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Research Article

Antimicrobial Activities and Metabolites Profiling of Actinobacteria from Rhizosphere Soil of *Erythrina variegata* from Mount Merapi National Park, Yogyakarta, Indonesia

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ABSTRACT

Actinobacteria are well known as a source of bioactive secondary metabolites, particularly antibacterial and antifungal agents. The increasing number of drug-resistant *Mycobacterium tuberculosis* strains has made tuberculosis more difficult to control and treat, highlighting the need to discover new antimycobacterial agents from natural sources, such as actinobacteria. In this study, a total of 17 actinobacteria isolates were collected from the rhizosphere soil of *Erythrina variegata*. Molecular identification based on 16S rRNA gene sequences showed that the majority of isolates (70.59 %) were identified as members of the genus *Streptomyces*. The antimycobacterial activities of crude extracts from these isolates were evaluated against drug-sensitive and drug-resistant strains of *Mycobacterium smegmatis* using the Resazurin Microplate Assay (REMA). Ten extracts exhibited activity against the drug-sensitive strain (WT-M), with five of them (MRS 8, MRS 11, MRS 15, MRS 16, and MRS 17) showing potent inhibition (>85 %). These five extracts also exhibited activity against drug-resistant strains, including rifampicin-resistant (RIF^R-M), isoniazid-resistant (INH^R-M), and multidrug-resistant (MDR-M) strains. Moreover, MRS 11 showed the strongest antibacterial and antifungal activities, inhibiting *Bacillus subtilis* (71.63 %), *Colletotrichum gloeosporioides* (63.51 %), and *Fusarium oxysporum* (44.12 %). Isolate MRS 11 was identified as *Streptomyces triticiradicis* NEAU -HE with 99.4 % similarity. Gas Chromatography–Mass Spectrometry (GC-MS) analysis of MRS 11 extract identified 18 compounds, including 1-nonadecene, E-15-heptadecenal, and hexadecanoic acid derivatives, which have been reported to exhibit antimicrobial activity. Future studies are required to include compound purification, structural elucidation, bioactivity testing, and genome-based biosynthetic gene cluster analysis.

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INTRODUCTION

Actinobacteria are Gram-positive bacteria with a high guanine and cytosine (G+C) content and are found in various terrestrial and aquatic environments (Barka et al. 2016). They are well known for producing a broad spectrum of bioactive compounds, including antibacterial and antifungal agents (Nair & Abraham 2020). Actinobacteria produce more than 55 % of antibiotics, and the majority of these antibiotics are derived from the genus *Streptomyces* (Nair & Abraham 2020; Ngamcharungchit et al. 2023). This biosynthetic potential is associated with the presence of various biosynthetic gene clusters (BGCs) involved in secondary metabolite production (Hu et al. 2018; Nair & Abraham 2020). *Streptomyces* species encode an average of approximately 30 BGCs per genome, highlighting their potential as prolific producers of bioactive secondary metabolites (Y. Lee et al. 2024).

Soil ecosystems, particularly the rhizosphere, harbour diverse actinobacterial strains with unique metabolic capabilities. The rhizosphere is the soil region around plant roots and is enriched with root exudates that play an essential role in regulating microbial diversity and activity (Zhalnina et al. 2018). These exudates provide a variety of compounds, including amino acids, organic acids, and secondary metabolites, which support microbial colonisation and influence both the function and composition of the soil microbial (Mavrodi et al. 2021; Hiremath et al. 2024). *Erythrina variegata*, commonly known as *dadap ayam* in the Indonesian vernacular language, is a plant species known for its rich phytochemical composition (Herlina et al. 2011).

Erythrina variegata contains alkaloids, flavonoids, and triterpenoids that are recognised as bioactive compounds. This plant has been reported to exhibit various biological activities such as antimicrobial, antioxidant, anti-inflammatory, and antidiabetic effects (Mohapatra et al. 2023; Fankam & Kuete 2024; Nguyen et al. 2024). Additionally, *E. variegata* has been reported to exhibit insecticidal (Baranitharan et al. 2019), anticancer (Vaishali Rai et al. 2017), and antimalarial activities (Herlina et al. 2011). Studies on actinobacteria from the rhizosphere soil of *E. variegata* may contribute to the discovery of novel antimicrobial agents due to their potential to produce bioactive compounds and their close association with rhizosphere environments.

