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Diversity, Molecular Identification and Antagonistic Potential of Culturable Tomato Rhizobiome against *Fusarium* Wilt across Tamil Nadu, India

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Authors' contributions

This work was carried out in collaboration between both authors. Author SJ designed the methodology, conducted the formal analysis, and prepared the original draft of the manuscript. Author DJC supervised the study and contributed to the investigation. Both authors read and approved the final manuscript.

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Abstract

Aims: To investigate the diversity and molecular identity of culturable bacterial and fungal microorganisms associated with tomato rhizosphere soils across Tamil Nadu, India, and evaluate their antagonistic potential against *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*).
Study Design: Laboratory-based isolation, characterisation, molecular identification and *In vitro* antagonism experiments.
Place and Duration of Study: Twenty tomato-growing locations across seven districts of Tamil Nadu, India, namely Coimbatore, Krishnagiri, Namakkal, Dharmapuri, Dindigul, Kallakurichi and Salem.
Methodology: Rhizosphere soil samples were collected from healthy tomato plants, and culturable microorganisms were isolated using selective and general-purpose media. A total of 32 bacterial and 13 fungal isolates were obtained. Bacterial isolates were characterised based on morphological and biochemical properties, while fungal isolates were examined through cultural and

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microscopic characteristics. Molecular identification was performed using 16S rRNA gene sequencing for bacteria and internal transcribed spacer (ITS) sequencing for fungi. The antagonistic activity of all isolates against *Fol* was assessed using dual-culture and inverted-plate assays, with four replications. Data were analysed using ANOVA, followed by Duncan's multiple range test (DMRT) at $P \leq 0.05$.

Results: Molecular characterisation identified 20 bacterial genera, with *Bacillus* (seven isolates) and *Pseudomonas* (four isolates) being predominant. The 13 fungal isolates represented 11 genera, including *Penicillium*, *Aspergillus*, *Trichoderma* and *Mortierella*. In the dual-culture assay, bacterial and fungal isolates exhibited maximum inhibition of 77.95% and 77.45%, respectively. In the inverted-plate assay, the highest inhibition was 76.73% for bacterial isolate TRB45 and 74.12% for fungal isolate TRF33. Notably, TRB45 and TRF33 consistently exhibited the highest inhibitory activity in both assays.

Conclusion: The study revealed considerable diversity among culturable tomato rhizosphere bacteria and fungi across Tamil Nadu and identified TRB45 and TRF33 as promising antagonistic isolates against *Fol*. Further greenhouse and field evaluations, along with investigations into their mechanisms of antagonism, are warranted to assess their potential for sustainable biological management of tomato *Fusarium* wilt.

Keywords: Tomato; rhizosphere microbiome; molecular identification; antagonistic activity; Fusarium wilt.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop cultivated worldwide and valued for its nutritional composition, particularly vitamins, minerals, dietary fibre and antioxidant compounds such as lycopene. Despite its agricultural and nutritional significance, tomato production is constrained by several biotic stresses, particularly soil-borne diseases that adversely affect crop growth and yield. Among these, *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), is a major disease affecting tomato cultivation. The pathogen infects plants through the root system, colonises vascular tissues and disrupts water and nutrient transport, resulting in yellowing, vascular browning and wilting. Its persistence in soil makes disease management challenging, highlighting the need for sustainable biological control strategies (Meshram and Adhikari, 2024; Mwaka *et al.,* 2026).

Jimoh and Afe (2024) evaluated *Rhizobium etli* strain CIAT 899 in a tomato *Fusarium* wilt management study, illustrating a further bacterial approach investigated for this disease.

The rhizosphere is a biologically active region surrounding plant roots that harbours diverse bacterial and fungal communities. Root exudates influence microbial recruitment and establish complex interactions between plants and soil microorganisms. These microbial communities contribute to nutrient acquisition, plant growth and protection against soil-borne pathogens through nutrient competition, antimicrobial metabolite production, hydrolytic enzyme secretion and induction of plant defence responses. Recent studies have demonstrated that interactions between bacterial and fungal rhizosphere communities play an important role in suppressing tomato *Fusarium* wilt (Zhou *et al.,* 2022; Jin *et al.,* 2024).

Zhao *et al*. (2026) reported inhibition of *Fol* by a three-strain bacterial consortium that was not observed with the corresponding individual or paired cultures, indicating that interactions among rhizosphere microorganisms can influence antagonistic activity.

Among rhizosphere-associated bacteria, genera such as *Bacillus*, *Pseudomonas*, *Streptomyces* and *Paenibacillus* are recognised for their plant growth-promoting and antagonistic properties. Their biocontrol potential is associated with the production of antibiotics, lipopeptides, siderophores, volatile organic compounds and extracellular enzymes. In addition, certain rhizobacteria promote plant growth through nutrient solubilisation and activation of host defence responses. These characteristics make beneficial bacteria promising candidates for the biological management of soil-borne diseases, including Fusarium wilt of tomato (Meshram and Adhikari, 2024; Modiba *et al.,* 2025).

Viana *et al*. (2024) observed strain-dependent direct and volatile-associated antagonism against *Fol* among tomato-associated *Bacillus* endophytes.

Tiendrebeogo *et al*. (2026) identified indigenous antagonistic bacteria, including *Bacillus* and *Pseudomonas*, in a study of tomato vascular wilt, providing further evidence for screening locally recovered isolates.

Similarly, rhizosphere-associated fungi contribute to soil microbial diversity, nutrient cycling and biological disease suppression. Beneficial fungal genera, particularly *Trichoderma* and *Penicillium*, can inhibit phytopathogens through mycoparasitism, competition, antimicrobial metabolite production and volatile-mediated inhibition. Previous studies have demonstrated the contribution of fungal communities and *Trichoderma*-associated interactions to the suppression of tomato Fusarium wilt (Zhou *et al.,* 2022; Bandara and Kang, 2024). Therefore, investigating both bacterial and fungal rhizosphere microorganisms may provide opportunities to identify diverse antagonists with complementary biocontrol potential.

The composition and functional diversity of rhizosphere microorganisms are influenced by geographical location, soil characteristics and environmental conditions. Recent investigations have also reported differences in bacterial and fungal communities between healthy and *Fusarium* wilt-affected tomato rhizospheres (Guo *et al.,* 2025). Hence, exploring indigenous microbial populations from different tomato-growing regions is important for identifying promising microorganisms adapted to local agroecological conditions.

