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Phenotypic Characterization and Antagonistic Potential of Mangrove-Associated *Streptomyces* spp. against *Sclerotium rolfsii*

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Authors' contributions

This work was carried out in collaboration between both authors. Author AA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KS managed the analyses of the study. Both authors read and approved the final manuscript.

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Abstract

Mangrove rhizosphere soils represent promising reservoirs of stress-adapted actinobacteria with potential applications in microbial biotechnology and sustainable plant disease management. However, the phenotypic diversity and antagonistic potential of culturable *Streptomyces* populations associated with mangrove vegetation along the Indian coast remain insufficiently explored. The present study aimed to characterise *Streptomyces* isolates obtained from mangrove rhizosphere soils and evaluate their antagonistic activity against *Sclerotium rolfsii*. Ten *Streptomyces* isolates were characterised based on colony morphology, microscopic features and biochemical properties. Scanning electron microscopy (SEM) was used to examine their morphological characteristics, including filamentous structures where observable. Biochemical characterisation included catalase activity, indole-3-acetic acid production,

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extracellular enzyme activities, carbon source utilisation and pigment production. Antagonistic activity against *S. rolfsii* was assessed using the dual culture technique. The isolates exhibited variation in colony texture, pigmentation and other morphological and biochemical characteristics. Among the isolates evaluated, $CpSr_1$ from Pichavaram recorded the highest inhibition of *S. rolfsii* mycelial growth (74.7%), followed by $VpSr_{10}$ (70.2%) and $MpSr_6$ (62.1%). Molecular identification based on 16S rRNA gene sequencing and phylogenetic analysis identified the $CpSr_1$ isolate as *Streptomyces rochei* (GenBank accession no. PZ622137). The findings highlight the phenotypic diversity and *In vitro* antagonistic potential of mangrove-associated *Streptomyces* isolates and identify promising candidates for further investigation of their application in biological disease management.

Keywords: Mangrove rhizosphere; Streptomyces rochei; Sclerotium rolfsii; tomato collar rot; biological control; 16S rRNA gene sequencing.

1. Introduction

Mangrove ecosystems are biologically diverse coastal environments that support complex interactions among plants, microorganisms and their surrounding habitats. These ecosystems experience distinctive environmental conditions, including high salinity, waterlogging, fluctuating pH and variable nutrient availability, which influence the structure and functional diversity of their associated microbial communities. In addition to stabilising shorelines and maintaining coastal biodiversity, mangroves play important roles in nutrient recycling and organic matter decomposition (Trégarot *et al.*, 2021; Wang & Gu, 2021). The mangrove rhizosphere is an important microbial habitat because root exudates and decomposing plant materials provide substrates that support diverse microbial populations. These microorganisms contribute to organic matter turnover, nutrient cycling and interactions that influence plant and ecosystem functioning (Alghamdi *et al.*, 2024; Nwachukwu *et al.*, 2021; Thatoi *et al.*, 2013).

Among the microbial groups associated with soil and plant rhizospheres, *Streptomyces* species are of particular interest because of their distinctive morphology and extensive metabolic capabilities. *Streptomyces* are filamentous, Gram-positive actinobacteria that typically develop substrate and aerial mycelia and produce spores in chains. They are widely recognised for their ability to synthesise diverse bioactive compounds, including antibiotics, antifungal and antibacterial metabolites, extracellular enzymes and siderophores. These properties have established the genus as an important resource for microbial biotechnology, with potential applications in agriculture, environmental processes and the discovery of biologically active compounds (Barka *et al.*, 2016). Their ability to produce hydrolytic enzymes also contributes to the decomposition of complex organic materials and supports their ecological role in soil environments.

Rhizosphere-associated *Streptomyces* species are of considerable interest in plant health research because selected strains can suppress phytopathogens through antimicrobial metabolite production, competition and the secretion of extracellular enzymes. Some strains may also contribute to plant growth through nutrient mobilisation and the production of biologically active substances. These properties make *Streptomyces* an important group for investigating potential microbial resources for biological disease management and sustainable crop production (Viaene *et al.*, 2016; Zhou *et al.*, 2022). However, these functions vary among strains, and their presence must be demonstrated through appropriate biochemical and functional assays rather than inferred solely from genus-level identification. Recent investigations of mangrove-derived *Streptomyces* have identified antifungal activity against plant pathogens, including *Fusarium oxysporum* f. sp. *cubense* and fungi associated with coffee leaf lesions, supporting the relevance of screening individual isolates for pathogen-specific antagonism (Hu *et al.*, 2026; Li *et al.*, 2026).