Mount Merapi National Park (Yogyakarta, Indonesia) was selected as the research site because it is one of the most active volcanoes in Indonesia. It is characterized by unique volcanic soil that may influence microbial diversity and secondary metabolite production. Volcanic eruptions have also been reported to affect vegetation structure, tree stands, carbon reserves, and soil nutrients in Mount Merapi National Park (Utami et al. 2023; Khan et al. 2024). Therefore, investigating actinobacteria from the rhizosphere of *E. variegata* in Mount Merapi National Park may lead to the discovery of unique strains of actinobacteria with novel biosynthetic potential. Additionally, no studies have reported the actinobacteria from the rhizosphere of *E. variegata* in this location, particularly in the Pakem Turi Resort area of Mount Merapi National Park.

According to the Global Tuberculosis Report 2024, an estimated 10.7 million new TB cases and approximately 1.23 million deaths were reported worldwide (WHO 2025). Eight countries account for 67 % of worldwide cases; among these, Indonesia ranks second after India, contributing approximately 10 % of the global TB burden (WHO 2025). Drug-resistant TB, particularly multidrug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB), remains a significant challenge in TB control. Globally, an estimated 390,000 MDR-TB/RR-TB cases have been reported, while in Indonesia, the number of MDR-TB/RR-TB cases is estimated to reach 9,573 cases (Ministry of Health of the Republic of Indonesia 2025; WHO 2025). These conditions underscore the need for the discovery of new anti-TB agents that

are effective against drug-resistant TB strains.

This study focused on the isolation of actinobacteria from the rhizosphere soil of *E. variegata* in Pakem Turi Resort of Mount Merapi National Park, Yogyakarta, Indonesia. The antimicrobial activities of the isolates were evaluated to screen for promising candidates against pathogenic microorganisms, particularly drug-resistant *M. smegmatis*. In addition, the secondary metabolites produced by these actinobacteria were analysed to identify potential bioactive compounds. Therefore, this study aimed to evaluate the antimicrobial activity of actinobacteria isolated from the rhizosphere of *E. variegata* and to profile their secondary metabolites.

MATERIALS AND METHODS

Materials

Rhizosphere soil samples were collected from *E. variegata* in Pakem Turi Resort, Mount Merapi National Park, Yogyakarta, Indonesia. Media used for microbial studies included humic-vitamin agar (HVA), Middlebrook 7H9 and 7H10 (MB7H9 and MB7H10), Mueller-Hinton broth (MHB), Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Yeast-Starch Agar (YSA), and Yeast Starch Broth (YSB). Antimycobacterial activity was evaluated using *M. smegmatis* mc2155 (WT-M), rifampicin-resistant *M. smegmatis* (RIF^R-M), isoniazid-resistant *M. smegmatis* (INH^R-M), and multidrug-resistant *M. smegmatis* (MDR-M) strains. The antimicrobial spectrum of potential isolates was assessed against *Bacillus subtilis* (InaCC B1), *Escherichia coli* (InaCC B5), and *Staphylococcus aureus* (InaCC B4). Antifungal activity was tested against *Colletotrichum gloeosporioides* (InaCC F304) and *Fusarium oxysporum* (InaCC F817). Antimycobacterial activity was evaluated using a resazurin-based assay (REMA), with fluorescence measured using a Varioskan LUX multimode microplate reader (Thermo Fisher, USA). Actinobacterial isolates were identified based on 16S rRNA gene sequences analysis, with the gene amplified by polymerase chain reaction (PCR). The secondary metabolite profiles of the potential isolates were analysed using gas chromatography-mass spectrometry (GC-MS).