Giri *et al*. (2026) screened tomato rhizosphere bacteria collected in Assam and reported differences in antagonistic activity among isolates, supporting regionally focused evaluation of microbial candidates.

Although previous studies have investigated rhizosphere microorganisms and their antagonistic activity, they do not establish the combined molecular diversity and comparative *In vitro* inhibitory activity of culturable bacterial and fungal isolates recovered from the tomato-growing locations surveyed in Tamil Nadu.

In this context, the present study was undertaken to characterise culturable bacterial and fungal rhizosphere-associated microorganisms isolated from tomato-growing regions across Tamil Nadu, India. The isolates were subjected to phenotypic, biochemical and microscopic characterisation, followed by molecular identification using 16S rRNA and internal transcribed spacer (ITS) gene sequencing. Furthermore, their antagonistic potential against *Fol* was evaluated through dual-culture and inverted-plate assays to assess direct and volatile-associated inhibitory activities. The findings aim to identify promising indigenous rhizosphere microorganisms for further investigation towards the sustainable biological management of tomato *Fusarium* wilt.

2. Materials and Methods

2.1 Collection of Rhizosphere Soil Samples

A field survey was conducted across 20 tomato-growing locations in seven districts of Tamil Nadu, India, namely Coimbatore, Krishnagiri, Namakkal, Dharmapuri, Dindigul, Kallakurichi, and Salem. Healthy tomato plants representing different cultivars and agroecological conditions were selected, and rhizosphere soil adhering to their roots was collected. Samples were transferred into sterile containers, transported under cool conditions and processed for microbial isolation.

2.2 Isolation of Rhizosphere Microorganisms

Soil samples were homogenized in sterile double-distilled water and serially diluted up to 10^{-7}. Appropriate dilutions were plated onto nutrient agar and tryptic soy agar for bacterial isolation, potato dextrose agar for fungal isolation and actinomycetes isolation agar for actinomycete recovery. Following incubation at 28 ± 2°C, morphologically distinct colonies were selected and purified through repeated subculturing. The purified cultures were designated as tomato rhizobacterial isolates (TRB44–TRB75) and tomato rhizosphere fungal isolates (TRF25–TRF37).

2.3 Morphological and Biochemical Characterization

Bacterial isolates were characterised based on colony pigmentation, form, margin, elevation, texture and surface characteristics. Gram staining and oxidase, catalase and methyl red tests were performed following standard procedures (Cappuccino & Welsh, 2017).

Fungal isolates were examined for colony pigmentation, texture, growth pattern, margin and sporulation. Microscopic characteristics, including hyphal septation, conidiophore structure, conidial morphology and reproductive structures, were examined using lactophenol cotton blue (LPCB) staining.

2.4 Molecular Identification of Bacterial Isolates

The 16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Frank *et al*., 2008). PCR was performed in a 25 µL reaction mixture containing 12.5 µL of 2× PCR Master Mix, 0.5 µL each of forward and reverse primers (10 µM), 2 µL of genomic DNA and 9.5 µL of nuclease-free water. Amplification was carried out with an initial denaturation at 95°C for 3 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 amplified products were resolved on 1% agarose gel and purified before sequencing.

2.5 Molecular Identification of Fungal Isolates

The internal transcribed spacer (ITS) region was amplified using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*., 1990). PCR was performed in a 25 µL reaction mixture containing 1× PCR buffer, 200 µM of each dNTP, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 1 U of Taq DNA polymerase and 50 ng of genomic DNA. The amplification programme consisted of initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were separated on 1.2% agarose gel and submitted for Sanger sequencing.

2.6 *In vitro* Evaluation of Antagonistic Activity against *Fol*

The antagonistic potential of the bacterial and fungal isolates against *Fol* was evaluated using dual-culture and inverted-plate assays.

2.6.1 Dual-culture Assay

A 9 mm mycelial disc from a 7-day-old *Fol* culture was placed on one side of a potato dextrose agar plate supplemented with streptomycin sulphate. Bacterial isolates were streak-inoculated opposite the pathogen, while fungal isolates were introduced as mycelial discs. Plates containing Fol alone served as controls. Following incubation at 28 ± 2°C for 4–5 days, pathogen mycelial growth was measured and compared with the untreated control.

2.6.2 Inverted-plate Assay

The pathogen and individual antagonists were cultured separately on their respective media. A 9 mm *Fol* mycelial disc was placed at the centre of the pathogen-containing plate, while the antagonist was inoculated onto a separate plate. Both plates were assembled face-to-face, sealed with parafilm and incubated at 28 ± 2°C for 4–5 days. The radial growth of Fol was measured and compared with the untreated control to determine volatile-associated inhibition.

2.7 Calculation of Percentage Inhibition

Percentage inhibition of *Fol* mycelial growth was calculated using the following formula:

$$\text{Percent Inhibition (\%)} = (C-T)/C \times 100.$$

Where *C* represents pathogen growth in the untreated control and *T* represents pathogen growth in the presence of the antagonist.

2.8 Statistical Analysis

Experiments were conducted with four replications. Data were subjected to analysis of variance (ANOVA), and treatment means were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$. Percentage inhibition values were arcsine square-root transformed before analysis, while original values were presented in the tables.

3. Results and Discussion

3.1 Phenotypic Characterization of Bacterial Rhizosphere Isolates

A total of 32 culturable bacterial rhizosphere isolates (TRB44–TRB75) from tomato-growing regions across Tamil Nadu exhibited considerable variation in colony morphology, including pigmentation, texture, growth pattern, aggregation and margin characteristics. Colony colours ranged from bluish-white, cream-white and ivory to pale yellow, tan and greyish-white, with growth varying from discrete colonies to dense and confluent patterns (Table 1; Fig. 1). Biochemical characterisation revealed that 18 isolates were Gram-positive and 14 were Gram-negative.

All isolates were catalase-positive, while 14 and nine isolates exhibited positive oxidase and methyl red reactions, respectively. These morphological and biochemical differences indicate phenotypic diversity among the culturable tomato rhizosphere bacteria. Similar diversity in the morphological, biochemical and functional characteristics of tomato-associated rhizobacteria has been reported by Chhetri *et al.* (2025) and Kaundal *et al.* (2025). Furthermore, studies by Jin *et al.* (2024) and Guo *et al.* (2025) highlighted the influence of root exudates and plant health status on tomato rhizosphere microbial community composition. Collectively, these findings emphasise the importance of characterizing indigenous rhizosphere bacteria to identify microbial resources with potential applications in tomato disease management.

