**Original Research Article**

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

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| --- |
| **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 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", holds significant economic and nutritional importance across tropical and subtropical regions. India is the largest producer of mango, contributing nearly 40% of the global production (Jeevanantham et al. 2024). Despite its commercial value and consumer demand, mango production is severely constrained by various biotic stresses, among which anthracnose, caused by *Colletotrichum gloeosporioides*, is one of the most devastating fungal diseases affecting both pre-harvest and post-harvest stages (Balamurugan et al. 2025). Anthracnose manifests as dark, sunken lesions on leaves, twigs, flowers, and fruits, ultimately leading to flower blight, fruit rot, and considerable yield loss. Under conducive conditions of high humidity and rainfall, the disease spreads rapidly, causing up to 60% post-harvest losses in some cultivars (Zakaria 2025).

Traditionally, the management of anthracnose has relied on the routine application of synthetic fungicides such as carbendazim, mancozeb, and copper oxychloride (Dofuor et al. 2023). While these chemicals offer short-term protection, their prolonged use has led to significant concerns including environmental contamination, residue accumulation, adverse effects on non-target organisms, and the emergence of fungicide-resistant pathogen strains (Iqbal et al. 2022).

In recent years, research has increasingly focused on sustainable and eco-friendly alternatives to chemical control. Several biological agents, including *Trichoderma*, *Pseudomonas*, *Bacillus*, and endophytic fungi, have been explored for their antagonistic activity against *C. gloeosporioides* (Suman et al. 2022). However, many of these agents face limitations such as inconsistent field performance, poor survivability in the rhizosphere, or narrow-spectrum activity. Among microbial biocontrol candidates, actinomycetes particularly *Streptomyces* spp. have attracted growing attention due to their prolific ability to synthesize antifungal metabolites, cell wall-degrading enzymes, siderophores, and volatile organic compounds (Kaur et al. 2023). Prior studies have demonstrated the effectiveness of *Streptomyces* isolates against various phytopathogens, but investigations targeting native *Streptomyces* from mango rhizospheres, specifically against mango anthracnose, remain limited (Khan et al. 2023).

In this context, the current investigation aimed to isolate and characterize native actinomycetes, with a focus on *Streptomyces* spp. from mango rhizosphere soils across different agro-climatic zones of Tamil Nadu and Puducherry. The study aimed to evaluate their morphological traits, in vitro antagonistic potential against *C. gloeosporioides*, and molecular identification of the most promising isolate. By identifying an effective native biocontrol agent, this research contributes to the development of sustainable disease management strategies and reduces reliance on hazardous chemical fungicides in mango cultivation.

**2. Materials and Methods**

**2.1 Isolation of Actinomycetes** (Prole et al. 2025)

Soil samples were collected from the rhizosphere of healthy mango (*Mangifera indica* L.) trees across prominent mango cultivation areas in Tamil Nadu and Puducherry. The collected samples were air-dried, pulverized and sieved to remove debris, then serially diluted up to 10-⁶ in sterile distilled water. Aliquots from 10-³ to 10-⁶ dilutions were aseptically plated on Actinomycetes Isolation Agar (AIA) using the soil dilution technique and incubated at 28°C for 7-14 days. Colonies exhibiting typical actinomycete morphology, characterized by filamentous growth and powdery or leathery texture were selected and purified using the single hyphal tip method. The purified isolates were maintained on AIA slants at 4 °C for further study. The isolates were designated as AUSA01 through AUSA10 for further morphological and molecular characterization.

**2.2 Morphological characterization of Actinomycetes**

The isolates of *Streptomyces* spp. were cultured on Actinomycetes Isolation Agar (AIA) plates, to assess their cultural and morphological characteristics. Macroscopic features such as colony shape, pigmentation, surface texture, and growth rate were recorded after incubation. Microscopic observations of spore morphology were conducted using a 100x I-Scope trinocular fluorescence microscope (Euromex, Netherlands, Model IS 3153 PLFi/3) equipped with a DC 20000i microscope camera and differential interference contrast (DIC) optics. Each isolates was examined in triplicate, and results were analyzed statistically to identify significant differences in growth and morphological features among isolates. For detailed structural characterization, mycelial and sporogenic features were further analyzed using Scanning Electron Microscopy (SEM), following the protocol described by Balamurugan et al. (2025). High-resolution SEM images were obtained from the Centralized Sophisticated Instrumentation Laboratory (CSIL), Department of Physics, Annamalai University. Observed morphological traits were compared with standard taxonomic descriptions outlined in *Bergey’s Manual of Systematic Bacteriology* (Kämpfer 2006) for presumptive identification of the isolates.