Methods

Culture conditions of microbial strains

The bacterial isolates tested (*B. subtilis*, *E. coli*, and *S. aureus)* were cultured on NA medium and incubated at 37 °C for 24 hours. *M.smegmatis* strains were grown on MB7H10 agar medium (Sigma-Aldrich) containing 0.5 % glycerol and 0.05 % Tween 80, and incubated at 37 °C for 24 hours (Arthur et al. 2019). For antifungal assays, *C. gloeosporioides* and *F. oxysporum* were cultured on PDA medium and incubated at 28 °C for 5 days.

Isolation and molecular identification of actinobacteria

Soil samples were collected from the rhizosphere of *Erythrina variegata* in Pakem Turi Resort of Mount Merapi National Park, Yogyakarta, Indonesia. The samples were placed in sterile plastic bags and air-dried at room temperature for 5 days. Actinobacteria were isolated using the SDS-Yeast Extract (SDS-YE) method as described by Hayakawa and Nonomura (1989). An aliquot of 100-150 µL of each diluted sample was spread onto HVA agar medium, which was supplemented with 50 µg L^{-1} cycloheximide and 20 µg L^{-1} nalidixic acid (Hayakawa & Nonomura 1987). The plates were incubated at 30 °C for 7–14 days. Colonies of actinomycetes were selected, purified on YSA medium, and incubated at 30 °C for 14 days. Pure isolates were preserved in 10 % glycerol at -80 °C.

Identification of actinobacterial isolates was performed based on 16S rRNA gene sequences. Genomic DNA was extracted using the phenol-

chloroform method following Putri and Sumerta (2020). The 16S rRNA gene sequences were amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Putri & Sumerta 2020). The 16S rRNA gene sequences of the isolates were analysed by comparison with sequences in public databases using the EzBioCloud platform (Yoon et al. 2017). Multiple sequence alignment of closely related species was conducted using CLUSTAL X, followed by phylogenetic tree construction using the neighbour-joining method (Saitou & Nei 1987) with 1,000 bootstrap replicates implemented in MEGA 11 software (Tamura et al. 2021).

Extraction of actinobacteria metabolites

Actinobacterial isolates were cultured in Yeast Starch Broth (YSB) medium in 300 mL Erlenmeyer flasks and incubated at 30 °C with shaking at 180 rpm for 14 days. After incubation, ethyl acetate was added to the culture at a 1:1 (v/v) ratio and shaken for 1 hour (Nurkanto et al. 2024). The ethyl acetate layer was evaporated using a rotary evaporator. These extracts were dissolved in dimethyl sulfoxide (DMSO) (Merck) and used for further analysis.

Antimycobacterial activity of actinobacteria isolates

The antimycobacterial activity was determined using the resazurin reduction assay (Nurkanto et al. 2024). The antimycobacterial activity of the actinobacterial extracts was tested against *M. smegmatis* mc^2155 strains, including WT-M, RIF^R-M, INH^R-M, and MDR-M. Each strain was cultured overnight in MB7H9 broth and then adjusted to a final concentration of 10^6–10^8 CFU mL^{-1}. A total of 2 µL of crude extract (concentration of 250 µg mL^{-1}) was added to 48 µL of bacterial suspension and incubated for 24 hours. The positive control contained two µL of DMSO and 48 µL of bacterial suspension, while the negative control contained two µL of DMSO and 48 µL of sterile test medium (M7H9). Bacterial growth was assessed using resazurin staining, and fluorescence was measured at 530 nm excitation and 590 nm emission using a Varioscan Lux fluorometer (Thermo Fisher, USA) (Nurkanto et al. 2024).

Antimicrobial spectrum activity of actinobacteria isolates

The antimicrobial spectrum of the potential isolates was assessed through bioassays against Gram-negative and Gram-positive bacteria as well as fungi. The antibacterial assay of the actinobacterial extracts was performed against *B. subtilis*, *E. coli*, and *S. aureus*. For antibacterial activity, the resazurin reduction assay was used, following the previously described method (Nurkanto et al. 2024). The antagonistic activity of actinobacterial isolates against *C. gloeosporioides* and *F. oxysporum* was evaluated using the dual culture agar method. Actinobacterial isolates were inoculated onto PDA plates at a distance of 2.5 cm from the fungal mycelium disc. The plates were then incubated at 28 °C for seven days, after which the inhibition zones were measured. The percentage of fungal colony growth inhibition was calculated using the formula Dc-Da/Dc x 100, where Dc is the diameter of fungal growth in the control group, and Da represents the diameter of fungal growth in the presence of the actinobacteria (Ghazala et al. 2022). The experiment was conducted in triplicate.