3.2 Phenotypic Characterisation of Fungal Rhizosphere Isolates

Thirteen culturable fungal rhizosphere isolates (TRF25–TRF37) exhibited considerable variation in cultural characteristics, including colony pigmentation, texture, growth pattern and sporulation. Colony colours ranged from olive-green, yellowish-green and greyish-brown to cream, salmon, white and dark brown, while textures varied from velvety and granular to powdery, woolly and cottony (Table 2; Fig. 2).

Microscopic examination revealed differences in hyphal morphology and reproductive structures. Septate hyphae were observed in TRF25, TRF27, TRF28, TRF29, TRF32, TRF33, TRF34 and TRF37, whereas TRF30 and TRF36 exhibited broad, predominantly aseptate hyphae with sporangiophores and sporangia. Other structures included conidiophores, phialides, chains of conidia, globose vesicles, curved septate conidia, rhizoids, stolons, asci and pigmented ascospores. These variations highlight the morphological diversity of the fungal isolates recovered from tomato rhizosphere soils.

Comparable fungal diversity was reported by Paulina Fatima Mary and Sagaya Giri (2016), who recovered 16 fungal species belonging to six genera from tomato rhizosphere soils in Tamil Nadu. Similarly, Kazerooni *et al.* (2017) documented fungal diversity in tomato rhizosphere soils under different farming systems. The ecological relevance of this diversity is illustrated by Coulibaly *et al.* (2022), who identified several tomato rhizosphere fungi, including *Trichoderma*, *Talaromyces*, *Penicillium* and *Clonostachys*, with antagonistic activity against *Sclerotium rolfsii*. Thus, morphological characterisation provides a preliminary basis for investigating the taxonomic diversity and potential functional roles of tomato-associated rhizosphere fungi.

Table 1. Phenotypic characterization of culturable bacterial rhizospheric microbes associated with tomato plants across Tamil Nadu

S. No.	Isolate	Colony characteristics	Bio chemical characteristics			
			Gram stain	Oxidase	Catalase	Methyl Red
1	TRB44	Bluish-white, finely granular growth with dense streak-associated development and irregular margins	−	+	+	+
2	TRB45	Tan to brownish growth with a dull surface and localized irregular, spreading patches	+	−	+	+
3	TRB46	Pale cream growth forming a broad, confluent layer with uneven peripheral outlines	−	−	+	+
4	TRB47	White, narrow streak-associated growth with smooth-looking margins and an isolated sinuous extension	−	+	+	−
5	TRB48	Translucent to whitish growth arranged in fine, parallel streaks with sparse isolated colonies	+	+	+	−
6	TRB49	Cream-white growth with uneven, clustered deposits and a mixture of dense and sparse streak regions	−	−	+	−
7	TRB50	Opaque ivory growth forming thick streaks and a few compact, lobed aggregates	+	−	+	−
8	TRB51	Pale bluish-white growth accompanied by numerous small, discrete, rounded colonies	+	−	+	−
9	TRB52	Abundant off-white growth with irregular, coalescing masses and uneven colony boundaries	+	−	+	+
10	TRB53	Beige to pale cream growth appearing as elongated, smooth-edged streaks	+	−	+	+
11	TRB54	Profuse cream-coloured growth with numerous raised-looking, irregular clusters of different sizes	+	+	+	−
12	TRB55	Fine cream-white growth with closely spaced pinpoint colonies and a narrow irregular extension	−	+	+	+
13	TRB56	Whitish growth with uneven, lobate aggregates interspersed among linear streaks	−	+	+	−
14	TRB57	Light cream growth forming continuous, slender streaks with a smooth, uniform appearance	+	−	+	−
15	TRB58	Pale white growth with diffuse, feathery edges and broad merging streaks	+	+	+	−
16	TRB59	Cream-white growth showing compact irregular patches and dense streak development	−	+	+	+
17	TRB60	Whitish to pale yellow growth with small clustered aggregates and an uneven distribution	−	−	+	−
18	TRB61	Heavy ivory-white growth with coarse, irregular masses concentrated along the inoculation lines	+	−	+	+
19	TRB62	White, narrow linear growth with sharply defined streak patterns and limited isolated growth	−	−	+	−
20	TRB63	Bluish-white, translucent growth with thin linear deposits and a relatively diffuse appearance	+	+	+	−
21	TRB64	Pale blue-white growth with fine streaks and a distinct curved, filament-like extension	+	−	+	−
22	TRB65	Numerous minute, cream-white colonies distributed along dense streaks with a small irregular extension	−	−	+	−
23	TRB66	Greyish-white, confluent growth with a finely textured appearance and indistinct margins	+	+	+	−
24	TRB67	Pale yellowish-cream growth forming broad, irregular patches with merging peripheral growth	−	−	+	+
25	TRB68	Opaque cream-white growth with several prominent, compact aggregates and uneven outlines	+	−	+	+
26	TRB69	Off-white growth with irregular, lobed deposits and dense streak-associated development	+	+	+	−
27	TRB70	Cream-coloured, thick growth forming broad, undulating masses along the streaked region	+	+	+	−
28	TRB71	Fine white colonies arranged in closely spaced rows with a narrow, curved extension	−	−	+	−
29	TRB72	Bluish-white, fine streak growth with a discrete curved extension and relatively uniform distribution	−	+	+	+
30	TRB73	Pale yellow to cream growth with scattered small colonies against a darker agar background	−	−	+	−
31	TRB74	Cream-white growth with a few compact, irregular aggregates and broad linear streaks	+	+	+	−
32	TRB75	Pale greyish-white growth with dense, merging streaks and a diffuse, uneven appearance	+	−	+	−

Table 2. Phenotypic assessment of culturable fungal rhizospheric microbes recovered from tomato plants across Tamil Nadu