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

The inhibitory activity of *Streptomyces* spp. against *C. gloeosporioides* was assessed using the dual culture technique described by Palaniyandi et al. (2011). A 9 mm mycelial disc from a seven days old *C. gloeosporioides* culture was placed approximately 1 cm from one edge of a Potato Dextrose Agar (PDA) plate. A loopful of 5 days old *Streptomyces* culture was streaked at a distance of 1 cm from the opposite edge on the same plate. Control plates were inoculated with the pathogen alone, without any antagonist. The plates were incubated at 28 ± 2°C for seven days under controlled conditions. The extend of mycelial growth inhibition of the pathogen was calculated using the formula described by Vincent (1947):

C-T

inhibition (%) = ……………… X 100

C

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

**2.3 Molecular characterization of Actinomycetes**

The most virulent isolate of *Streptomyces* spp. was cultured in Ken Knight & Munaier’s broth (HiMedia laboratories Pvt. Ltd., Mumbai, Maharashtra, India) and incubated at 30°C for a duration of 48 hours. After incubation, 1 mL of culture was centrifuged at 12,000 rpm for 10 minutes at ambient temperature. The resulting pellet was 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. Genomic DNA was extracted using phenol: chloroform: isoamyl alcohol and chloroform: phenol: isoamyl alcohol, followed by ethanol precipitation with 3 M NaCl (Gill et al. 2025).

The quality and integrity of the extracted DNA were confirmed by electrophoresis on a 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 performed in a Veriti™ Thermal Cycler (Applied Bio systems™, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) under the following conditions: initial denaturation at 95 °C for 5 minutes; 29 cycles of denaturation at 94 °C for 30 seconds, annealing at 52 °C for 30 seconds, and extension at 72 °C for 45 seconds; followed by a final extension at 72 °C for 10 minutes.

Amplified products were confirmed on 1% agarose gel using a 1 kb ladder and visualized under UV light. Bidirectional sequencing of the purified PCR products was performed using an ABI 3730xl Genetic Analyzer (Applied Bio systems™, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Sequence homology was determined using NCBI BLAST tool. The obtained sequence was submitted to the GenBank database, and phylogenetic analysis was using the maximum likelihood method in MEGA 11 (Vigneshwaran et al. 2025).

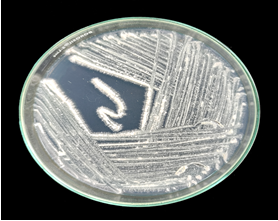
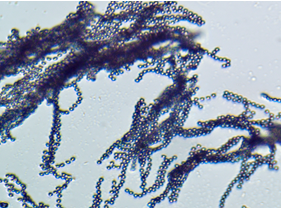
**3. Results and Discussion**

**3.1. Morphological characterization of different isolates of actinomycetes**

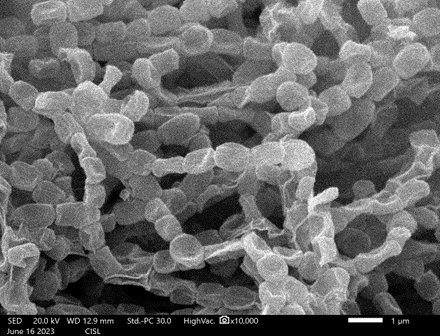
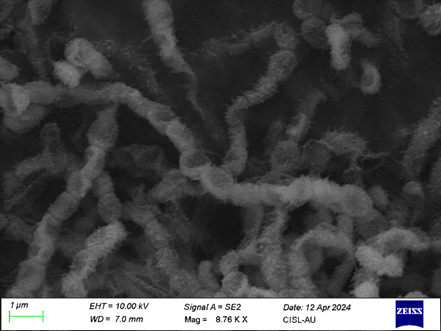
A total of ten actinomycetes strains were isolated from rhizospheric soil samples collected from various mango growing regions of Tamil Nadu and Puducherry. These isolates were designated as AUSA1 to AUSA10, were cultured on Actinomycetes Isolation Agar (AIA) medium, and incubated 10 days to assess their cultural and morphological characteristics. Each isolate exhibited distinct features, including variations in mycelial pigmentation, colony shape, surface texture, spore morphology and growth rate.

The aerial mycelium displayed a range of pigmentation from white-grey to light grey, while the substrate mycelium varied in colour from whitish-grey, yellow, red, greyish-yellow, and brownish-yellow. Colony morphologies included round forms with concentric lines, radiating patterns, feather-like structures, irregular shapes, and double-ring configurations. Surface textures ranged from smooth to powdery, and all isolates exhibited rod-shaped spores with smooth surfaces. Growth rates were categorized as slow, moderate, good or very good (Table 1 & Fig 1, 2, 3a and 3b).

Previous studies have emphasized that the filamentous growth and sporulation patterns of actinomycetes, particularly *Streptomyces* spp., play a crucial role in their colonization of plant hosts and competitiveness in the rhizosphere (Shepherdson et al. 2023). These morphological traits are vital for differentiating *Streptomyces* from other genera of spore-forming actinomycetes (Taddei et al. 2006). Actinomycetes typically exhibit well-developed aerial and substrate mycelia, observable under microscopy (Nguyen et al. 2025). Furthermore, variations in spore size, shape and arrangement – such as the formation of spore chains provide additional taxonomic markers (Hassan et al. 2025). Differences in colony pigmentation, texture, and morphology on selective media continue to be essential criteria in the presumptive identification and classification of actinomycetes (Helmi 2025).