Profiling of antimicrobial compounds

The potential isolate was cultured in YSB medium for 14 days and incubated at 30 °C in a shaking incubator at 180 rpm for 14 days. The culture broth was extracted with ethyl acetate at a 1:1 (v/v) ratio and shaken overnight at 120 rpm. The ethyl acetate phase was separated and concentrated using a rotary evaporator. Volatile compounds from the ethyl acetate extract were analysed

using GC-MS (GCMS-QP 2010 Ultra, Shimadzu, Japan). Compounds of secondary metabolites were identified based on mass spectral comparison with the National Institute of Standards and Technology (NIST) database (Version 11) (Masrukhin et al. 2021).

RESULTS AND DISCUSSION

Isolation and identification of actinobacteria from the rhizosphere soil of *Erythrina variegata*

Seventeen actinobacterial isolates were obtained from the rhizosphere soil of *E. variegata* and identified based on 16S rRNA gene analysis. These isolates exhibited a diversity of colony colours. Among them, only two isolates produced soluble pigments. Isolate MRS 2 produced brown pigment, whereas isolate MRS 12 produced yellow pigment. Based on 16S rRNA gene analysis revealed that these isolates were assigned to the genera *Streptomyces* and *Kitasatospora*. The majority of the isolates were classified within the genus *Streptomyces*, accounting for 12 of the 17 isolates (70.59 %) (*Table 2).* Additionally, three isolates were identified as *Kitasatospora*, namely *K. purpeofusca* (MRS 6 and MRS 7) and *K. misakiensis (*MRS 12). Two isolates, MRS 4 and MRS 15, could not be identified based on 16S rRNA gene sequence. However, morphological characteristics supported their classification within the genus *Streptomyces* (Table 1).

The majority of isolates obtained from the rhizosphere soil of *E. variegata* were members of the genus *Streptomyces*. This finding was consistent with the adaptability of *Streptomyces* to diverse soil environments, including organically rich soils under moderate to low disturbance, such as those found in the Pakem Turi Resort area. Utami et al. (2023) reported that the secondary forests (stations A and B) in the Pakem Turi resort showed high carbon stocks under low to moderate disturbance, whereas Station C in the Cangkringan Resort, which was highly disturbed, had lower carbon stocks.

The predominance of *Streptomyces* observed in this study is consistent with previous reports, as this genus is among the most widely distributed and frequently encountered actinobacteria. The genus is also the most extensively studied, with approximately 750 validly published species and more than 7,000 deposited 16S rRNA gene sequences, reflecting its high taxonomic diversity (Heike et al. 2025; LPSN 2025).

Antimycobacterial activity of actinobacteria isolates from the rhizosphere soil of *Erythrina variegata*

Mycobacterium smegmatis remains widely used as a surrogate, particularly in primary assays, due to its rapid growth rate, similarities in genetic and physiological, and cell wall structures to *M. tuberculosis* (Lelovic et al. 2020; Sundarsingh et al. 2020; Sparks et al. 2023). Antibiotic-resistant *M. smegmatis* strains have been used in studies to understand resistance mechanisms and develop new drugs (Lelovic et al. 2020). Resistance to rifampicin, isoniazid, and multidrug-resistant TB treatments in *M. tuberculosis* is primarily linked to mutations in the *rpoB* gene (for rifampicin resistance) and the *inhA* gene (for isoniazid resistance) (Arthur et al. 2019). The resazurin microplate assay (REMA) was employed in this study as a fast, cost-effective, and reliable method to measure bacterial growth through fluorescence detection (Rakhmawatie et al. 2019).