S. No.	Isolate	Colony characteristics	Microscopic characteristics
1	TRF25	Olive-green to yellowish-green, velvety colonies with dense sporulation and concentric growth zones	Septate hyphae, branched penicillate conidiophores, phialides and chains of globose conidia
2	TRF26	Greyish-brown, velvety colonies with a dark central region and pale cream margins	Septate hyphae, reproductive structures and ascospores require microscopic confirmation
3	TRF27	Black, granular colonies with dense sporulation and scattered dark patches	Septate hyphae, conidiophores with globose vesicles, phialides and globose conidia
4	TRF28	Yellowish-green, powdery colonies with irregular sporulating patches and darker central zones	Septate hyphae, conidiophores with globose vesicles, phialides and chains of rough-walled conidia
5	TRF29	Dark olive-brown, velvety colonies with a black central region and pale peripheral growth	Pigmented, septate hyphae, geniculate conidiophores and curved, transversely septate conidia
6	TRF30	White to pale yellow, cottony colonies with concentric growth zones and a slightly raised centre	Broad, predominantly aseptate hyphae, sporangiophores and sporangia
7	TRF31	Cream to pale buff, cottony colonies with diffuse margins and a dark central region	Broad, hyaline hyphae; sporangiophores and sporangia-like reproductive structures
8	TRF32	Greenish-grey, woolly colonies with dense radial mycelial growth and a pale central region	Septate hyphae, sexual structures and ascospores require microscopic confirmation
9	TRF33	Cream to salmon-coloured, compact colonies forming irregular, raised sporulating patches	Septate hyphae, branched conidiophores, phialides and conidia
10	TRF34	Cream to pale yellow, powdery colonies with irregular growth patches and darker intervening regions	Septate hyphae, branched penicillate conidiophores, metulae, and chains of conidia
11	TRF35	Greyish-brown, cottony colonies with irregular white aerial mycelium and scattered dark regions	Microscopic reproductive structures, colony appearance alone is insufficient for diagnosis
12	TRF36	Dark brown to black, densely sporulating colonies with a granular surface and pale outer margins	Broad, predominantly aseptate hyphae, rhizoids, stolons, sporangiophores and sporangia
13	TRF37	Rapidly spreading, white cottony colonies with dense aerial mycelium and a pale-yellow central region	Septate hyphae, globose to subglobose ascomata bearing characteristic hairs, asci and pigmented ascospores



Fig. 1. Colony morphology of bacterial rhizospheric isolates recovered from tomato plants across Tamil Nadu

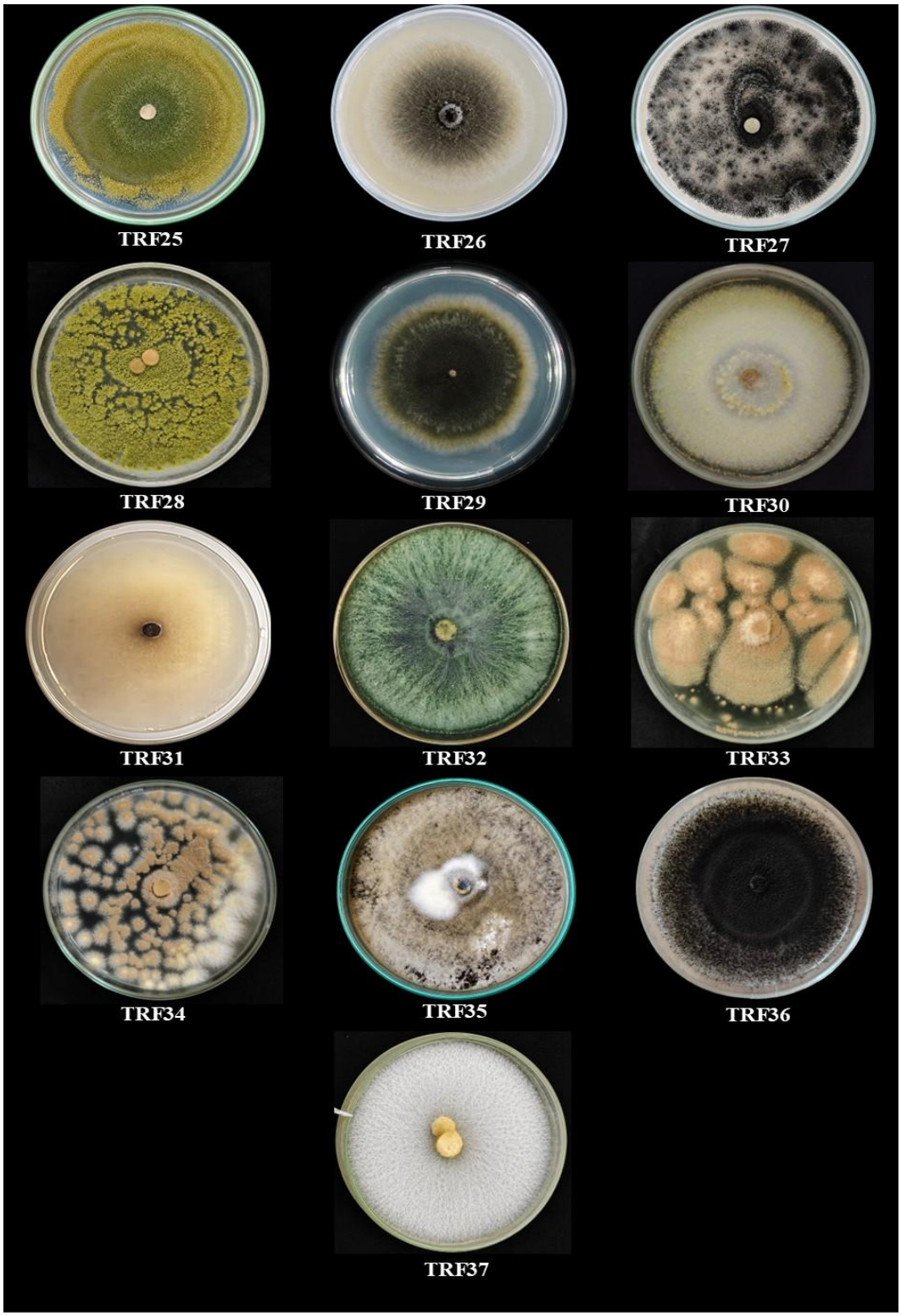

Fig. 2. Colony morphology of fungal rhizospheric isolates recovered from tomato plants across Tamil Nadu

3.3 Molecular Identification of Bacterial Rhizosphere Isolates

The 32 bacterial isolates were identified through 16S rRNA gene sequencing and BLASTn analysis against the NCBI GenBank database (Table 3). Sequence lengths ranged from 1,312 to 1,526 bp, with 100% query coverage and sequence identity and E-values of 0.0. The sequences were deposited in GenBank under accession numbers PZ501777–PZ501808.