**Fig. 1. Pure Culture of *S. albofaciens*  Fig. 2. Microscopic observations of *S. albofaciens* (100 x magnification)**



**Fig. 3a Fig. 3b**

**Fig. 3a & 3b. SEM** **showing spore chain morphology and spore surface ornamentation of *S. albofaciens***

**3.2. Evaluation of *in vitro* efficacy of different isolates of actinomycetes against *C. gloeosporiodes* (Dual culture)**

All ten *Streptomyces* isolates (AUSA1 to AUSA10) were evaluated for their antagonistic efficacy against *C. gloeosporioides* using the dual culture technique on PDA medium (Table 2). Among the isolates, 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, which showed 46.24% inhibition. All isolates significantly suppressed the radial growth of the pathogen compared to the control, confirming their antagonistic potential under in vitro conditions. These results align with findings reported by Lai et al. (2024), who demonstrated that actinomycetes exhibit strong antifungal activity against *C. gloeosporioides,* primarily through the production of hydrolytic enzymes degrade the fungal cell wall. Similarly, Li et al. (2024) reported that volatile organic compounds (VOCs) produced by *S. corchorusii* exhibited in vitro mycelial inhibition of 50.77 per cent against *C. gloeosporioides* infecting Strawberries.

**3.3 Molecular characterization of virulent isolate of *S. albofaciens* (AUSA3)**

Ten actinomycetes isolates (AUSA1 to AUSA10) were screened for their antagonistic efficacy against mango anthracnose. Among the isolates AUSA3, exhibited the highest inhibition against *C. gloeosporiodes* and was thus selected for molecular identification. Genomic DNA from AUSA3 was extracted and subjected to PCR amplification using universal 16S rRNA primers 27F and 1492R. The resulting amplicon was approximately 909 bp, confirming 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 was subsequently submitted to the GenBank database under accession number PV603281. The identification was consistent with earlier studies. Duangupama et al. (2024), reported the isolation of *Streptomyces* from the rhizosphere of mango trees and confirmed its identity using PCR with the use of 16S rRNA specific forward F243 and A3R, which yielded as 650 bp amplicon. Similarly, Singh et al. (2024) identified *S. albofaciens* strain MS38, using 16S rRNA sequencing, generating an amplicon size of 1489 bp. Zainal Abidin et al. (2016) also described five effective antagonistic actinomycete isolates, identified as *Streptomyces* spp., producing approximately 1250 bp amplicons using the same primer pair. The identification of *S. albofaciens* is commonly confirmed through comparative sequence analysis of the 16S rDNA sequence offers insights into evolutionary relationships and aids in the accurate classification of bacterial isolates (Rai et al. 2025).

**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. gloeosporiodes* in dual culture bioassays. Molecular delineation *via.* 16S rRNA gene sequencing authenticated AUSA3 as *S. albofaciens*.

**Disclaimer (Artificial Intelligence)**

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 | AUSA1 | White | Red | Round with radiated | Rough | Smooth | Moderate | Rod shaped |
| 2. | Valapadi | AUSA2 | Grey | Brownish yellow | Irregular | Rough | Smooth | Good | Rod shaped |
| 3. | Kattukottai | AUSA3 | White | Bright yellow | Round | Powdery | Smooth | Very Good | Rod shaped |
| 4. | Shoolagiri | AUSA4 | White | Light Yellow | Round with lines | Smooth | Smooth | Slow | Rod shaped |
| 5. | Sivapuri | AUSA5 | Light grey | Greyish yellow | Round | Powdery | Smooth | Slow | Rod shaped |
| 6. | Papparapatti | AUSA6 | White | Moderate yellow | Two round circles | Smooth | Smooth | Good | Rod shaped |
| 7. | Kaveripattinam | AUSA7 | White | Greyish yellow | Double ring | Smooth | Smooth | Slow | Rod shaped |
| 8. | Nannilam | AUSA8 | White | Light grey | Round | Smooth | Smooth | Good | Rod shaped |
| 9. | Ayothiyampattinam | AUSA9 | Light grey | Yellowish grey | Round with feathers | Rough | Smooth | Moderate | Rod shaped |
| 10. | Kollidam | AUSA10 | Grey | Pale yellow | Round with feathers | Powdery | smooth | Good | Rod shaped |

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

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl.no** | **Isolate** | **Mycelial growth**  **(mm)** | **Percent inhibition over control** |
| T1 | AUSA1 | 35.66 (37.42) | 60.37 |
| T2 | AUSA2 | 42.70 (41.53) | 73.90 |
| T3 | AUSA3 | 18.25 (26.21) | 79.72 |
| T4 | AUSA4 | 25.04 (30.85) | 72.17 |
| T5 | AUSA5 | 33.34 (36.03) | 62.95 |
| T6 | AUSA6 | 38.71 (39.21) | 56.98 |
| T7 | AUSA7 | 21.27 (28.34) | 76.36 |
| T8 | AUSA8 | 23.49 (29.84) | 72.48 |
| T9 | AUSA9 | 46.58 (43.76) | 48.24 |
| T10. | AUSA10 | 30.52 (34.32) | 66.08 |
| T11. | 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)