

Original Research Article

Exploring Native Actinomycetes of *Streptomyces albofaciens* for the Sustainable Management of Mango Anthracnose

Abstract

Mango (*Mangifera indica* L.), a commercially significant *climacteric* fruit, is highly susceptible to anthracnose incited by *Colletotrichum gloeosporioides*. In pursuit of sustainable alternatives to chemical fungicides, the present study involved the isolation and characterization of native actinomycetes, particularly *Streptomyces* spp., from mango rhizosphere soils across Tamil Nadu and Puducherry. Ten isolates (AUSA₁-AUSA₁₀) were morphologically and microscopically characterized, with AUSA₃ exhibiting the highest antagonistic activity against *C. gloeosporioides* in dual culture assays, recording 79.72% mycelial inhibition. Scanning Electron Microscopy revealed smooth, rod-shaped spores, while molecular identification through 16S rRNA gene sequencing confirmed AUSA₃ as *Streptomyces albofaciens* with 100% similarity. These findings suggest that native *S. albofaciens* exhibits strong potential as an eco-friendly biocontrol agent against mango anthracnose.

Aims: To isolate, characterize, and evaluate native *Streptomyces* spp. from mango rhizosphere soils for their antagonistic potential against *Colletotrichum gloeosporioides*, the causal agent of mango anthracnose.

Study design: Experimental study involving in vitro and molecular evaluation of actinomycete isolates.

Place and Duration of Study: Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Tamil Nadu, and Rice Research Station, Ambasamudram, Tamil Nadu, conducted between July 2023 and May 2024

Methodology: Ten actinomycete isolates (AUSA₁-AUSA₁₀) were obtained using the soil dilution plate method on Actinomycetes Isolation Agar from mango rhizosphere soils of Tamil Nadu and Puducherry. Isolates were morphologically characterized by macroscopic and microscopic features. Antagonistic activity against *C. gloeosporioides* was evaluated through dual culture assays on PDA. The most effective isolate, AUSA₃, was further characterized using Scanning Electron Microscopy and molecularly identified via 16S rRNA gene sequencing. DNA was extracted using phenol:chloroform:isoamyl alcohol protocol, and PCR amplification was performed with universal primers (27F and 1492R).

Results: Among the isolates, AUSA₃ exhibited the highest mycelial growth inhibition (79.72%) of *C. gloeosporioides*, followed by AUSA₇ (76.36%). Morphologically, AUSA₃ displayed a bright yellow substrate mycelium, powdery surface, and rod-shaped smooth spores. SEM analysis confirmed its spore structure. Molecular identification revealed 100% sequence similarity to *Streptomyces albofaciens*, and the sequence was submitted to GenBank (Accession No. PV603281).

Conclusion: The study confirms that native *Streptomyces albofaciens* (AUSA₃) possesses strong in vitro antagonistic activity against *C. gloeosporioides* and holds promise as a sustainable biocontrol agent for mango anthracnose management. Its deployment could reduce

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dependence on synthetic fungicides and promote eco-friendly disease control strategies in mango cultivation.

Keywords: *Streptomyces albofaciens*, Actinomycetes, Mango anthracnose, *Colletotrichum gloeosporioides*.

Introduction

Mango (*Mangifera indica* L.), acclaimed as the "King of fruits", is a major tropical climacteric fruit crop in the family Anacardiaceae. It is globally valued for its distinctive organoleptic properties and high phytonutrient content (Jeevanantham et al. 2024). However, mango is highly susceptible to a wide range of biotic and abiotic stresses across phenological stages, which adversely affect physiological functions, yield, and post-harvest quality (Balamurugan et al. 2025). For many years, safeguarding mango trees against anthracnose diseases has traditionally depended on the extensive use of synthetic fungicides, a practice deeply implemented in agricultural conventions (Iqbal et al. 2022). In response to escalating concerns over chemical residues and pathogen resistance, the quest for eco-friendly solutions to manage mango anthracnose has become more urgent than ever (Dofuor et al. 2023). In this context, the microbiota have attracted considerable interest for their role in promoting plant health and improving nutrient dynamics (Suman et al. 2022). Among these beneficial microbes, actinomycetes particularly *Streptomyces* spp. stand out for their ecological versatility and remarkable biocontrol potential (Khan et al. 2023). These Gram-positive, filamentous actinobacteria are prolific producers of secondary metabolites including antibiotics, siderophores, hydrolytic enzymes, and antifungal compounds. *Streptomyces* spp. apply on foliage, outcompete pathogens, and secrete bioactive molecules, positioning them as promising candidates for the sustainable management of mango anthracnose (Kaur et al. 2023). In this context, the current investigation aimed to isolate and characterize native *Streptomyces* spp. from diverse agro-climatic zones of Tamil Nadu and Puducherry, and identification of potent isolates for the management of mango anthracnose.

