



RESEARCH ARTICLE

Bioprospecting *Trichoderma* spp. from diverse ecological niches for antifungal activity against soilborne pathogens

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Abstract

The growing threat of soilborne plant pathogens and the risks associated with chemical fungicides make the use of sustainable biocontrol alternatives essential. *Trichoderma* species are ubiquitous soil-inhabiting fungi commonly associated with decomposing plant residues and are widely recognised for their potential as effective biocontrol agents in sustainable agriculture. In the present study 7 indigenous *Trichoderma* spp. isolates were retrieved out of rhizosphere soils of different agro-ecological zones of India and thoroughly combined against 6 major soil borne pathogens like *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *ciceri*, *Alternaria brassicicola*, *Curvularia geniculata*, *Phomopsis vexans* and *Ustilaginoidea virens* in this study. It was found in dual culture assays that isolates TriB (Bareilly) and TriJO (Jobner) had better and broad-spectrum inhibitory action (up to 84 %) against all the pathogens tested. The strains were also sturdy in physiological adaptive capacity in 8 nutrient media demonstrating the formation capability. Their morphology was characterised as belonging to *Trichoderma asperellum* and *Trichoderma harzianum* clades, which are generally known to be effective in biocontrol along with internal transcribed spacer (ITS)-based molecular identification and phylogenetic analysis. The high degree of antagonism is attributed to synergistic mechanisms such as mycoparasitism, production of antibiotics, nutrient competition, and activation of host plant defense responses (induced systemic resistance, ISR). The results of this experiment both TriB and TriJO are noteworthy candidates in the development of formulation of region-specific biopesticides. Future studies will emphasise field validation and metabolomic profiling, followed by formulation standardisation for use in integrated disease management (IDM) programs across diverse cropping systems.

Keywords: biocontrol; indigenous isolates; ITS sequencing; mycoparasitism; rhizosphere fungi; sustainable agriculture

Introduction

Since global population is projected to exceed 9.7 billion by 2050 and there is greater demand for food, it is vital to enhancement of crop productivity and lower environmental damage (1). Soilborne (SB) fungi including *Fusarium oxysporum*, *Ustilaginoidea virens*, *Alternaria brassicicola*, *Curvularia geniculata* and *Phomopsis vexans* threaten sustainable agriculture by damaging crops and decreasing their yield because of diseases such as wilt, seedling blight and root rot (2–5). At present, most farmers use synthetic fungicides to manage fungal problems, but these can cause harm to the environment, create ecological instability and allow resistant pathogens to appear (6–9). As a result of these drawbacks, biological control has received a lot of attention as an environmentally friendly, low-cost and reliable method (10–12). Among microbes that control other microbes, species from genus *Trichoderma* (Hypocreales, Ascomycota) are well known for

helping control pathogens, boost plant growth and increase crop responsiveness to various environmental stresses. The mechanisms these fungi use to combat pathogens include stealing nutrients and space from them, secretion of antibiotic compounds, cell wall degrading enzymes, negatively affecting growth of pathogens and boosting the plants defense system (13–17). Furthermore, *Trichoderma* spp. makes soil nutrients usable by roots by dissolving them, releasing siderophores and secreting indole-3-acetic acid (IAA), an indole phytohormone that promotes root growth and the uptake of essential minerals (5, 14, 16, 18).

Although *Trichoderma* species offer several benefits as biocontrol agents, their effectiveness varies among strains and is strongly influenced by agroecological conditions, necessitating region-specific evaluation (19–21). Many commercial bioformulations are based on non-native or generic strains that often show inconsistent

performance across locations, highlighting a research gap in the identification, characterisation and molecular study of locally adapted *Trichoderma* spp. isolates (22–24). Advances in internal transcribed spacer (ITS)-based molecular identification now allow accurate discrimination of closely related species, revealing hidden diversity and supporting the selection of promising strains for commercial use (4, 25, 26). However, studies integrating morpho-molecular characterisation with functional antagonistic assays against multiple pathogens remain limited, particularly in the Indian context (23, 27). Therefore, the present study evaluated 7 *Trichoderma* spp. strains from different regions of India for their biocontrol potential by assessing growth on various culture media, antagonistic activity against 6 soilborne fungal pathogens and species confirmation through morphological traits supported by ITS sequencing, with the aim of developing effective biofungicidal formulations suitable for Indian agriculture.