Mangrove rhizosphere soils may provide an underexplored source of *Streptomyces* populations adapted to saline, waterlogged and nutrient-variable environments. The environmental conditions of these habitats may influence the phenotypic characteristics and functional potential of the microorganisms that inhabit them. Although mangrove-associated actinobacteria have attracted attention for their ecological and biotechnological importance, the culturable diversity of *Streptomyces* across different coastal locations remains insufficiently documented in several regions. In particular, systematic information on the morphological and biochemical variability of mangrove-associated *Streptomyces* populations from coastal Tamil Nadu and the Union Territory of Puducherry and Karaikal is limited. Further investigations are therefore necessary to document the diversity of recoverable isolates and identify candidates for subsequent taxonomic and functional evaluation (Mamangkey *et al.*, 2021; Zhou *et al.*, 2022). Studies of coastal environments in southern India have also documented antimicrobial activity in actinobacteria recovered from mangrove soils in Andhra Pradesh and marine sediments from the Tamil Nadu coast (Rani & Golla, 2026; Selvamani *et al.*, 2024).

Morphological and biochemical characterisation is a useful preliminary approach for investigating the diversity of *Streptomyces* isolates obtained from environmental samples. Characteristics such as colony pigmentation, substrate and aerial mycelial development, colony texture, sporulation and biochemical responses can help distinguish isolates and document phenotypic variation. Tests involving enzyme production, substrate utilisation and pigment formation can provide additional information about their biochemical characteristics. Although these observations are valuable for preliminary grouping and characterisation, molecular analysis is required to strengthen taxonomic identification, while functional assays are necessary to establish specific biocontrol or plant growth-promoting capabilities.

A specific gap addressed by the present investigation is the combined assessment of phenotypic variability and *In vitro* antagonism against *Sclerotium rolfsii*, the pathogen associated with tomato collar rot, among culturable *Streptomyces* isolates from the selected mangrove rhizosphere sites.

Therefore, the present study was undertaken to isolate and characterise *Streptomyces* populations associated with mangrove rhizosphere soils collected from different coastal locations of Tamil Nadu and the Union Territory of Puducherry and Karaikal. The study aimed to assess the morphological and biochemical variability of the recovered isolates and evaluate their antagonistic activity

against *S. rolfsii*, where supported by the experimental investigations. Molecular characterisation of a selected isolate was also performed to support its identification. The findings provide baseline information on the phenotypic diversity of mangrove-associated *Streptomyces* and identify promising isolates for further investigation of their potential applications in biological disease management and microbial biotechnology.

2. Experimental Details

2.1 Study Area and Sample Collection Sites

Rhizosphere soil samples were collected from ten different sampling locations across mangrove ecosystems in Tamil Nadu and the Union Territories of Puducherry and Karaikal, India, during 2024–2025. The sampling locations included Pichavaram, Koliyampalayam, North Pichavaram, T.S. Pettai and Muthupet in Tamil Nadu; Ariyankuppam, Arikamedu, Murungapakkam and Veerampattinam in Puducherry; and Karaikal Beach in Karaikal. One rhizosphere soil sample was collected from each location, resulting in a total of ten samples. The collected samples were placed in sterile polythene bags, labelled appropriately, transported to the laboratory and used for the isolation of mangrove-associated *Streptomyces* spp. The selected sampling sites represented ecologically distinct mangrove habitats distributed across the southeastern coast of India. Rhizosphere soil was collected from the root zones of dominant mangrove plant species such as *Avicennia marina, Rhizophora mucronata*, *Rhizophora apiculata, Acanthus ilicifolius* and *Excoecaria agallocha*.

2.2 Collection of Rhizosphere Soil Samples

Soil samples were collected from the root-adhering soil zone of mangrove plants from a depth of 5–15 cm. Soil adhering directly to the roots was gently shaken off and collected using sterile spatulas. Each sample was placed separately in sterile polythene bags and transported to the laboratory. Standard rhizosphere soil sampling and storage protocols were followed according to Cappuccino and Sherman (2014).