2. Materials and Methods

2.1 Isolation of Actinomycetes

Soil samples from the rhizosphere of healthy mango trees across were collected from prominent mango cultivation areas in Tamil Nadu and Puducherry. Ten *Streptomyces* spp. were isolated using the soil dilution plate technique on Actinomycetes Isolation Agar (AIA). Samples were air-dried, pulverized, sieved, and serially diluted up to 10^{-6} in sterile distilled water. Aliquots

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from 10⁻³ to 10⁻⁶ dilutions were aseptically plated and incubated at 28°C for 7-14 days. Colonies exhibiting typical actinomycetes morphology were subcultured using the single hyphal tip method and preserved on slants at 4°C. Isolates were characterized based on macroscopic, microscopic, and molecular parameters to confirm their taxonomic identity.

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2.2 Morphological characterization of Actinomycetes

The morphological characterizations of actinomycetes involved both macroscopic and microscopic evaluations. Macroscopically, isolates were assessed based on aerial and substrate mycelium pigmentation, colony morphology, and growth rate (Eppard et al. 1996). Microscopic analysis using the slide culture method enabled observation of spore morphology and arrangement under a light microscope at 100× magnification. For detailed structural analysis, spore surface features and chain arrangements were further examined using Scanning Electron Microscopy (SEM). Morphological traits were compared with standard taxonomic descriptions provided in *Bergey's Manual of Systematic Bacteriology* (Kämpfer 2006) for presumptive identification.

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2.3 Evaluation of invitro efficacy of different isolates of actinomycetes against *C. gloeosporioides* (Dual culture)

The inhibitory activity of *Streptomyces* spp. against *C. gloeosporioides* was assessed using the dual culture technique (Palaniyandi et al. 2011). Nine mm disc from a seven days old *C. gloeosporioides* culture was placed 1 cm from one edge of a PDA plate, and a loopful of five days old *Streptomyces* culture was streaked at a distance of 1 cm from the opposite edge. Control plates were inoculated with the pathogen alone. Cultures were incubated at 28 ± 2°C for seven days under controlled conditions. The inhibition percentage was calculated using (Vincent 1947) formula:

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Where C = Pathogen growth in control; T = Pathogen growth in dual culture (Kunova et al. 2016)

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2.4 Molecular characterization of Actinomycetes

The virulent isolate of *Streptomyces* spp. was cultured in Ken Knight & Munaier's broth and incubated at 30°C for a duration of 48 hours. After the incubation period, 1 mL of culture was centrifuged at 12,000 rpm for 10 minutes at ambient temperature. The resulting pellet was

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resuspended in 0.85% NaCl solution, centrifuged again, and treated with 600 µL of lysis buffer and 7 µL of Proteinase K at 65 °C for 1 hour. Extraction of genomic DNA carried out using phenol: chloroform: isoamyl alcohol and chloroform: phenol: isoamyl alcohol, followed by ethanol precipitation with 3 M NaCl. DNA quality was confirmed via electrophoresis on 0.8% agarose gel using 1× TBE buffer and a 100 bp ladder, visualized under UV light. PCR amplification of the 16S rRNA gene was carried out using universal primers 27F and 1492R in a 25 µL reaction containing Taq buffer with MgCl₂, dNTPs, Taq polymerase, and template DNA. Thermal cycling in a Veriti™ Thermal Cycler included initial denaturation at 95 °C for 5 minutes; 29 cycles of denaturation (94 °C, 30 sec), annealing (52 °C, 30 sec), and extension (72 °C, 45 sec); followed by a final extension at 72 °C for 10 minutes. Amplified products were confirmed on 1% agarose gel alongside a 1 kb ladder and visualized under UV light. Sequencing was performed bidirectionally using an ABI 3730xl Genetic Analyzer, and homology was determined using NCBI BLAST. The sequence was submitted to the GenBank repository and phylogenetic relationships were inferred using the maximum likelihood method implemented in MEGA 11.