Table 3. Sequence-based molecular characterisation of culturable bacterial rhizospheric microbes associated with tomato across Tamil Nadu

S. No.	Isolate	Sequence length (bp)	Closest match	GenBank accession
1	TRB44	1,378	*Achromobacter marplatensis*	PZ501777
2	TRB45	1,432	*Streptomyces griseus*	PZ501778
3	TRB46	1,335	*Pseudomonas simiae*	PZ501779
4	TRB47	1,348	*Martinezella rhizogenes*	PZ501780
5	TRB48	1,312	*Paenibacillus polymyxa*	PZ501781
6	TRB49	1,458	*Pseudomonas fluorescens*	PZ501782
7	TRB50	1,504	*Bacillus subtilis*	PZ501783
8	TRB51	1,384	*Microbacterium testaceum*	PZ501784
9	TRB52	1,400	*Bacillus cereus*	PZ501785
10	TRB53	1,440	*Micromonospora maris*	PZ501786
11	TRB54	1,425	*Micromonospora rosaria*	PZ501787
12	TRB55	1,429	*Devosia riboflavina*	PZ501788
13	TRB56	1,394	*Acidovorax radicis*	PZ501789
14	TRB57	1,526	*Streptomyces avermitilis*	PZ501790
15	TRB58	1,436	*Bacillus mycoides*	PZ501791
16	TRB59	1,478	*Luteolibacter pohnpeiensis*	PZ501792
17	TRB60	1,485	*Dyella japonica*	PZ501793
18	TRB61	1,421	*Actinospica acidiphila*	PZ501794
19	TRB62	1,477	*Mesorhizobium loti*	PZ501795
20	TRB63	1,442	*Enterococcus faecalis*	PZ501796
21	TRB64	1,487	*Arthrobacter* sp.	PZ501797
22	TRB65	1,345	*Myroides odoratimimus*	PZ501798
23	TRB66	1,504	*Bacillus paramycoides*	PZ501799
24	TRB67	1,428	*Pseudomonas fluorescens*	PZ501800
25	TRB68	1,474	*Bacillus thuringiensis*	PZ501801
26	TRB69	1,432	*Glycomyces harbinensis*	PZ501802
27	TRB70	1,455	*Bacillus amyloliquefaciens*	PZ501803
28	TRB71	1,474	*Myroides marinus*	PZ501804
29	TRB72	1,494	*Dokdonella koreensis*	PZ501805
30	TRB73	1,418	*Pseudomonas aeruginosa*	PZ501806
31	TRB74	1,438	*Bacillus licheniformis*	PZ501807
32	TRB75	1,459	*Nocardioides plantarum*	PZ501808

Table 4. Sequence-based molecular identification of culturable fungal rhizospheric microbes associated with tomato across Tamil Nadu

S. No.	Isolate	Sequence length (bp)	Closest match	GenBank Accession
1	TRF25	534	*Penicillium griseofulvum*	PZ507486
2	TRF26	554	*Pseudeurotium ovale*	PZ507487
3	TRF27	572	*Aspergillus niger*	PZ507488
4	TRF28	531	*Aspergillus flavus*	PZ507489
5	TRF29	560	*Curvularia geniculata*	PZ507490
6	TRF30	593	*Mucor irregularis*	PZ507491
7	TRF31	611	*Mortierella polygonia*	PZ507492
8	TRF32	570	*Poaceascoma filiforme*	PZ507493
9	TRF33	568	*Trichoderma gamsii*	PZ507494
10	TRF34	564	*Penicillium oxalicum*	PZ507495
11	TRF35	436	*Powellomyces hirtus*	PZ507496
12	TRF36	538	*Rhizopus schipperae*	PZ507497
13	TRF37	517	*Chaetomium globosum*	PZ507498

The isolates represented 20 bacterial genera, of which *Bacillus* (seven isolates) and *Pseudomonas* (four isolates) were predominant. *Streptomyces*, *Micromonospora* and *Myroides* were represented by two isolates each, while the remaining 15 genera were represented by single isolates. Phylogenetic analysis further supported the sequence-based assignments, showing the clustering of isolates with their closest reference taxa (Fig. 3).

Similar taxonomic diversity has been reported in tomato-associated bacterial communities by Anzalone *et al.* (2021). Nicotra *et al.* (2024) also identified diverse bacterial genera, including *Bacillus*, *Pseudomonas*, *Paenibacillus* and *Microbacterium*, among tomato-associated bacterial isolates. The predominance of *Bacillus* and *Pseudomonas* in the present collection is noteworthy, as members of these genera have been investigated for their antagonistic potential against plant pathogens, including *Fusarium oxysporum* (Prashar *et al.,* 2013). However, 16S rRNA sequencing has limited resolution for distinguishing certain closely related bacterial species (Bartoš *et al.,* 2024).