Materials and Methods

Collection of soil samples and isolation of *Trichoderma* spp.

Soil samples were collected from rhizosphere regions across 7 ecologically diverse agro-zones of India to capture potential genetic and functional variability in *Trichoderma* populations. The specific locations and their coordinates are presented in Table 1.

Table 1. Specific locations and their coordinates of soil samples

Isolate code	Location	State	Latitude (°N)	Longitude (°E)
TriA	Anand	Gujarat	22.532752	72.971365
TriB	Bareilly	Uttar Pradesh	28.255299	79.199941
TriCO	Coimbatore	Tamil Nadu	11.853786	77.715176
TriCOO	Cooch Behar	West Bengal	26.096340	89.537202
TriGO	Gopalganj	Bihar	26.441084	84.096562
TriJO	Jobner	Rajasthan	26.944054	75.324219
TriLU	Ludhiana	Punjab	30.896006	75.807829

Samples were taken from 5–15 cm soil depth in the rhizosphere of healthy plants, placed in sterile polyethylene bags and transported to laboratory under cold conditions (4 °C) for immediate processing (5, 20).

Isolation and purification of *Trichoderma* spp.

Trichoderma spp. was isolated by using soil dilution plate technique. One gram of soil was placed in sterile distilled water and was serially diluted down to 10^{-5} . Aliquots of 0.1 mL were transferred into potato dextrose agar enriched with $100 \mu\text{g mL}^{-1}$ of streptomycin, to reduce the risk of bacterial contamination. *Trichoderma* spp. cultures were inoculated at 28 ± 1 °C for up to 7 days and colonies that appeared were sub-cultured and kept on potato dextrose agar (PDA) slants using standard methods (4, 7).

Morphological and microscopic characterisation

Isolates were examined for their colony colour, the texture of their colonies, the speed at which they grew and whether they sporulated. The shape of conidia, the placement of phialides and the branching of conidiophores were investigated with staining using lactophenol cotton blue. We applied taxonomic labels using morphology keys (28) and used the updated names (25, 29).

Cultural evaluation on different media

Each isolate was cultured on 8 synthetic media: PDA, potato carrot agar (PCA), Czapek Dox agar (CDA), yeast extract agar (YEA), starch agar (SA), carrot meal agar (CMA), nutrient agar (NA) and Rose Bengal agar (RBA) to evaluate growth performance. A 5 mm mycelial disc from a 5

day-old culture was placed at center of each Petri dish with 3 replications in completely randomized design (CRD) and incubated at 28 ± 1 °C. Cumulative radial growth was determined by measuring the colony diameter along 2 perpendicular lines, calculating the average (mm) and recording it daily for 7 days (30, 31).

Antagonistic activity: Dual culture assay

Dual culture assays were conducted to evaluate antagonistic activity of *Trichoderma* isolates against 6 key soilborne fungal pathogens: *F. oxysporum* f. sp. *lycopersici* (Fol), *F. oxysporum* f. sp. *ciceri* (Foc), *A. brassicicola* (Ab), *C. geniculata* (Cg), *P. vexans* (Pv) and *U. virens* (Uv). Pathogens were previously isolated and identified from diseased crops at Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh. A 5 mm mycelial disc of *Trichoderma* and test pathogen were inoculated 60 mm apart on opposite ends of PDA plates. Plates were incubated at 28 ± 1 °C and radial growth of pathogens was measured at 2, 4 and 6 days after inoculation (DAI). Percent inhibition (I) was calculated (32).