2.3 Isolation, Identification and Maintenance of the Pathogen

The fungal pathogen associated with collar rot of tomato was isolated from naturally infected tomato plants exhibiting typical collar rot symptoms. Isolation was performed using the tissue-segment method on potato dextrose agar (PDA), following the general procedure described by Mahato *et al.* (2017). The isolated fungus was purified and maintained as a pure culture for subsequent experiments. Preliminary identification was based on cultural and morphological characteristics, including mycelial growth and sclerotial characteristics. Molecular identification was subsequently performed by amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. The obtained sequence was compared with reference sequences available in the NCBI database using BLASTn. The sequence was deposited in GenBank under accession number PZ535494. The confirmed isolate was maintained on PDA medium for subsequent experimental studies.

2.4 Isolation of *Streptomyces* spp.

Isolation of *Streptomyces* spp. was performed using the serial dilution plating method with selective actinomycete media. Soil suspensions were prepared as described above, and serial dilutions were plated on actinomyces isolation agar (AIA) (Goodfellow & Williams, 1983; Shirling & Gottlieb, 1966). The inoculated plates were incubated at 28 ± 2°C for 5–10 days, as *Streptomyces* colonies generally develop more slowly than typical bacterial colonies. Colonies exhibiting characteristic chalky, powdery, leathery or filamentous appearances with aerial and substrate mycelium were selected as presumptive *Streptomyces* isolates.

2.5 Morphological and SEM Characterisation of *Streptomyces* Isolates

Purified *Streptomyces* isolates were characterised based on their colony morphology and cultural characteristics using standard microbiological procedures. The colony parameters recorded included shape, size, margin, elevation, surface texture, opacity, colour and pigmentation. Particular attention was given to colony texture, including powdery, chalky, leathery or velvety appearances; development of aerial and substrate mycelia; colour of aerial and substrate mycelia; and pigment production. These characteristics were assessed to document phenotypic variability among the isolates. For scanning electron microscopy (SEM), a 3-day-old culture of the selected *Streptomyces* isolate CpSr1 was collected for morphological examination. The specimens were fixed using 2.5% glutaraldehyde and subsequently dehydrated through a graded ethanol series. The dehydrated specimens were mounted on SEM stubs and coated with a thin conductive layer before examination. The surface morphology, filamentous structures and other cellular characteristics were examined using a JEOL JSM-IT200 scanning electron microscope at 13,000× magnification. SEM micrographs were recorded with a scale bar of 1 µm to document the morphological characteristics of the selected isolate.

2.6 Biochemical Characterisation of *Streptomyces* Isolates

2.6.1 Indole-3-acetic Acid

Indole-3-acetic acid (IAA) production was evaluated following the methods described by Gopalakrishnan *et al.* (2011). IAA production was tested using test tubes containing ISP medium 2 amended with L-tryptophan. Isolates producing IAA were confirmed by the appearance of pink colouration in the broth medium (Khamna *et al.*, 2009).

2.6.2 Carbon Source Utilisation

The ability of the selected *Streptomyces* isolates to utilise different carbon sources was evaluated using a suitable carbon utilisation medium supplemented separately with D-fructose and D-xylose. The inoculated media were incubated under suitable conditions, and colour change or growth response was recorded to determine carbon source utilisation, expressed as positive (+) or negative (−).

2.6.3 Extracellular Lytic Enzyme

The extracellular lytic enzyme activities of all isolates were evaluated by assessing their ability to degrade cellulose and chitin. Cellulolytic activity was tested on carboxymethyl cellulose (CMC) agar containing 1 g $(NH_4)_2$ SO_4, 1 g $MgSO_4 \cdot 7H_2O$, 1 g $CaCl_2 \cdot 2H_2O$, 0.2 g $FeCl_3$, 1 g K_2HPO_4, 2 g casitone, 15 g carboxymethyl cellulose, 6 g agar and 1 L distilled water. Chitinolytic activity was determined using a medium supplemented with 10 g chitin $[(C_8H_{13}NO_5)_n]$, 6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl, 20 g NaCl, 0.05 g yeast extract, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g agar and 1 L distilled water. The inoculated plates were maintained at 28 °C for 7 days, after which enzymatic activity was assessed based on the formation of a clear hydrolysis zone or visualised using 1% Congo red staining. All assays were performed with three replicates.