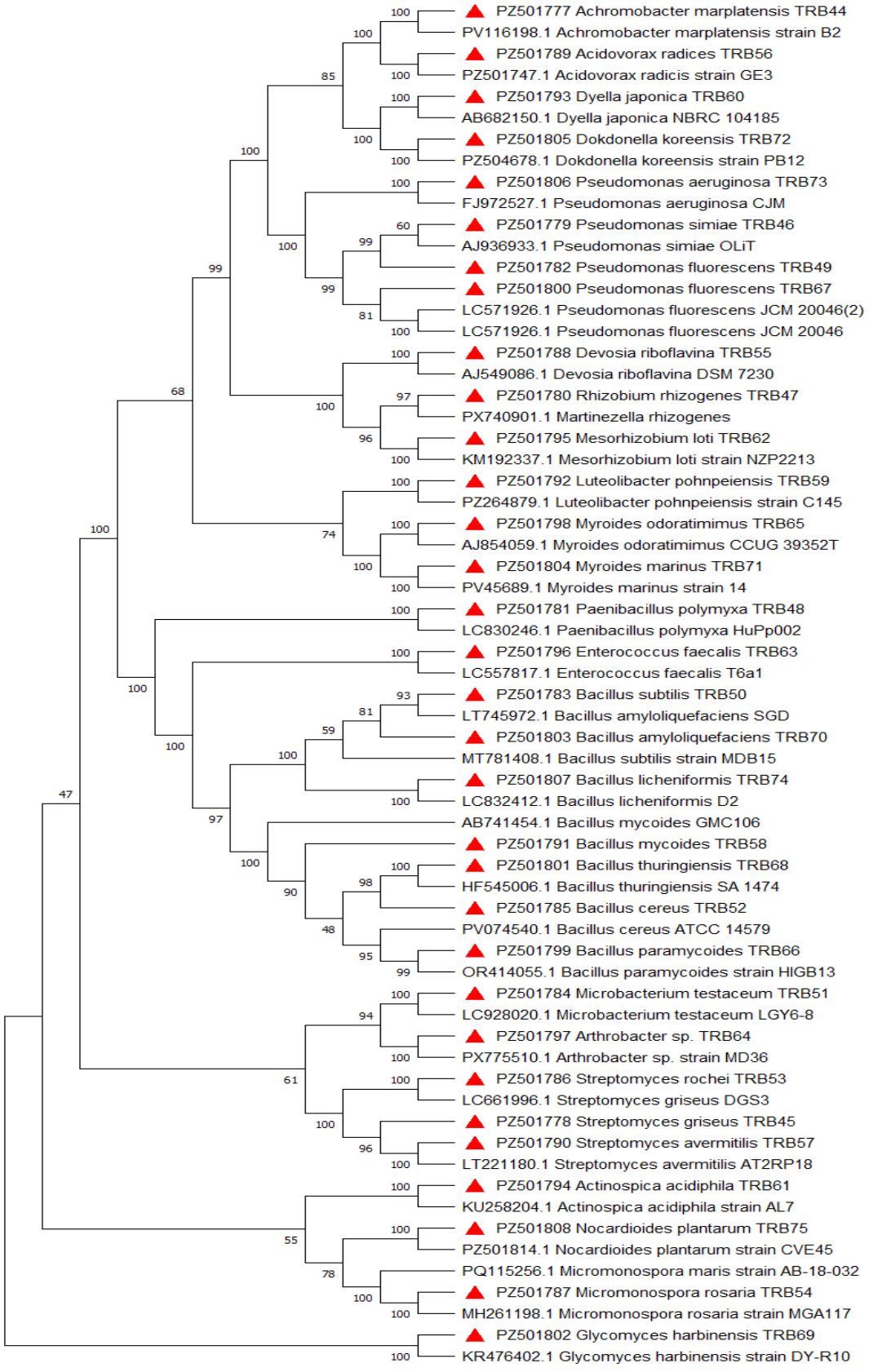

Fig. 3. Phylogenetic tree of rhizospheric bacterial isolates based on 16S rRNA gene sequences

3.4 Molecular Identification of Fungal Rhizosphere Isolates

The 13 fungal rhizosphere isolates were identified using ITS region sequencing and BLASTn analysis against the NCBI GenBank database (Table 4). Sequence lengths ranged from 436 to 611 bp, with 100% query coverage and sequence identity and E-values of 0.0. The isolates represented 11 genera, including *Penicillium* (two isolates), *Aspergillus* (two isolates), *Pseudeurotium*, *Curvularia*, *Mucor*, *Mortierella*, *Poaceascoma*, *Trichoderma*, *Powellomyces*, *Rhizopus* and *Chaetomium*. The sequences were deposited in GenBank under accession numbers PZ507486–PZ507498. Phylogenetic analysis further supported the BLASTn-based assignments by showing the clustering of isolates with their closely related reference taxa (Fig. 4).

Comparable fungal diversity in tomato rhizospheres has been reported by Kazerooni *et al.* (2017), who identified genera such as *Aspergillus*, *Penicillium*, *Mortierella* and *Chaetomium*. Similarly, Chen *et al.* (2026) demonstrated that soil type influenced fungal community composition in tomato rhizospheres, with variations in the occurrence of *Aspergillus*, *Penicillium*, *Trichoderma*, *Mortierella* and *Curvularia*. These studies highlight the diversity of tomato-associated fungi and the influence of environmental conditions on their distribution.

The recovery of *Trichoderma* and *Penicillium* is particularly relevant because selected members of these genera have demonstrated antagonistic activity against soil-borne pathogens. For instance, Coulibaly *et al.* (2022) reported antagonistic fungi, including *Trichoderma* and *Penicillium*, from tomato rhizosphere soils against *Sclerotium rolfsii*. However, their presence in the present collection does not necessarily indicate biocontrol activity, which requires confirmation through functional screening.

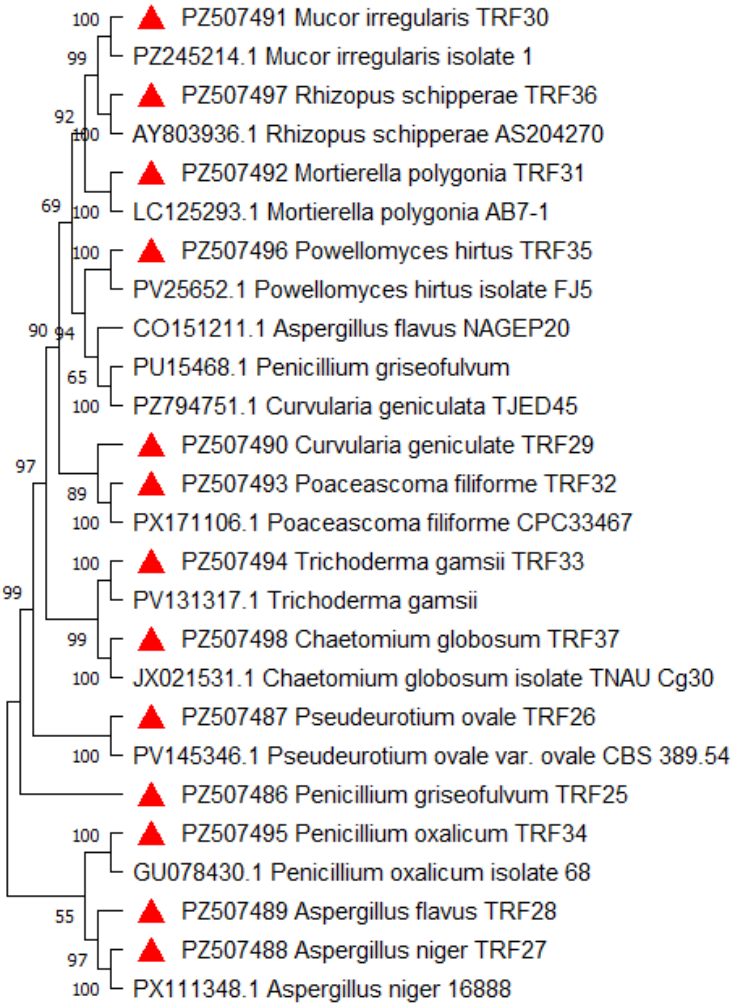

Fig. 4. Phylogenetic tree of rhizospheric fungal isolates based on ITS rDNA sequences