$$\text{Percent inhibition (I)} = (C - T / C) \times 100$$

Eqn. 1

Where, C = radial growth of pathogen in control,

T = radial growth in presence of *Trichoderma*

Molecular identification

Fungal genomic DNA was extracted from 7-day-old cultures using cetyltrimethylammonium bromide (CTAB) method. Internal transcribed spacer (ITS) region was amplified using universal primers ITS1 and ITS4. PCR products were visualised on agarose gel, purified and sequenced commercially. BLASTn analysis was used to compare sequences against the National Center for Biotechnology Information (NCBI) GenBank database for species-level identification (17, 25, 33).

Statistical analysis

All experiments were laid out in a CRD with 3 replications per treatment. The data on radial growth and inhibition percentage were analysed using ANOVA in SPSS v22.0. Differences among treatment means were separated using coefficient of variation (CV) test at 1 % level of significance ($p \leq 0.01$) (7, 20).

Results and Discussion

Growth performance of *Trichoderma* isolates on different media

All 7 *Trichoderma* isolates demonstrated significant variation in mycelial growth when cultured on different nutrient media as depicted in Table 2. Among the 8 tested substrates, PDA supported the most robust growth (mean radial growth: 22.86 mm/day), closely followed by modified PDA and carrot meal agar. In contrast, water agar and nutrient agar exhibited restricted growth (mean < 14 mm/day). These differences can be attributed to the variation in available carbon, nitrogen and micronutrient sources among media types, which differentially affect mycelial development and sporulation (4, 27). The observed media-dependent growth patterns are consistent with earlier reports, who highlighted that PDA enhances sporulation due to optimal sugar content and osmotic balance (34). Moreover, previous results emphasised that media like carrot meal agar also promote pigment production and secondary metabolite synthesis in *Trichoderma* spp., which are important for biocontrol efficacy (20). Understanding such physiological traits is vital for scaling up production of *Trichoderma*-based bioformulations (7).

Table 2. Antagonistic potential of *Trichoderma* isolates against different isolate of *Trichoderma* on mycelial growth of different soilborne (SB) pathogens tested through dual culture techniques

Isolate name	Mycelial growth inhibition* (%)					
	Cg	Uv	Fol	Foc	Ab	Pv
TriA	47.69±3.08 ^e	45.16±0.80 ^e	56.67±2.18 ^c	46.83±4.23 ^d	55.35±1.40 ^f	21.43±0.00 ^f
TriB	80.51±1.78 ^a	82.03±1.38 ^a	70.48±1.65 ^a	70.24±0.84 ^a	84.42±0.40 ^a	67.62±3.59 ^b
TriCO	67.69±0.00 ^c	68.66±3.48 ^c	65.72±2.47 ^b	64.39±2.24 ^b	62.33±1.40 ^c	58.09±1.65 ^c
TriCOO	47.69±1.54 ^e	72.35±0.00 ^b	57.14±0.00 ^c	55.61±2.24 ^c	56.74±1.40 ^{ef}	46.19±0.83 ^e
TriGO	74.87±2.35 ^b	72.35±2.77 ^b	59.52±4.13 ^c	50.24±1.47 ^d	59.07±0.80 ^{de}	50.00±0.00 ^d
TriJO	81.03±0.89 ^a	58.99±2.88 ^d	72.86±1.43 ^a	73.17±2.24 ^a	77.16±2.83 ^b	71.91±2.18 ^a
TriLU	56.41±2.35 ^d	58.53±1.39 ^d	65.24±2.18 ^b	58.54±2.23 ^c	60.47±2.13 ^{cd}	53.33±2.97 ^d
Control	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^d	0.00±0.00 ^e	0.00±0.00 ^e	0.00±0.00 ^e
SE(m)	1.06	1.16	1.25	1.30	0.89	1.12
SE(d)	1.49	1.65	1.77	1.84	1.27	1.58
CV (%)	3.21	3.52	3.87	4.31	2.72	4.20

*Cell values are mean of 3 replications ± SD; Transformed mean in parenthesis; Treatments with same letter grouping are not significantly different ($p \leq 0.01$). (Cg.: *Curvularia geniculata*; Uv.: *Ustilagoidea vires*; Fol.: *Fusarium oxysporum* f. sp. *Lycopersici*; Foc.: *F. oxysporum* f. sp. *ciceri*; Ab.: *Alternaria brassicicola*; Pv.: *Phomopsis vexans*).

