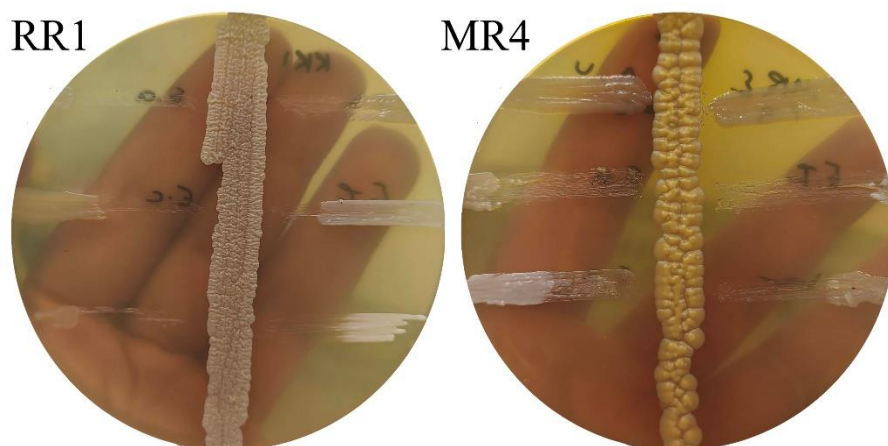


Fig 2. Representative images of the cross-streak assay illustrating the antimicrobial activity of actinomycete isolates RR1 and MR4 against the test microorganisms on TSA.

The differences between ISP2 and TSA are noteworthy. For example, RR12 inhibited *E. coli* by 80% on ISP2 but showed no measurable inhibition of this organism on TSA. Conversely, RR29 completely inhibited MSSA and MRSA on TSA, whereas inhibition of these targets on ISP2 was only 20% for each. Such differences suggest that the expression of antagonistic activity depended on the culture conditions [21]. Differences in nutrient availability may affect isolate growth and the production, accumulation, or diffusion of inhibitory substances. Nevertheless, because metabolite production and biomass were not independently quantified in this experiment, the precise basis of the medium-dependent responses cannot be established from the inhibition percentages alone.

3.3. Agar-Well Diffusion

The agar-well diffusion assay detected antimicrobial activity in the CFS of seven of the nine selected isolates (Table 1). RR1 exhibited the broadest activity, inhibiting all seven test microorganisms, with inhibition zones ranging from 12 to 19 mm. The largest zone was observed against MSSA (19 mm), followed by *C. albicans* (18 mm) and *L. monocytogenes* (16 mm). MR4 produced the largest inhibition zones in the assay, measuring 20 mm against both MSSA and MRSA, but showed no detectable inhibition of the other five targets. RR13 and RR26 each inhibited six of the seven

Table 1. Antimicrobial activity of cell-free culture supernatants from selected actinomycete isolates determined by agar-well diffusion.

Isolate	<i>E. coli</i>	<i>P. aeruginosa</i>	MSSA	MRSA	<i>E. faecalis</i>	<i>L. monocytogenes</i>	<i>C. albicans</i>
RR1	15 ± 1	12 ± 2	19 ± 2	15 ± 3	14 ± 1	16 ± 2	18 ± 1
RR6	13 ± 1	-	17 ± 1	10 ± 1	-	-	-
RR12	-	-	12 ± 2	8 ± 0	16 ± 2	-	-
RR13	11 ± 1	-	13 ± 2	11 ± 1	16 ± 2	13 ± 2	12 ± 2
RR15	-	-	-	-	-	-	-
RR22	-	-	-	-	-	-	-
RR26	13 ± 2	10 ± 2	14 ± 1	14 ± 0	-	13 ± 2	13 ± 1
RR29	9 ± 1	-	10 ± 1	10 ± 1	-	-	-
MR4	-	-	20 ± 2	20 ± 0	-	-	-

Values are inhibition-zone diameters in mm; Dashes indicate no measurable zone recorded. MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.