3.5 *In vitro* Evaluation of Antifungal Activity against *Fol*

The antagonistic activity of the bacterial and fungal rhizosphere isolates against *Fol* was evaluated using dual-culture and inverted-plate assays. Considerable variation in mycelial growth inhibition was observed among the tested isolates in both assays (Table 5).

Table 5. Evaluation of antifungal activity of culturable tomato rhizospheric microbes against *Fol*

S. No.	Dual culture assay						Inverted plate assay					
	TRB isolates	Mycelial growth (mm)	Inhibition over control (%)	TRF isolates	Mycelial growth (mm)	Inhibition over control (%)	TRB isolates	Mycelial growth (mm)	Inhibition over control (%)	TRF isolates	Mycelial growth (mm)	Inhibition over control (%)
1	TRB44	81.54^{v} (64.55)	09.40	TRF25	61.42^{gh} (51.60)	31.75	TRB44	78.36^{u} (62.27)	12.93	TRF25	62.48^{hi} (52.22)	30.57
2	TRB45	19.87^{a} (26.47)	77.95	TRF26	66.29^{h} (54.50)	26.34	TRB45	20.94^{a} (27.23)	76.73	TRF26	65.17^{i} (53.83)	27.58
3	TRB46	83.29^{w} (65.87)	07.45	TRF27	55.37^{cde} (48.08)	38.47	TRB46	82.54^{y} (65.30)	08.28	TRF27	56.82^{e} (48.91)	36.86
4	TRB47	62.10^{k} (52.00)	31.00	TRF28	57.86^{ef} (49.52)	35.71	TRB47	60.73^{j} (51.19)	32.52	TRF28	59.34^{g} (50.38)	34.06
5	TRB48	59.22^{h} (50.31)	34.20	TRF29	83.20^{j} (65.80)	07.55	TRB48	57.86^{gh} (49.52)	35.71	TRF29	81.46^{k} (64.49)	09.48
6	TRB49	86.19^{y} (68.18)	04.23	TRF30	54.53^{c} (47.59)	39.41	TRB49	85.32^{aa} (67.47)	05.20	TRF30	33.71^{c} (27.12)	40.32
7	TRB50	25.29^{b} (23.44)	47.45	TRF31	55.19^{cd} (47.97)	38.67	TRB50	26.58^{b} (23.03)	48.24	TRF31	55.83^{d} (48.34)	37.96
8	TRB51	74.20^{q} (59.47)	17.55	TRF32	81.29^{i} (64.37)	09.67	TRB51	72.63^{r} (58.45)	19.30	TRF32	79.54^{j} (63.10)	11.62
9	TRB52	34.97^{c} (37.85)	38.92	TRF33	20.29^{a} (26.77)	77.45	TRB52	33.46^{c} (30.98)	40.60	TRF33	23.29^{a} (28.85)	74.12
10	TRB53	70.14^{o} (56.87)	22.06	TRF34	59.64^{f} (50.55)	33.73	TRB53	68.75^{o} (56.01)	23.61	TRF34	58.26^{f} (49.75)	35.26
11	TRB54	57.54^{g} (49.33)	36.06	TRF35	56.37^{e} (48.65)	37.36	TRB54	55.92^{ef} (48.39)	37.86	TRF35	57.14^{ef} (49.10)	36.51
12	TRB55	55.12^{cd} (47.93)	38.75	TRF36	60.42^{g} (51.01)	32.86	TRB55	54.38^{d} (47.51)	39.57	TRF36	61.73^{h} (51.78)	31.41
13	TRB56	84.15^{x} (66.53)	06.50	TRF37	29.40^{b} (26.83)	40.88	TRB56	83.67^{z} (66.16)	07.03	TRF37	28.10^{b} (26.53)	41.46
14	TRB57	56.20^{e} (48.56)	37.55	Control	90.00^{k} (71.56)	-	TRB57	57.21^{g} (49.14)	36.43	Control	90.00^{l} (71.56)	-
15	TRB58	65.32^{m} (53.92)	27.42	C.D.	9.18	-	TRB58	64.18^{m} (53.23)	28.68	C.D.	4.81	-
16	TRB59	60.14^{i} (50.85)	33.17	S.E.(d)	3.06	-	TRB59	59.46^{i} (50.45)	43.93	S.E.(d)	1.60	-
17	TRB60	55.62^{cde} (48.22)	38.20	-	-	-	TRB60	54.86^{de} (47.78)	39.04	-	-	-
18	TRB61	62.95^{kl} (52.50)	30.05	-	-	-	TRB61	63.72^{l} (52.96)	29.2	-	-	-
19	TRB62	77.29^{s} (61.53)	14.12	-	-	-	TRB62	76.84st (61.23)	14.62	-	-	-
20	TRB63	78.45^{t} (62.34)	12.83	-	-	-	TRB63	77.63^{stu} (61.77)	13.74	-	-	-
21	TRB64	79.80^{tu} (63.29)	11.33	-	-	-	TRB64	80.24^{w} (63.60)	10.84	-	-	-
22	TRB65	59.50^{hi} (50.47)	33.88	-	-	-	TRB65	58.92^{h} (50.13)	34.53	-	-	-
23	TRB66	80.56^{u} (63.83)	10.48	-	-	-	TRB66	79.68^{v} (63.20)	11.46	-	-	-
24	TRB67	67.15^{n} (55.02)	25.38	-	-	-	TRB67	66.42^{n} (54.58)	26.20	-	-	-
25	TRB68	56.39^{f} (48.67)	37.34	-	-	-	TRB68	55.64^{e} (48.23)	38.17	-	-	-
26	TRB69	82.11^{vw} (64.97)	08.76	-	-	-	TRB69	81.73^{x} (64.69)	09.18	-	-	-
27	TRB70	63.15^{l} (52.62)	29.83	-	-	-	TRB70	62.48^{k} (52.22)	30.57	-	-	-
28	TRB71	70.54^{op} (57.12)	21.62	-	-	-	TRB71	69.82^{p} (56.67)	22.42	-	-	-
29	TRB72	56.82^{fg} (48.91)	36.86	-	-	-	TRB72	56.37^{f} (48.65)	37.36	-	-	-
30	TRB73	76.50^{r} (61.00)	15.00	-	-	-	TRB73	75.28^{s} (60.18)	16.35	-	-	-
31	TRB74	61.20^{j} (51.47)	32.02	-	-	-	TRB74	60.85^{jk} (51.26)	32.38	-	-	-
32	TRB75	72.63^{p} (58.45)	19.30	-	-	-	TRB75	71.56^{q} (57.77)	20.48	-	-	-
33	Control	90.00^{z} (71.56)	-	-	-	-	Control	90.00^{ab} (71.56)	-	-	-	-
	C.D.	5.45	-	-	-	-	C.D.	5.67	-	-	-	-
	S.E.(d)	1.81	-	-	-	-	S.E.(d)	1.89	-	-	-	-