Antagonistic potential of *Trichoderma* isolates against soilborne pathogens through dual culture technique

Seven *Trichoderma* isolates were tested against 6 economically significant soilborne pathogens on how they can interact with each other in the dual culture method to isolate pathogenic properties or potential antagonists as depicted in Table 2 and Supplementary Fig. 1. Two isolates (TriB (Bareilly) and TriJO (Jobner)) were found to be the most effective among those tested because of showing the maximum mycelial growth inhibition in all target pathogens in each test. In particular, TriB possessed the strongest inhibitory activity against *A. brassicicola* (84.42 %), *U. vires* (82.03 %) and *C. geniculata* (80.51 %), whereas TriJO had excellent antagonistic effects against the *C. geniculata* (81.03 %), *F. oxysporum* f.sp. *ciceri* (73.17 %) and *P. vexans* (71.91 %). This was much higher as compared to other isolates implying an antifungal spectrum wider and adaptable in TriB and TriJO. Their effectiveness points to strong biocontrol features, presumably due to an interdependence of mycoparasitism, antibiosis, competitive exclusion and the stimulation of resistance in host plants. The mechanisms behind this potent inhibition are well-documented. Mycoparasitism refers to antagonistic activity of *Trichoderma* species against pathogens and thus includes direct hyphal contact, hyphal coiling, penetration and digestion of pathogen hyphae with the help of chitinases, B-1,3 glucanases and proteases (17, 33). The consistently high inhibition observed in TriB and TriJO supports the proposed hypothesis, indicating that these isolates likely possess a robust enzymatic capacity contributing to their strong antagonistic activity. This correlates with the growing literature on genomic analyses indicating the growth of the number of carbohydrate-active enzyme (CAZyme) families in virulent *Trichoderma* strains (24, 25). Along with the enzymatic lysis, *Trichoderma* spp. synthesises a set of secondary metabolites, among which peptaibols, gliotoxins, viridin, volatile organic compounds (VOC) including 6-pentyl-2H-pyran-2-one (6PP) inhibit growth, sporulation and viability of phytopathogens, to a great extent (14, 31). Compounds are particularly important in attacking necrotrophic fungi including the *Alternaria* and *Fusarium* fungi that can avoid host immune detection. Also, TriB and TriJO can probably block the pathogens by competitive exclusion, whereby available niches are filled at a high rate and essential nutrients needed by the pathogen to multiply are consumed. This competitiveness in the ecology is supported by their active radial growth on a varied variety of media (20, 35, 36).