microorganisms. RR13 was active against *E. coli*, MSSA, MRSA, *E. faecalis*, *L. monocytogenes*, and *C. albicans*, with zones of 11–16 mm, whereas RR26 inhibited *E. coli*, *P. aeruginosa*, both *S. aureus* phenotypes, *L. monocytogenes*, and *C. albicans*, with zones of 10–14 mm. More restricted activity was observed for RR6, RR12, and RR29. RR6 inhibited *E. coli*, MSSA, and MRSA, producing zones of 13, 17, and 10 mm, respectively. RR12 inhibited MSSA (12 mm), MRSA (8 mm), and *E. faecalis* (16 mm), while RR29 produced smaller zones against *E. coli* (9 mm), MSSA (10 mm), and MRSA (10 mm). No detectable inhibition was observed with the CFS of RR15 or RR22 against any of the tested microorganisms.

The CFS results broadly supported the cross-streak findings for some isolates, particularly RR1, whose activity extended across both Gram-positive and Gram-negative bacteria and the fungal target. However, the two assays did not produce identical inhibition profiles for all isolates. For example, RR12 inhibited *E. coli* by 80% in the cross-streak assay on ISP2, yet its CFS showed no detectable inhibition of this organism in the agar-well diffusion assay. Such discrepancies are not unexpected because cross-streak

assays evaluate antagonism in the presence of a growing producer strain, whereas CFS-based assays assess the inhibitory activity of extracellular material recovered under specific cultivation conditions. Differences in compound production, concentration, stability, and diffusion through agar may therefore contribute to variation between the assays.

The absence of detectable inhibition by RR15 and RR22 should consequently be interpreted in relation to the assay conditions rather than as definitive evidence that these isolates lack antimicrobial biosynthetic potential. Conversely, the presence of inhibition zones does not identify the active compounds or establish their mechanisms of action.

3.4. Molecular Characterization of Isolates

The 16S rRNA gene sequences of the four selected isolates (RR1, RR13, RR29, and MR4) were assembled from forward and reverse sequencing reads, yielding sequences of 1,388, 1,463, 1,463, and 1,494 nucleotides, respectively. The accession numbers and BLAST results are summarized in Table 2.

Table 2. 16S rRNA gene sequence similarity of the identified actinomycete isolates to their closest reference sequences in GenBank.

Strain (Acc. No.)	Closest reference matches (Acc. No.)	Coverage (%)	Identity (%)
RR1, RR13, RR29 (QB063951-3)	<i>Streptomyces amritsarensis</i> 2A (NR_126204)	100	100
	<i>Streptomyces yangpuensis</i> Fd2-tb (NR_148776)	100	99.93
	<i>Streptomyces flavotricini</i> NBRC 12770 (NR_112268)	100	99.86
	<i>Streptomyces antibioticus</i> JCM 4620 (NR_043348)	100	100
MR4 (QB063950)	<i>Streptomyces griseoruber</i> CSSP408 (NR_115370)	100	99.28
	<i>Streptomyces cyaneochromogenes</i> MK-45 (NR_170501)	100	98.99



Comparison with sequences available in the NCBI database revealed that RR1, RR13, and RR29 shared 100% sequence identity with *Streptomyces amritsarensis*, whereas MR4 shared 100% identity with *Streptomyces antibioticus*. The Neighbor-Joining phylogenetic analysis further supported the placement of all four isolates within the genus *Streptomyces* (Figure 3). Nevertheless, species-level assignments should be considered provisional because closely related *Streptomyces* species may share highly similar or identical 16S rRNA gene sequences. Recent genome-based studies have highlighted discrepancies between 16S rRNA-based clustering and genome-based taxonomy, emphasizing the limitations of this marker for

definitive species delineation [22,23]. Further confirmation could be obtained through genome-based comparisons, including average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH). The close 16S rRNA gene sequence similarity of RR1, RR13, and RR29 to *S. amritsarensis* is noteworthy in light of previous reports of antimicrobial activity in strains assigned to this species. Sharma et al. (2014) characterized a novel lipopeptide from *S. amritsarensis* with activity against several Gram-positive bacteria, including *Bacillus subtilis*, *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, and methicillin-resistant *S. aureus* [24].