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3. Results and Discussion

3.1. Morphological characterization of different isolates of actinomycetes

A total of ten actinomycetes strains were isolated from rhizospheric soil samples gathered across multiple regions of Tamil Nadu and Puducherry. Each isolate exhibited distinct morphological features, including mycelial pigmentation, colony shape, surface texture, growth rate, and spore morphology. The *Streptomyces* spp. isolates were cultured on Actinomycetes Isolation Agar (AIA) medium, and traits were recorded after 10 days of incubation. These isolates were designated as AUSA₁ to AUSA₁₀. Colony pigmentation varied, with aerial mycelium ranging from white-grey to light grey, and substrate mycelium showing shades such as whitish-grey, yellow, red, greyish-yellow, and brownish-yellow. Colony shapes included round with concentric lines, radiating, feather-like, irregular, and double-ring forms. Surface textures ranged from smooth to powdery, and spores were rod-shaped with smooth surfaces. Growth rates were categorized as slow, moderate, good or very good (Table 1 & Fig 1, 2 and 3). (Shepherdson et al. 2023) further highlighted that the filamentous growth and spore formation in actinomycetes, particularly *Streptomyces* spp., contribute to their unique plant colonization strategies compared to sessile bacteria. These morphological traits remain essential in distinguishing *Streptomyces* from other spore-forming actinomycetes (Taddei et al. 2006).

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Fig. 1. Pure Culture of *S. albobacillus*

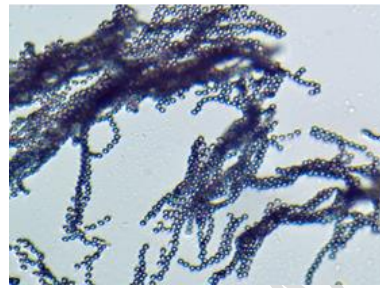


Fig. 2. Microscopic observations of *S. albobacillus*

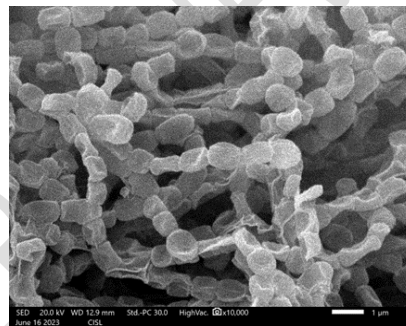
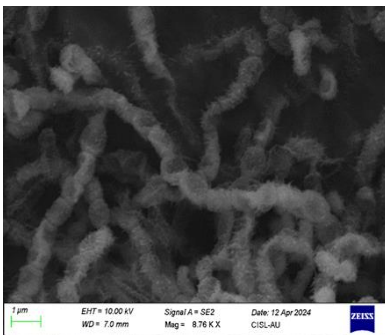


Fig. 3. SEM observation of *S. albobacillus*

3.2. Evaluation of *invitro* efficacy of different isolates of actinomycetes against *C. gloeosporioides* (Dual culture)

All ten *Streptomyces* isolates were evaluated for their antagonistic efficacy against *C. gloeosporioides* was assessed by means of the dual culture technique on PDA medium (Table 2). Among them, AUSA₃ exhibited the highest inhibition of mycelial growth, recording 79.72% inhibition, followed by AUSA₇ with 76.36%. The least inhibition was observed in AUSA₉ (46.24%). All isolates significantly suppressed the radial growth of the pathogen compared to the control, with AUSA₃ emerging as the most effective antagonist. The finding (Lai et al. 2024), revealed that actinomycetes exhibited significant antagonistic activity against *C. gloeosporioides* by hydrolyzing of the fungal cell wall. In vitro efficacy of biocontrol agents against *C. gloeosporioides* revealed that the isolate have recorded maximum mycelial inhibition of 50.77 per cent over control (Li et al. 2024).

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3.2 Confirmation and Molecular characterization of virulent isolate of *S. albofaciens* (AUSA₃)