Seventeen extracts obtained from actinobacterial isolates were evaluated for their antimycobacterial activity. Ten extracts (MRS 2, MRS 3, MRS 5, MRS 8, MRS 9, MRS 11, MRS 13, MRS 15, MRS 16, and MRS 17) exhibited inhibitory activity against WT-M (Table 2). Among these, five extracts (MRS 8, MRS 11, MRS 15, MRS 16, and MRS 17) demonstrated broad-spectrum activity by inhibiting all tested *M. smegmatis* strains, including WT-

Table 1. Identification of actinobacteria isolates from the rhizosphere soil of *Erythrina variegata* based on 16S rRNA gene sequencing.

Code	Top-hit taxon	Top-hit strain	Similarity (%)
MRS 1	*Streptomyces durhamensis*	NRRL B-3309	98.97
MRS 2	*Streptomyces cirratus*	NRRL B-3250	99.78
MRS 3	*Streptomyces* sp.	-	-
MRS 4	*Streptomyces olivochromogenes*	DSM 40451	99.56
MRS 5	*Streptomyces lutosisoli*	NEAU-QTH3-11	99.30
MRS 6	*Kitasatospora purpeofusca*	NRRL B-1817	99.53
MRS 7	*Kitasatospora purpeofusca*	NRRL B-1817	99.55
MRS 8	*Streptomyces shenzhenensis subsp. oryzicola*	MZ901363.1	99.64
MRS 9	*Streptomyces adustus*	WH-9	99.78
MRS 10	*Streptomyces lydicamycinicus*	NBRC 110027	99.93
MRS 11	*Streptomyces triticiradicis*	NEAU-H2	99.40
MRS 12	*Kitasatospora misakiensis*	NBRC 12891	99.45
MRS 13	*Streptomyces* sp.	-	-
MRS 14	*Streptomyces fuscichromogenes*	m16	99.26
MRS 15	*Streptomyces gramineus*	JR-43	99.49
MRS 16	*Streptomyces recifensis*	NBRC 12813	98.74
MRS 17	*Streptomyces lydicus*	ATCC 25470	99.70

M, RIF^R-M, INH^R-M, and MDR-M. Extracts from isolates MRS 16 and MRS 17 showed the strongest potent inhibitor activity among all tested extracts (Table 2). Based on these results, five potential isolates (MRS 8, MRS 11, MRS 15, MRS 16, and MRS 17) were selected for further antimicrobial screening against bacterial (Gram-negative and Gram-positive) and fungal pathogens, due to their strong antimycobacterial activity.

All potential antimycobacterial isolates (MRS 8, MRS 11, MRS 15, MRS 16, and MRS 17) belonged to the genus *Streptomyces*, based on 16S rRNA gene sequence analysis. The sequence similarities of the isolates to their closest type strains were as follows: MRS 8 to *Streptomyces shenzhenensis* subsp. *oryzicola* MZ901363.1 (99.64 %), MRS 11 to *Streptomyces triticiradicis* NEAU-H2 (99.40 %), MRS 15 to *Streptomyces gramineus* JR-43 (99.49 %), MRS 16 to *Streptomyces recifensis* NBRC 12813 (98.74 %), and MRS 17 to *Streptomyces lydicus* ATCC 25470 (99.70 %). Four isolates (MRS 8, MRS 11, MRS 15, and MRS 17) shared >99 % 16S rRNA gene sequence similarity with their closest type strains, suggesting a close relationship with known *Streptomyces* species. In contrast, MRS 16 exhibited 98.74 % similarity to *S. recifensis*, indicating that it may represent a potential novel species. However, further analyses, including polyphasic taxonomy approaches, are required to confirm its taxonomic status.