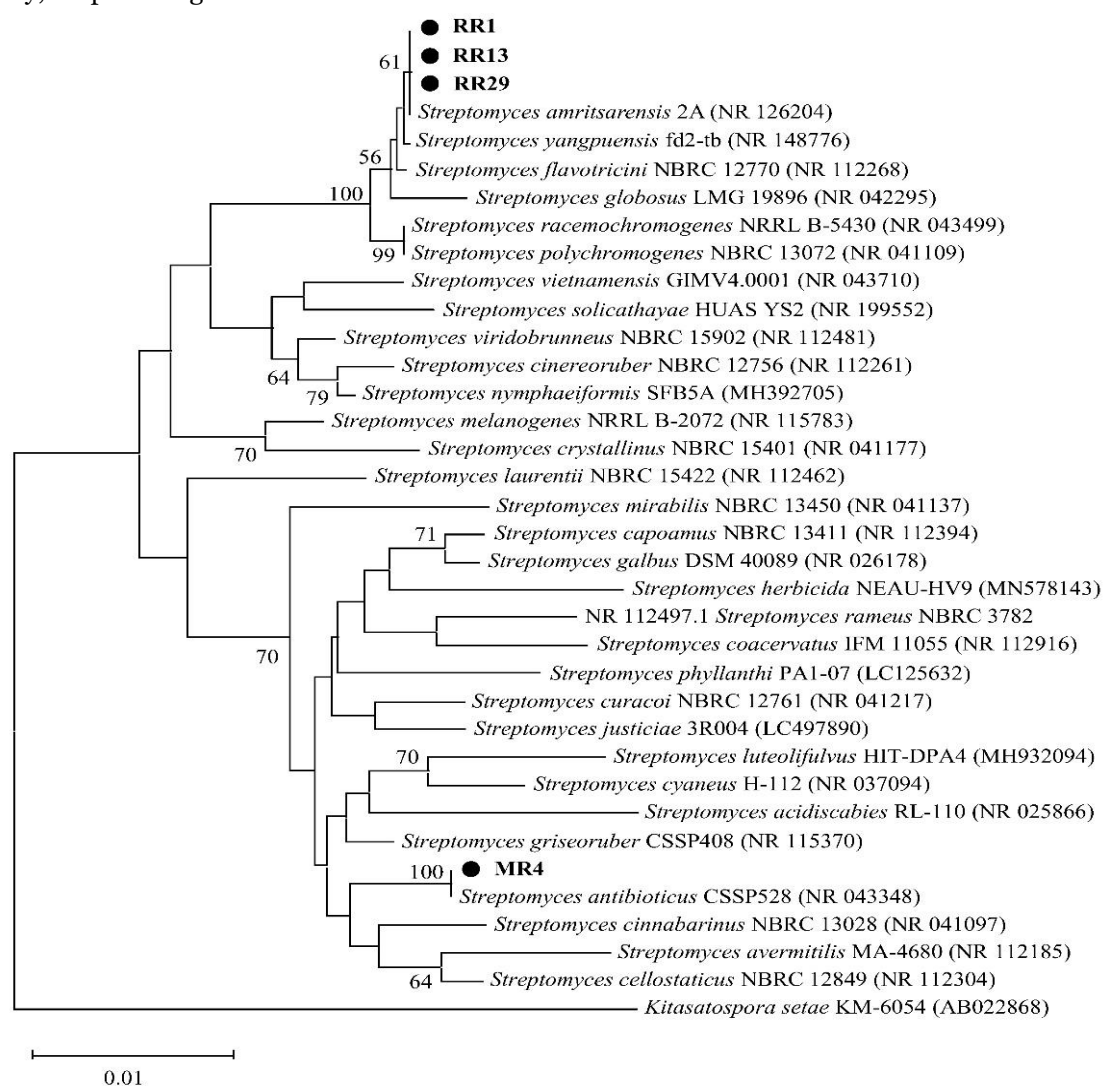


Fig 3. Phylogenetic tree reconstructed using the Neighbor-Joining method based on 16S rRNA gene sequences, showing the phylogenetic positions of isolates RR1, RR13, RR29, and MR4 among closely related species of the genus *Streptomyces*. Bootstrap values based on 1,000 replicates are shown at branch nodes when greater than 50%. Branch lengths are proportional to the evolutionary distances calculated using the Tamura three-parameter model. The analysis included 1,383 positions in the final dataset. The scale bar represents 0.01 nucleotide substitutions per site.