Ten actinomycetes isolates (AUSA₁ to AUSA₁₀) collected and efficacy against anthracnose was tested. Among the isolates AUSA₃, found to be more effective against anthracnose. The genomic DNA of *S. albofaciens* (AUSA₃), was processed through PCR amplification using universal primers 27F and 1492R. The resulting amplicon was approximately 909 bp, verifying successful amplification of the targeted 16S rRNA gene region. Sequence analysis and BLAST search on the NCBI database validated the identity of the isolate, revealing 100% nucleotide similarity with *S. albofaciens*. The partial sequence has been deposited in GenBank under accession number PV603281. Similar finding were observed by many researchers (Duangupama et al. 2024), reported that Actinomycetes were isolated from the rhizosphere soils of mango trees and identified as Streptomyces by PCR amplification with the use of 16S rRNA specific forward (F243) and reverse (A3R) primers which resulted in an amplicon size primer of 650 bp. The *Streptomyces* strain was identified using 16S rRNA gene sequencing as *S. albofaciens* (MS38), it amplifies at an amplicon size of 1489 bp (Singh et al. 2024). (Zainal Abidin et al. 2016), reported that the five effective antagonistic actinomycete isolates, identified as *Streptomyces* spp. were subjected to 16s rRNA gene amplification using gene specific primers namely F243 and A3R primers. Approximately 1250 bp amplicon was obtained for all the isolates.

4. Conclusion

This investigation elucidates the phenotypic variability among *Streptomyces* spp. isolates procured from diverse agroecological zones across Tamil Nadu and Puducherry. The aerial mycelia exhibited pigmentation ranging from white-grey to pale grey, while the substrate mycelia presented a spectrum of colours including whitish-grey, yellow, red, greyish-yellow, and brownish-yellow. Colonies displayed distinct morphotypes, such as concentric-ringed, radiating, feather-like, irregular, and double-ring configurations. Notably, isolate AUSA₃ exhibited marked antagonistic efficacy against *C. gloeosporioides* in dual culture bioassays. Molecular delineation via 16S rRNA gene sequencing authenticated AUSA₃ as *S. albofaciens*.

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

Competing Interests

The authors have no competing interests to disclose.

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Table 1: Morphological characterization of different isolates of actinomycetes

S. No	Place of collection	Isolate no	Aerial mass colour	Colour of substrate mycelium	Colony Shape	Colony Surface	Spore surface morphology	Speed of growth	Spore shape
1.	Nallathur	AUSA ₁	White	Red	Round with radiated	Rough	Smooth	Moderate	Rod shaped
2.	Valapadi	AUSA ₂	Grey	Brownish yellow	Irregular	Rough	Smooth	Good	Rod shaped
3.	Kattukottai	AUSA ₃	White	Bright yellow	Round	Powdery	Smooth	Very Good	Rod shaped
4.	Shoolagiri	AUSA ₄	White	Light Yellow	Round with lines	Smooth	Smooth	Slow	Rod shaped
5.	Sivapuri	AUSA ₅	Light grey	Greyish yellow	Round	Powdery	Smooth	Slow	Rod shaped
6.	Papparapatti	AUSA ₆	White	Moderate yellow	Two round circles	Smooth	Smooth	Good	Rod shaped
7.	Kaveripattinam	AUSA ₇	White	Greyish yellow	Double ring	Smooth	Smooth	Slow	Rod shaped
8.	Nannilam	AUSA ₈	White	Light grey	Round	Smooth	Smooth	Good	Rod shaped
9.	Ayothiyampattinam	AUSA ₉	Light grey	Yellowish grey	Round with feathers	Rough	Smooth	Moderate	Rod shaped
10.	Kollidam	AUSA ₁₀	Grey	Pale yellow	Round with feathers	Powdery	smooth	Good	Rod shaped

Table 2: Evaluation of *invitro* efficacy of different isolates of actinomycetes against *C. gloeosporiodes* (Dual culture)

Sl.no	Isolate	Mycelial growth (mm)	Percent inhibition over control
T ₁	AUSA ₁	35.66 (37.42)	60.37
T ₂	AUSA ₂	42.70 (41.53)	73.90
T ₃	AUSA ₃	18.25 (26.21)	79.72
T ₄	AUSA ₄	25.04 (30.85)	72.17
T ₅	AUSA ₅	33.34 (36.03)	62.95
T ₆	AUSA ₆	38.71 (39.21)	56.98
T ₇	AUSA ₇	21.27 (28.34)	76.36
T ₈	AUSA ₈	23.49 (29.84)	72.48
T ₉	AUSA ₉	46.58 (43.76)	48.24
T ₁₀ .	AUSA ₁₀	30.52 (34.32)	66.08
T ₁₁ .	Control	90.00	-
C.D. at 5 %		3.01	
S. Em. ±		1.00	

*Mean of three replications, Values in the parentheses are arcsine- transformed values. Values in the column followed by common letters are not differ significantly by DMRT (p=0.05)

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