All five isolates belong to the genus *Streptomyces* and have previously been reported to possess potential bioactive compounds. *Streptomyces shenzhenensis* subsp. *oryzicola* was initially isolated as an endophyte from a leaf sheaths and tissues of *Oryza sativa* KDML 105 in Bangkok, Thailand. Genome mining of *S. shenzhenensis* subsp. *oryzicola* revealed its potential for antibiotic production as well as plant growth–promoting traits (Kaewkla et al. 2022). The remaining four species of *Streptomyces* are also known for their bioactive compounds from various literature sources. *Streptomyces triticiradicis* has been reported to exhibit potent antifungal activity (Yu et al. 2020). Similarly, *Streptomyces gramineus*, originally isolated from the rhizosphere soil of *Sasa borealis*, is known to produce antibiotics (Lee et al. 2012). *Streptomyces recifensis* has also been documented as an effective biocontrol agent, capable of inhibiting fire blight disease caused by *Erwinia amylovora* (S.I. Lee et al. 2024). Finally, *Streptomyces lydicus* is a well-known species with broad-spectrum antimicrobial activitiy and as a biocontrol agent (Wang et al. 2022). These findings

Table 2. Inhibition percentage of antimycobacterial activity of actinobacteria isolates against **WT-M,** RIF^R-M, INH^R-M, and MDR-M.

Code	WT-M. (%)	INH^R-M (%)	RIF^R-M (%)	MDR-M (%)
MRS 2	49.43	-	-	-
MRS 3	69.67	-	-	-
MRS 5	74.58	-	-	-
MRS 8	87.00	80.7	97.8	89.1
MRS 9	76.43	-	-	-
MRS 11	9.3	86.43	93.0	93.2
MRS 13	23.29	-	-	-
MRS 15	95.55	68.66	100	98.71
MRS 16	100	80.69	97.76	99.11
MRS 17	100	95.06	100	98.87

support the well-established role of *Streptomyces* species as a source of antibiotics (Hutchings et al. 2019; Lee et al. 2019).

Streptomyces and other actinobacteria are recognised for their ability to produce antituberculosis compounds. Several widely used anti-TB drugs, including streptomycin, rifampicin, and kanamycin, are derived from *Streptomyces*. Other anti-TB compounds from actinobacteria have also been reported, including caprazamycin B (Igarashi et al. 2003), ecumicin (Lee & Suh 2016), chrysomycin A (Ni et al. 2021), streptomycobactin, kitamycobactin, and amycobactin (Quigley et al. 2020), as well as rufomycins (Choules et al. 2019).

Antimicrobial activity spectrum of the selected isolates

The selected extracts were further evaluated for their antimicrobial activity against *B. subtilis*, *E. coli*, and *S. aureus* using the resazurin assay. The results demonstrated a range of antibacterial activities. None of the extracts exhibited inhibitory activity against *E. coli*, suggesting that the antimicrobial compounds were more effective against Gram-positive bacteria, which is consistent with the typical activity of many actinobacterial-derived antibiotics (Hutchings et al. 2019). All extracts demonstrated inhibition of *B. subtilis*, while only two extracts inhibited *S. aureus*. Among the tested isolates, MRS 11 exhibited the highest inhibition, with 71.63 % against *B. subtilis* (Table 3).

The five selected isolates were also tested for their antifungal activity against *C. gloeosporioides* and *F. oxysporum* using the dual culture agar method. The results showed that only two isolates exhibited inhibitory effects against these fungal pathogens. Isolate MRS 11 showed the most potent antifungal activity, with growth inhibition of 63.51 % against *C. gloeosporioides* and 44.12 % against *F. oxysporum*. MRS 17 also demonstrated antifungal activity, inhibiting *C. gloeosporioides* by 50 % and *F. oxysporum* by 27.94 % (Table 3). In contrast, MRS 8, MRS 15, and MRS 16 did not exhibit any inhibitory activity against either fungal strain. The antifungal activity observed against *C. gloeosporioides* and *F. oxysporum* suggests that these isolates produce secondary metabolites with antifungal properties. These results demonstrate the broad antimicrobial potential of the five isolates, with MRS 11 showing the most potent antibacterial activity, and both MRS 11 and MRS 17 exhibiting the most potential antifungal activity.