2.6.4 Catalase Test

A few drops of a freshly grown *Streptomyces* suspension were placed on a sterile glass slide, followed by the addition of one drop of 3% hydrogen peroxide. The appearance of immediate bubbling or effervescence, resulting from the release of oxygen, was recorded as a positive catalase reaction, confirming the presence of the catalase enzyme (Hafezi & Khamar, 2024).

2.7 Dual Culture Assay against *S. rolfsii*

The antagonistic activity of *Streptomyces* (CpSr1–VpSr10) isolates against *S. rolfsii* was evaluated using the dual culture technique following Dennis and Webster (1971). A loopful of each bacterial antagonist was streaked on NA + PDA medium opposite to a 5 mm mycelial disc of a 7-day-old *S. rolfsii* culture. The plates were incubated at 28 ± 2°C for 7 days with three replications, after which the radial growth of the pathogen was measured. The percentage inhibition of *S. rolfsii* mycelial growth was calculated using the following formula:

Per cent inhibition = (C-T)/C x 100

Where, C- Diameter of mycelial growth of the pathogen in control
T- Diameter of mycelial growth in the treatment

2.8 Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted from actively growing cultures of the selected *Streptomyces* isolate using a standard bacterial genomic DNA extraction protocol. The bacterial 16S rRNA gene was amplified using universal primers (Weisburg *et al.*, 1991) 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACT-3'). PCR amplification was performed in a 25 µL reaction mixture containing 2× PCR master mix, 50–100 ng genomic DNA, 10 pmol of each primer and nuclease-free water. The amplification consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 90 s, with a final extension at 72 °C for 10 min. The consensus sequences were compared with reference sequences in the NCBI GenBank database using BLASTn, and closely related sequences were retrieved for phylogenetic analysis. Multiple sequence alignment was performed using ClustalW (Thompson *et al.*, 1994), followed by phylogenetic reconstruction using the neighbour-joining method in MEGA 12 (Kumar *et al.*, 2024).

2.9 Statistical Analysis

Data on radial mycelial growth and percentage inhibition were subjected to analysis of variance (ANOVA), and treatment means were compared using the critical difference (CD) at the 5% significance level ($p \leq 0.05$). Statistical analyses were performed using OPSTAT software, and results were presented as mean values with standard errors or standard deviations, as appropriate.

3. Results and Discussion

3.1 Morphological Characterisation and SEM Analysis of *Streptomyces* Isolates

The *Streptomyces* isolates were distinguished by colony texture, including powdery, chalky, velvety and leathery growth forms. Aerial mycelium colour was mainly white and grey, whereas substrate mycelium pigmentation included cream ($CpSr_1$, $TsSr_4$, $AkSr_7$ & $VpSr_{10}$), yellow ($KpSr_2$ & $AmSr_8$), brown ($NpSr_3$, $KbSr_5$ & $MkSr_9$) and yellowish brown (MpSr6). Diffusible pigment production was observed in isolates $CpSr_1$, $KpSr_2$, $NpSr_3$, $KbSr_5$, $MpSr_6$, $AmSr_8$ and $MkSr_9$, while the remaining isolates ($TsSr_4$, $AkSr_7$ and $VpSr_{10}$) were non-pigmented (Table 1). All isolates were Gram-positive and produced filamentous mycelia with distinct spore chain arrangements, including spiral, straight and flexuous forms (Fig. 1). SEM analysis of $CpSr_1$ revealed elongated, oval to cylindrical spore-like structures arranged in chains and clusters (Fig. 2). The individual structures exhibited predominantly smooth surfaces, with variations in shape and size. Some spores appeared closely associated, forming aggregated and chain-like arrangements. These observations support the characteristic sporulation morphology of *Streptomyces* spp. These environmental conditions create strong selective pressures that favour microorganisms with broad metabolic adaptability and stress tolerance. Recent microbial ecological studies have confirmed that mangrove sediments and rhizospheres harbour complex and functionally distinct bacterial communities with important ecological roles in nutrient exchange and plant health (Ye *et al.*, 2023).