Means of four replicates, transformed values are given in parentheses. Identical superscript letters indicate non-significant differences (DMRT, $p \leq 0.05$)

3.5.1 Dual-culture Confrontation Assay

In the dual-culture assay, the bacterial isolates exhibited inhibition ranging from 4.23 to 77.95%. Among them, TRB45 recorded the highest inhibition (77.95%). The lowest inhibition was recorded for TRB49 (4.23%). Among the fungal isolates, inhibition ranged from 7.55 to 77.45%. TRF33 recorded the highest inhibition (77.45%), whereas TRF29 exhibited the lowest inhibition (7.55%). The untreated control recorded 90.00 mm mycelial growth. The antagonistic interactions between selected bacterial and fungal isolates and *Fol* are illustrated in Fig. 5.

This finding is supported by Pandiyan *et al.* (2026), who recovered *Streptomyces* isolates from tomato rhizosphere soils and demonstrated their antagonistic activity against important soil-borne pathogens, including *Fol*. More recently, Mattei *et al.* (2026) reported strain-dependent inhibition of *Fol* by tomato-associated *Streptomyces* strains in dual-culture assays, further highlighting the potential of this genus in tomato disease management.

In a separate groundnut study conducted in Tamil Nadu, Jeevanantham *et al*. (2026) evaluated indigenous fungal and bacterial antagonists against the stem rot pathogen, illustrating the broader relevance of screening locally sourced biocontrol candidates. Similarly, Buldu and Yıldırım (2026) reported considerable variation in the antagonistic activity of soil-derived *Trichoderma* isolates against *Fol*, with selected isolates exhibiting up to 100% inhibition in dual-culture assays. These studies support the potential of indigenous *Trichoderma* species as candidates for the biological management of tomato *Fusarium* wilt.



Fig. 5. Antagonistic activity of rizospheric isolates against *Fol* in dual-culture assay

3.5.2 Inverted-plate Assay

The inverted-plate assay revealed variable inhibition of *Fol* growth among the bacterial (5.20–76.73%) and fungal (9.48–74.12%) isolates. TRB45 and TRF33 exhibited the highest inhibition (76.73 and 74.12%, respectively), whereas TRB49 (5.20%) and TRF29 (9.48%) showed the lowest activity. The untreated control recorded 90.00 mm mycelial growth. Notably, TRB45 and TRF33 were the most effective isolates in both antagonistic assays (Table 5; Fig. 6).



Fig. 6. Antagonistic activity of rhizospheric isolates against *Fol* in inverted plate assay

The observed inhibition suggests the involvement of volatile-mediated antagonism. Buldu and Yıldırım (2026) demonstrated volatile-associated inhibition of the tomato wilt pathogen by selected fungal antagonists using a dual-plate assay. Similarly, Du *et al.* (2024) reported that acetoin and 2-heptanol produced by *Bacillus subtilis* N-18 inhibited *F. oxysporum* f. sp. *radicis-lycopersici* and reduced disease development in tomato. These findings highlight the potential role of microbial volatile organic compounds in suppressing *Fusarium* pathogens. Overall, TRB45 and TRF33 are promising candidates for further biocontrol studies, although headspace GC–MS and compound-specific bioassays are required to identify the metabolites responsible for the observed inhibition.

4. Conclusion

This investigation characterised culturable bacterial and fungal microorganisms associated with tomato rhizosphere soils collected from 20 locations in seven districts of Tamil Nadu. In total, 32 bacterial and 13 fungal isolates were recovered. Sequence-based identification indicated 20 bacterial genera, including *Bacillus and Pseudomonas*, and 11 fungal genera. These observations demonstrate taxonomic diversity within the cultured collection rather than the complete rhizosphere microbiome. The dual-culture and inverted-plate assays revealed variation in inhibition of *Fusarium oxysporum f. sp. lycopersici* among isolates. TRB45, identified as *Streptomyces griseus*, showed the highest bacterial inhibition in both assays (77.95% and 76.73%, respectively). TRF33, identified as *Trichoderma gamsii*, showed the highest fungal inhibition (77.45% and 74.12%, respectively). The consistency of their responses across the two laboratory assays supports prioritising these isolates for subsequent investigation. However, inhibition measured under controlled culture conditions does not establish disease suppression in tomato plants. Further greenhouse and field assessments, accompanied by investigations of antagonistic metabolites and mechanisms, are necessary to determine whether these isolates could contribute to the biological management of tomato Fusarium wilt.

5. Limitations

The study was limited to culturable microorganisms from 20 locations across seven districts of Tamil Nadu. Identification based on 16S rRNA and ITS sequencing may not provide definitive species-level resolution. Moreover, *In vitro* assays did not establish the specific mechanisms of antagonism or confirm efficacy under greenhouse and field conditions. Further studies involving advanced molecular identification, metabolite profiling and plant-based evaluations are required to validate the biocontrol potential of the promising isolates.

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Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. ChatGPT, Claude and Perplexity were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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