Based on molecular identification, isolate MRS 11 was identified as *Streptomyces triticiradicis* NEAU-H2, whereas MRS 17 was identified as *Streptomyces lydicus* ATCC 25470 (Figure 1). *Streptomyces triticiradicis* has been reported to exhibit potent antifungal activity against several phytopathogens, including *Colletotrichum cassiicola*, *C. orbiculare*, *Exserohilum turcicum*, and *Sclerotinia sclerotiorum*, with inhibition rates of 48.4 - 69.0 %. This antifungal activity has been linked to a biosynthetic gene cluster with 63 % similarity to the antifungal compound natamycin (Yu et al. 2020). Meanwhile, *Streptomyces*

Table 3. Antibacterial and antifungal activity of potential actinobacteria isolates against test bacteria.

New code	Antibacterial (%)			Antifungal (%)	
	B. subtilis	*E. coli*	*S. aureus*	*C. gloeoporides*	*F. oxysporum*
MRS 8	43.8	-	23.44	-	-
MRS 11	71.63	-	-	63.51	44.12
MRS 15	30.2	-	30.94	-	-
MRS 16	14.3	-	-	-	-
MRS 17	25.1	-	-	50	27.94

lydicus is a species recognised for its broad-spectrum antimicrobial potential and has been extensively studied as a biocontrol agent (Wang et al. 2022). Notably, both isolates demonstrated activity against bacterial pathogens as well as fungi. Isolate MRS 11 was selected for further investigations, including metabolite profiling using GC–MS.

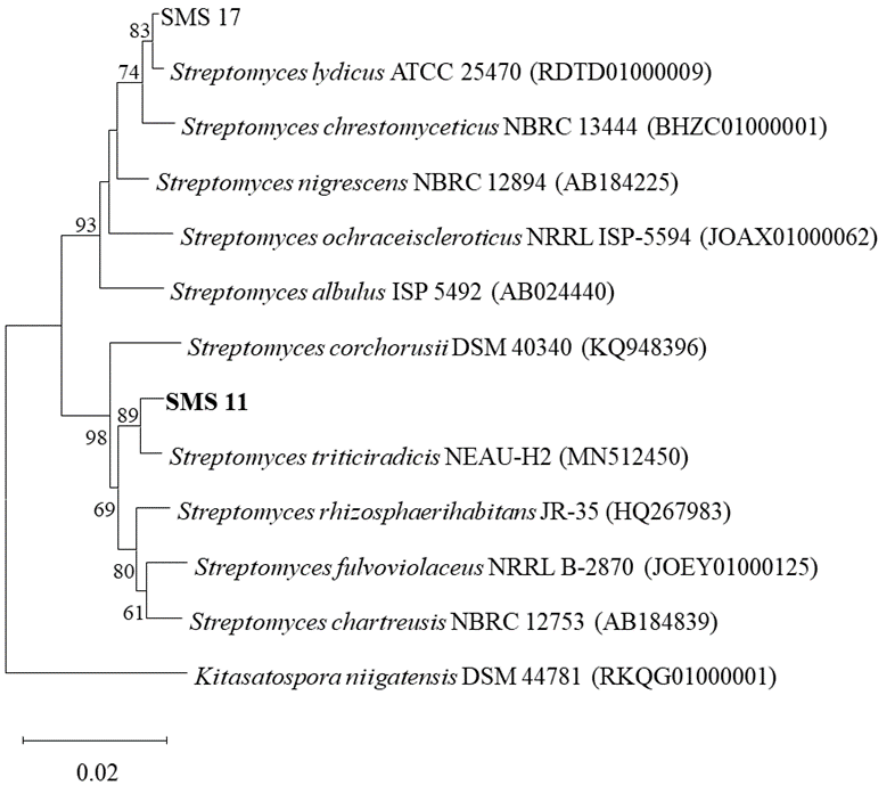

Figure 1. Phylogenetic tree of potential isolates SMS 11 and SMS 17, isolated from the rhizosphere soil of *Erythrina variegata* of Mount Merapi National Park, and their closely related type strains based on 16S rRNA gene sequences in the 1000 bootstrap replications. *Kitasatospora niigatensis* DSM 44781 was used as the outgroup