The *Streptomyces* isolates exhibited significant variation in colony texture, aerial mycelium colour, substrate mycelium pigmentation, pigment production and spore arrangement. These characteristics remain important phenotypic parameters for preliminary differentiation of actinomycetes and reflect differences in secondary metabolism. Pigment production in *Streptomyces* is frequently associated with biosynthesis of antibiotics, siderophores and other specialised metabolites. Taha and Abd El-Hafez (2025) similarly reported considerable variation in aerial mycelium colour, pigment production and spore chain morphology among pathogenic *Streptomyces* isolates recovered from potato scab lesions. The present findings also support earlier reports that mangrove habitats are rich sources of novel *Streptomyces* taxa. Law *et al.* (2019) described *Streptomyces monashensis* from Malaysian mangrove soil and demonstrated its distinct phenotypic profile together with bioactive potential. Likewise, several other new species such as *Streptomyces avicenniae* and *Streptomyces xiamenensis* have been reported from mangrove rhizosphere and sediment, respectively (Xiao *et al.*, 2009; Xu *et al.*, 2009), indicating that coastal wetlands are hotspots of actinobacterial diversity and taxonomic novelty.

Table 1. Morphological and microscopic characteristics of *Streptomyces* isolates

Isolate code	Colony texture	Aerial mycelium colour	Substrate mycelium colour	Pigment production	Margin	Gram reaction	Filamentous growth	Spore arrangement
$CpSr_1$	Powdery	White	Cream	Present	Entire	Positive	Present	Spiral
$KpSr_2$	Chalky	Grey	Yellow	Present	Undulate	Positive	Present	Straight
$NpSr_3$	Velvety	White	Brown	Present	Entire	Positive	Present	Flexuous
$TsSr_4$	Leathery	Grey	Cream	Absent	Lobate	Positive	Present	Spiral
$KbSr_5$	Powdery	White	Brown	Present	Entire	Positive	Present	Flexuous
$MpSr_6$	Chalky	Grey	Yellowish brown	Present	Undulate	Positive	Present	Spiral
$AkSr_7$	Velvety	White	Cream	Absent	Entire	Positive	Present	Straight
$AmSr_8$	Powdery	Grey	Yellow	Present	Filamentous	Positive	Present	Spiral
$MkSr_9$	Chalky	White	Brown	Present	Undulate	Positive	Present	Flexuous
$VpSr_{10}$	Leathery	Grey	Cream	Absent	Entire	Positive	Present	Spiral



Fig. 1. Axenic culture of *Streptomyces* spp. ($CpSr_1$)

3.2 Biochemical Characterisation of Streptomyces Isolates

The biochemical characterisation of ten mangrove-associated *Streptomyces* isolates revealed that all isolates were positive for catalase activity. IAA production was observed in nine isolates, while extracellular cellulase activity was detected in nine isolates and chitinase activity in seven isolates. All isolates utilised D-fructose, whereas D-xylose utilisation was observed in nine isolates. Pigment production was recorded in seven isolates, indicating variation in the biochemical and physiological characteristics among the isolates (Table 2). A recent meta-analysis demonstrated the relevance of chitinolytic microorganisms as biological control agents against plant pathogens, supporting the use of chitinase activity as an important preliminary screening trait (Das *et al.*, 2024). The cellulase and chitinase activities among the present *Streptomyces* isolates are also comparable with the findings of Zhang *et al.* (2024). A chitinase-producing *S. cellulosae* strain was shown to suppress *R. solani* and was subsequently developed into a bioformulation for the management of peanut damping-off and root rot (Abo-Zaid *et al.*, 2024). Similarly, *Streptomyces* strains possessing combinations of cellulase, chitinase and other plant-beneficial traits have demonstrated antagonistic activity against

phytopathogenic fungi, indicating that extracellular hydrolytic enzymes may contribute to the multifaceted biocontrol capacity of actinomycetes. The biochemical profiles demonstrated that the *Streptomyces* isolates possessed diverse and potentially complementary traits associated with plant growth promotion and pathogen suppression. The variation in IAA and the frequent occurrence of cellulase and chitinase activities among *Streptomyces* isolates provide useful criteria for selecting strains for subsequent antagonistic and plant-based assays.