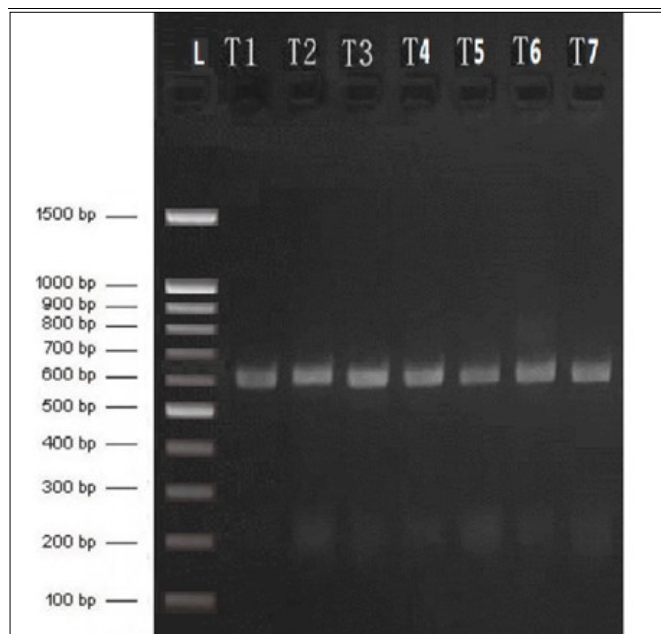
Another important aspect of *Trichoderma*-based biocontrol is its ability to activate host plant defense mechanisms through the induction of ISR. This process involves the stimulation of key hormonal signaling pathways, including salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), which enhances the plants resistance against subsequent pathogen attacks (8, 18). TriB and TriJO can also help to regulate oxidative stress (16) showed that *T. asperellum* boosted tomato resistance to *Fusarium* wilt through activation of antioxidant enzyme activity. This was proved on the molecular level as TriB and TriJO are both identified as belonging to the *T. asperellum* and *T. harzianum* respectively, two of most commercially valuable as they are both adaptable and efficient. Internal transcribed spacer (ITS) markers-based phylogenetic analysis has resolved taxonomy effectively, but considering the nature of cryptic species in *T. harzianum* complex, in the future, the analysis should be extended through multiple loci gene sequencing (e.g., TEF1- α , RPB2) (4, 37, 38). Geared to practical application, the high biocontrol potential and broad spectrum of TriB and TriJO makes them applicably adaptive into locally adapted biofungicides. The fact that they have different agroecological origins could have a role in their versatility in metabolism and rhizospheric capability (7). Considering that fungicide resistance poses an even more prominent threat today, environmental issues have grown and sustainable agriculture must be achieved, these isolates would be promising in terms of implementation in integrated disease management (IDM) regimes (3, 33). To sum up, the current work confirms great antagonistic potential of TriB and TriJO, which makes both star candidates in terms of commercial development. These results highlight the need of region-wise screening and functional validation of native isolates to come up with efficient, environmentally safe approach to the management of soilborne diseases. These can be made to manifest their potential in the agricultural systems through additional research on field-level validation, formulation technology and multi-season efficacy tests.

Molecular identification and taxonomic validation

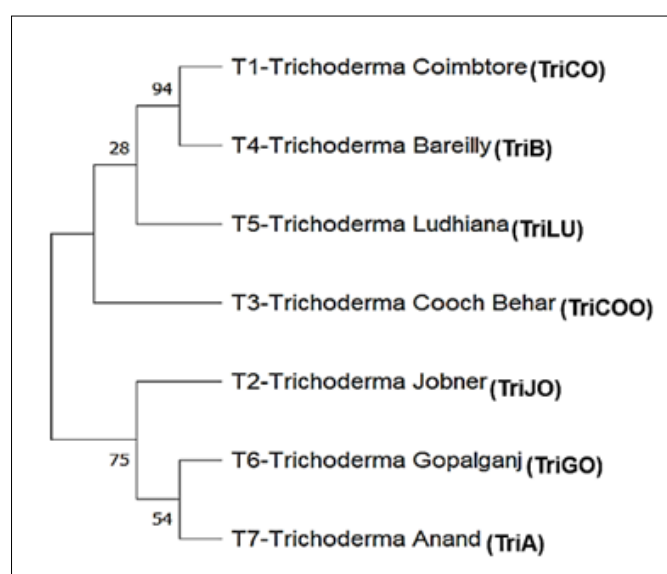
Molecular characterisation using ITS sequencing revealed that isolates belonged primarily to the *T. asperellum*, *T. harzianum* and *T. asperelloides* clades, consistent with their antagonistic performance profiles as depicted in Table 3 and Fig. 1. The ITS region has long been considered the gold standard for fungal barcoding (25, 39) and its use in this study ensures reliable identification. Phylogenetic placement of the isolates in clades with known biocontrol efficacy supports their potential utility in commercial applications