Metabolite profiling of potential actinobacteria isolates using GC-MS

The metabolite profiles of the potential actinobacterial isolate MRS 11 was analysed using GC-MS. GC-MS analysis identified 18 compounds in the crude extract of isolate MRS 11. The identified compounds in the crude extract of MRS 11 contained 1-nonadecene, E-15-heptadecenal, tricosyl trifluoroacetate, hexadecanoic acid methyl ester, n-hexadecanoic acid, tricosyl heptafluorobutyrate, undecane, 5-octadecene (E), n-propyl 11-octadecenoate, hexadecane, nonane (2,6-dimethyl-), pentadecane, heptacosane, methyl stearate, tridecane, and methyl cis-12-octadecenoate. Five of the most abundant compounds in MRS 11, which have been reported to exhibit antimicrobial activity, are listed in Table 4.

1-Nonadecene ($C_{19}H_{38}$) was observed as two distinct peaks at RT 18.61 min and 19.95 min, both known for their antimicrobial activity (Kumari et al. 2019; Patil & Jadhav 2021). E-15-Heptadecenal ($C_{17}H_{32}O$) displayed antimi-

Table 4. Identification of major antimicrobial compounds in MRS 11 using GC-MS.

Compound	Molecular Formula	SI value (%)	RT (min)	Area (%)	Activity	References
1-Nonadecene	$C_{19}H_{38}$	95	18.61	9.31	antimicrobial	Kumari et al. 2019; Patil & Jadhav 2021.
1-Nonadecene	$C_{19}H_{38}$	95	19.95	8.95	antimicrobial	Kumari et al. 2019; Patil & Jadhav 2021.
E-15-Heptadecenal	$C_{17}H_{32}O$	96	17.13	6.85	antimicrobial	Supardy et al. 2012
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	94	18.14	3.29	antibacterial, antifungal	Patil & Jadhav 2021; Shaaban et al. 2021.
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	94	18.43	3.02	antimicrobial	Kumari et al. 2019; Lykholat et al. 2023.

crobial properties (Supardy et al. 2012). Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) is known for antibacterial and antifungal effects (Abubacker & Deepalakshmi 2013; Patil & Jadhav 2021; Shaaban et al. 2021). Additionally, n -Hexadecanoic acid ($C_{16}H_{32}O_2$) is also known to possess antimicrobial activity (Ravi & Krishnan 2016; Kumari et al. 2019; Lykholat et al. 2023). These findings indicate that MRS 11 is rich in unsaturated hydrocarbons and fatty acids, which likely affect its antimicrobial and antifungal efficacy. Further studies are needed to isolate the active compounds, determine their structures, and confirm their bioactivities. Sequencing the genome of MRS 11 and analysing its biosynthetic gene clusters will help explain how these compounds are produced.

CONCLUSIONS

Seventeen actinobacterial isolates were successfully obtained from the rhizosphere of *E. variegata*, and the majority of the isolates belonged to the genus *Streptomyces*. Five isolates (MRS 8, MRS 11, MRS 15, MRS 16, and MRS 17) exhibited potent antimycobacterial activity against both drug-sensitive and drug-resistant *M. smegmatis* strains. Among these, MRS 11 also showed the most potent antibacterial activity against *B. subtilis* and antifungal effects against *C. gloeosporioides* and *F. oxysporum*. GC-MS analysis of the extract from isolate MRS 11 revealed the presence of unsaturated hydrocarbons (such as 1-nonadecene, E-15-heptadecenal) and fatty acids (such as n-hexadecanoic acid and hexadecanoic acid methyl ester), which are likely responsible for its broad-spectrum antimicrobial and antifungal activity. Further studies, including compound isolation, structural elucidation, and bioactivity testing, are needed to identify the metabolites responsible for the observed antimicrobial effects.

AUTHOR CONTRIBUTION

All authors contributed equally to this study. A.L.P. was responsible for the research design, data collection and analysis, and manuscript preparation. Y.L., I.R., and A.N. contributed to the research design and supervised the overall research process.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

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