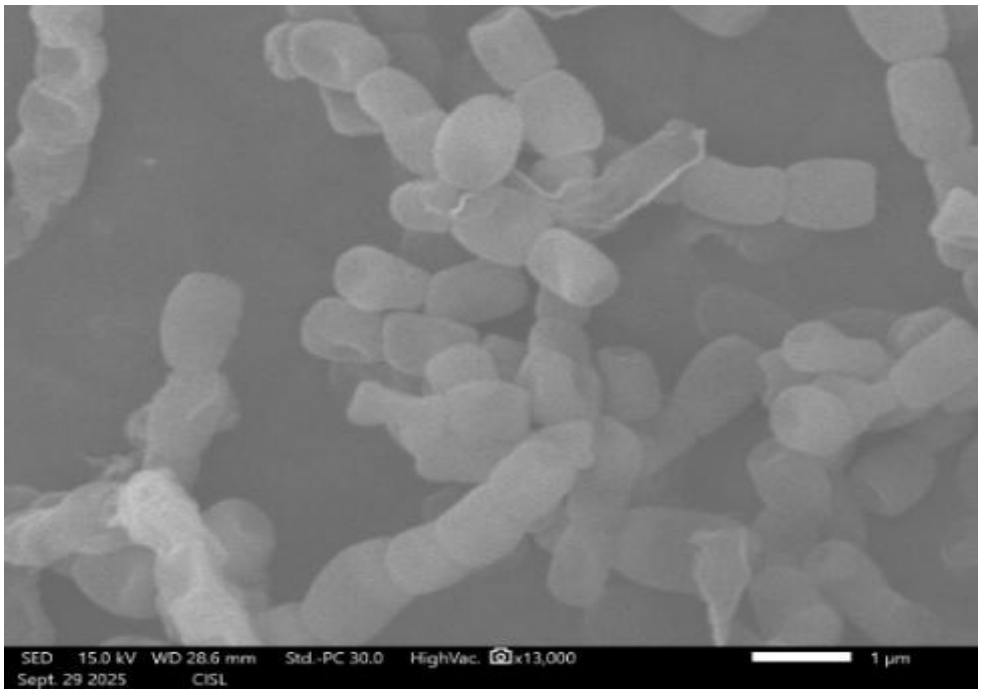

Fig. 2. Chains of spore *Streptomyces* spp. ($CpSr_1$)

Table 2. Biochemical characterization of *Streptomyces* spp. isolates

Isolate Code	Catalase test	IAA	Extra cellular lytic activities		Carbon sources			Pigment Production
			Cellulase	Chitinase	Cellulose	D-Fructose	D-Xylose	
$CpSr_1$	+	+	+	+	+	+	+	+
$KpSr_2$	+	+	-	+	+	+	+	+
$NpSr_3$	+	+	+	+	+	+	+	+
$TsSr_4$	+	+	+	+	+	+	+	-
$KbSr_5$	+	-	+	-	+	+	+	+
$MpSr_6$	+	+	+	+	+	+	+	+
$AkSr_7$	+	-	+	+	+	+	+	-
$AmSr_8$	+	+	-	-	+	-	-	+
$MkSr_9$	+	+	-	-	+	+	+	+
$VpSr_{10}$	+	+	+	+	+	+	+	-

(+) - Positive, (-) - Negative

3.3 Antagonistic Activity of *Streptomyces* spp. against *S. rolfsii*

The antagonistic activity of the mangrove-associated *Streptomyces* isolates against *S. rolfsii* varied, indicating differences in their ability to suppress the mycelial growth of the pathogen under *In vitro* conditions. Among the isolates tested, $CpSr_1$ recorded the highest mycelial growth inhibition of 74.7%, followed by $VpSr_{10}$ (70.2%) and $MpSr_6$ (62.1%), whereas AkSr7 exhibited the lowest inhibition (50.0%). In the untreated control, the pathogen showed a radial growth of 90.0 mm (Table 3; Fig. 3a and b). The comparatively high inhibition exhibited by $CpSr_1$ identifies it as a promising candidate for further investigation of its antagonistic potential against *S. rolfsii*. The results are consistent with previous studies demonstrating the antagonistic potential of *Streptomyces* against *S. rolfsii*. Ruangwong *et al.* (2022) reported that *Streptomyces albulus* Z1-04-02 inhibited the mycelial growth of *S. rolfsii* by approximately 74.3% in a dual culture assay. Similarly, Huyen *et al.* (2023) reported 63.59% inhibition of *S. rolfsii* by *Streptomyces corchorusii* L72. These findings support the potential of selected *Streptomyces* isolates to inhibit the growth of this soil-borne fungal pathogen.