Table 3. Molecular identification of *Trichoderma* isolates based on ITS region sequencing and NCBI accession numbers

Isolate designation	Place of collection	Species identified	NCBI accession ID
TriA	Anand (Gujarat)	<i>Trichoderma asperelloides</i> isolate HBT small subunit	PP897276
TriB	Bareilly (Uttar Pradesh)	<i>Trichoderma asperellum</i> isolate H531/ <i>Trichoderma</i> sp. CC-2016 strain	PP897273
TriCO	Coimbatore (Tamil Nadu)	<i>Trichoderma asperellum</i> isolate jZ3	PP897231
TriCOO	Cooch Behar (West Bengal)	<i>Trichoderma asperellum</i> isolate H531/ <i>Trichoderma harzianum</i> isolate Th-4	PP897272
TriGO	Gopalganj (Bihar)	<i>Trichoderma asperellum</i> isolate T18	PP897275
TriJO	Jobner (Rajasthan)	<i>Trichoderma asperellum</i> clone SF_146	PP897271
TriLU	Ludhiana (Punjab)	<i>Trichoderma asperellum</i> isolate jz-3/ <i>Trichoderma viride</i> strain PF1	PP897274

**Fig. 1.** Polymerase chain reaction (PCR) amplification of internal transcribed spacer (ITS) region from genomic DNA of *Trichoderma* isolates (T1–T7) showing ~600 bp amplicons. Lane L: 100 bp DNA ladder.

(Fig. 2 and Supplementary Fig. 2). The findings are supported by similar molecular works (5, 24), where ITS sequencing successfully delineated functional *Trichoderma* groups for region-specific deployment. The species-level resolution within the *T. harzianum* complex may require multilocus sequencing (e.g., TEF1- α , RPB2).

**Fig. 2.** Phylogenetic tree of *Trichoderma* isolates (TriA–TriJO) constructed using maximum likelihood method based on Tamura-Nei model in MEGA11. Bootstrap values (%) are shown at branch nodes, indicating clustering confidence based on ITS sequence alignment of 631 bp.

Conclusion

The biocontrol potential of 7 indigenous *Trichoderma* isolates has been thoroughly studied and TriB (Bareilly) and TriJO (Jobner) have been found to have outstanding broad-spectrum antagonistic activity against a wide array of soilborne (SB) pathogens, such as, *Fusarium oxysporum* (f. sp. *lycopersici* and *ciceri*), *Alternaria brassicicola*, *Curvularia geniculata*, *Phomopsis vexans* and *Ustilaginoidea virens*. Their high level of efficacy has been attributed to a number of synergistic mechanisms, such as mycoparasitism, synthesis of antifungal metabolites (e.g., peptaibols, gliotoxins), competitive exclusion and stimulation of induced systemic resistance (ISR) pathways in plants. Phylogenetic analysis with high clade support confirmed molecular identification as the most effective isolates, that were *Trichoderma asperellum* and *Trichoderma harzianum*, sequencing of internal transcribed spacer (ITS). The different physiological flexibility of these isolates, in differential growth on different nutrient media, also further propounds their appropriateness as commercial bioformulations. The results do not only improve the knowledge base regarding strain-specific interactions and antagonistic behavior but also spot regionally adjusted candidates of integrated disease management (IDM). Future research should focus on greenhouse and field validation, detailed metabolomic and genomic characterisation, optimisation of formulation technologies and large-scale production strategies. Such efforts will facilitate the development of effective, eco-friendly biofungicides based on native *Trichoderma* spp. isolates for sustainable agricultural disease management.

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Authors' contributions

PCT carried out the experiment, took observations and analysed the data. SKB guided the research by formulating the research concept, and DKC, PK approved the final manuscript. SK, RK participated in the design of the study and performed the statistical analysis reviewed the VS, MM contributed by imposing the experiment and helped edit, summarise and revise the manuscript. M and BSM helped to summarise and revise the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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