In a separate study, Sharma et al. reported broad-spectrum antimicrobial activity associated with the species, including activity against Gram-positive and Gram-negative bacteria and fungi [25]. Other investigations have also reported anti-fungal metabolites and additional bioactive products from particular *S. amritsarensis* strains [26-28]. These findings provide a rationale for further investigating the antimicrobial potential of RR1, RR13, and RR29, particularly given their observed activity against staphylococci. Similarly, several bioactive secondary metabolites have been reported from strains identified as *S. antibioticus*, the closest 16S rRNA gene sequence match for MR4. These include oleandomycin and related derivatives, as well as phenazine compounds reported from particular strains [29-32]. Geiger et al. (1988), for example, characterized phenazines produced by strain TŮ 2706, including (–)-saphenamycin and saphenic acid, which exhibited antibacterial activity [29]. Chlorothricin has also been investigated in *S. antibioticus* DSM 40725, with activity reported against selected Gram-positive bacteria but not against *P. aeruginosa* or *C. albicans* under the tested conditions [33]. Collectively, these studies illustrate the diversity of bioactive metabolites associated with this species and the variability of their antimicrobial spectra. The pronounced inhibition of MSSA and MRSA by MR4 is therefore of interest; however, it does not demonstrate that this isolate produces the previously reported compound.

The antimicrobial profiles observed in this study also highlight differences among the selected isolates. RR1 showed the broadest activity in the cell-free supernatant assay, whereas MR4 exhibited a more selective profile, with inhibition zones of 20 mm against both MSSA and MRSA. These findings identify RR1 as a promising candidate for further investigation of broad-spectrum antimicrobial activity and MR4 as a candidate for more focused studies of anti-staphylococcal activity. Nevertheless, inhibition-zone measurements represent preliminary screening outcomes and should not be interpreted as standardized susceptibility results or evidence of clinical efficacy. Differences in strain-level biosynthetic capacity, cultivation conditions, metabolite concentration, stability, and diffusion may all contribute to the observed activity profiles.

4. CONCLUSION

This study highlights the antimicrobial potential of rhizosphere-derived actinomycetes associated with rosemary (*S. rosmarinus*) and spearmint (*M. spicata*). By combining cross-streak screening on two culture media with agar-well diffusion assays of cell-free culture supernatants, the study provides complementary evidence of antimicrobial activity and demonstrates that the observed inhibitory profiles vary among isolates, target microorganisms, and cultivation conditions. Notably, the isolates whose 16S rRNA gene sequences were most closely related to *S. amritsarensis* and *S. antibioticus* exhibited promising inhibitory activities, supporting further investigation of these strains as potential sources of antimicrobial compounds.

Future studies should focus on optimizing culture conditions to enhance antimicrobial activity, characterizing the active compounds through bioassay-guided fractionation and analytical techniques such as LC–MS/MS, and determining their mechanisms of action. Assessment of minimum inhibitory and bactericidal or fungicidal concentrations, cytotoxicity toward relevant mammalian cells, and activity against a broader collection of clinical isolates would help establish the potency and selectivity of the most promising candidates. Whole-genome sequencing and genome mining could also reveal biosynthetic gene clusters associated with secondary-metabolite production and clarify the biosynthetic potential of the selected strains. Overall, this study provides a foundation for the systematic characterization of rhizosphere-associated *Streptomyces* and the future exploration of their antimicrobial potential.

5. DECLARATIONS

5.1. Acknowledgements

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5.2. Conflict of Interest

The authors declare no conflicts of interest.



5.3. Authors Contributions

MK: Methodology, Data acquisition, Analysis, and interpretation, Resources, Writing—original draft preparation; SN: Study design, Methodology; NA: Conceptualization, Study design, Project administration, Writing Review & Editing.

5.4. Using Artificial Intelligent Chatbots

AI-assisted tools were used solely for translation and language editing of the manuscript. The authors reviewed and revised the text and take full responsibility for the accuracy and integrity of the manuscript.

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