The antagonistic activity of *Streptomyces* may be associated with the production of antimicrobial secondary metabolites and extracellular enzymes capable of affecting fungal growth. Abo-Zaid *et al.* (2021) demonstrated that *S. cellulosae* Actino 48

suppressed *S. rolfsii* and produced chitinase; scanning electron microscopy also revealed abnormalities and damage to fungal hyphae during antagonistic interactions. Huyen *et al.* (2023) reported chitinase production by *S. corchorusii* L72. These studies provide plausible explanations for the antagonistic activity observed in the present investigation. However, the specific mechanisms responsible for the inhibition exhibited by CpSr1, VpSr10 and MpSr6 cannot be established from the dual culture assay alone and require further investigation of enzyme activity, culture filtrates and antifungal metabolites. Overall, the present findings demonstrate the *In vitro* antagonistic potential of mangrove-associated *Streptomyces* isolates and provide a basis for further evaluation of promising strains for the biological management of diseases caused by *S. rolfsii*.

Table 3. **Antagonistic activity of *Streptomyces* spp. isolates against *S. rolfsii* in dual culture**

Isolate	*Streptomyces* spp.	
	Radial growth of *S. rolfsii* (mm)*	**Mycelial growth inhibition (%)**
$CpSr_1$	22.7	74.7
$KpSr_2$	36.5	59.4
$NpSr_3$	40.8	54.7
$TsSr_4$	43.2	51.0
$KbSr_5$	38.4	56.7
$MpSr_6$	34.1	62.1
$AkSr_7$	45.0	50.0
$AmSr_8$	37.2	58.7
$MkSr_9$	41.5	53.9
$VpSr_{10}$	26.8	70.2
Control	90.0	--
SE(d)	0.7	--
CD ($p \leq 0.05$)	2.1	--

**Mean of three replications*



Fig. 3. Antagonistic activity of *Streptomyces* spp. against *S. rolfsii.* a.) Control b.) *Streptomyces* spp. ($CpSr_1$) vs *S. rolfsii*

3.4 Molecular Identification and Phylogenetic Analysis of *Streptomyces* Isolates

Molecular characterisation of the selected *Streptomyces* isolate was performed using 16S rRNA gene sequencing. The isolate yielded a 900 bp partial 16S rRNA gene sequence, which exhibited 98–99% sequence similarity to *Streptomyces rochei* sequences in the NCBI BLASTn database. Phylogenetic analysis with reference sequences further supported the taxonomic placement of the isolate (Fig. 4). The obtained sequence was deposited in GenBank under accession number PZ622137, providing an accessible record for future comparative studies. Similar molecular approaches have been used to identify antagonistic *Streptomyces* isolates, including *Streptomyces* sp. SP5, which showed 100% sequence similarity to *S. rochei* NRRL B-2410 (Devi *et al.*, 2022). However, 16S rRNA gene sequencing has limited discriminatory power among closely related *Streptomyces* species (Law *et al.*, 2018). Therefore, the present isolate is closely related to *S. rochei*, but further confirmation using multilocus sequence analysis or genome-based methods would strengthen species-level identification. Its molecular characterisation, together with the observed antagonistic activity against *Sclerotium rolfsii*, supports further evaluation of its potential as a biological control agent. The 16S rRNA gene is widely used for bacterial identification and phylogenetic analysis; however, its ability to distinguish closely related *Streptomyces* species may be limited (Church *et al.*, 2020). Mispelaere *et al.* (2024) also highlighted the limitations of 16S rRNA gene sequencing for species-level identification within *Streptomyces* and the value of additional molecular approaches, including multilocus sequence analysis and whole-genome comparisons. Therefore, based on the sequence similarity and phylogenetic evidence obtained, the isolate

was identified as *Streptomyces rochei*, although further molecular analysis would be required for definitive species-level confirmation.

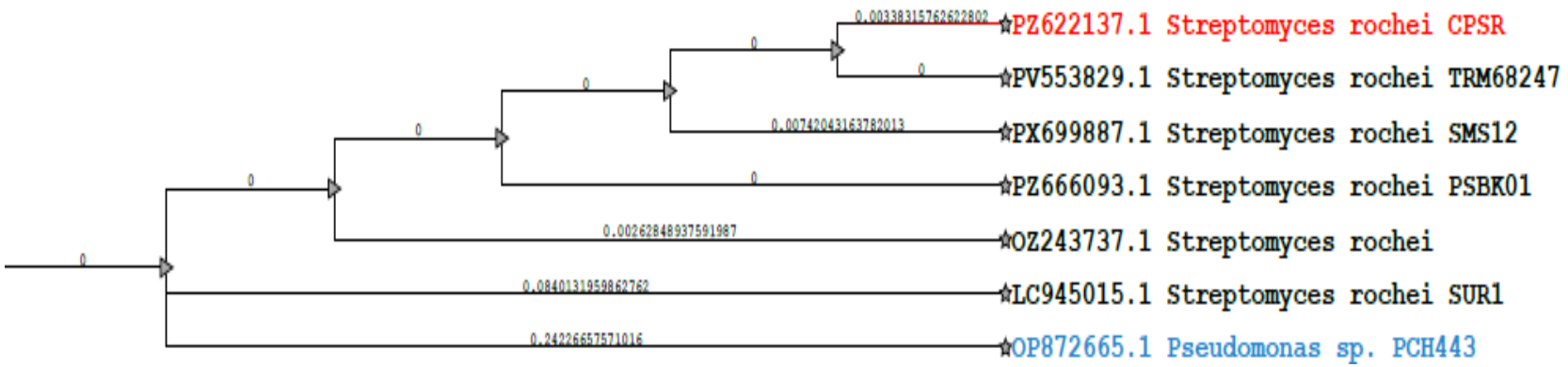

Fig. 4. Phylogenetic analysis of *Streptomyces rochei* ($CpSr_1$)

4. Limitations of the Study and Future Perspectives

Although the present study demonstrated the *In vitro* antagonistic potential of mangrove-associated *Streptomyces* isolates against *S. rolfsii*, certain limitations need to be considered. The investigation was restricted to ten culturable isolates, which may not represent the complete diversity of *Streptomyces* populations associated with mangrove rhizosphere ecosystems. Furthermore, the antagonistic activity was evaluated primarily through dual culture assays, and the effectiveness of the promising isolates under greenhouse and field conditions remains to be established. Although the production of extracellular lytic enzymes was investigated, the specific contribution of these enzymes and antifungal secondary metabolites to pathogen inhibition was not experimentally confirmed. Molecular identification based on partial 16S rRNA gene sequencing provides preliminary taxonomic evidence; however, further confirmation using multilocus sequence analysis or whole-genome sequencing would strengthen species-level identification. Future investigations should therefore focus on characterising antifungal metabolites, elucidating the mechanisms of antagonism, evaluating plant growth-promoting effects and developing stable formulations for the biological management of tomato collar rot.

5. Conclusion

The present investigation demonstrated morphological and biochemical variability among ten Streptomyces isolates recovered from mangrove rhizosphere soils of Tamil Nadu, Puducherry and Karaikal. Differences in colony texture, mycelial pigmentation, spore arrangement and biochemical characteristics reflected the phenotypic diversity of the recovered isolates. The occurrence of catalase activity, indole-3-acetic acid production and extracellular hydrolytic enzyme activities provided useful information for preliminary functional screening. All ten isolates exhibited antagonistic activity against *Sclerotium rolfsii* under *In vitro* conditions, with $CpSr_1$ recording the greatest inhibition, followed by $VpSr_{10}$ and $MpSr_6$. Molecular characterisation of $CpSr_1$ revealed a partial 16S rRNA gene sequence of 900 bp with 98–99% similarity to Streptomyces rochei, supporting its preliminary taxonomic identification. Nevertheless, further molecular analysis is necessary for definitive species-level confirmation. The findings indicate that mangrove rhizosphere soils represent a potential source of antagonistic Streptomyces isolates. Among the isolates investigated, $CpSr_1$ warrants further evaluation to establish its antagonistic mechanisms and effectiveness under greenhouse and field conditions. Such investigations would contribute to assessing its suitability for the biological management of tomato collar rot.

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Competing Interests

Authors have declared that no competing interests exist.

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