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Full Length Research Paper

Characterization of Indian native isolates of *Trichoderma* spp. and assessment of their bio-control efficiency against plant pathogens

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A study was intended to corroborate the positive relatedness of molecular and morphological characters with antagonistic ability of *Trichoderma* species. On the basis of morphological and cultural characteristics, the *Trichoderma* isolates were identified as *T. virens* (11 isolates), *T. asperellum* (15), *T. harzianum* (14) and *T. longibrachiatum* (32). This result was in concordance with the result obtained from the DNA sequence data analysis of internal transcribed spacer 1 and 2 region (ITS1 and ITS2) and the elongation factor 1-alpha gene (*tef1*). The phylogenetic analyses of the above two marker loci sequences were done. *Trichoderma* isolates were not fairly separated by ITS 1 and 2 regions analysis whereas these were clearly separated with *tef1* sequences analysis into different clusters. Comparative sequence analyses suggest that *tef1* is a better marker to distinguish *Trichoderma* species because of greater transition/transversion ratio (1.32) and evolutionary divergence (1.965). *T. virens* (Vn) and *T. harzianum* (Th) were assessed for their mycoparasitic effect on soil borne plant pathogens, *Rhizoctonia solani, Pythium aphanidermatum, Fusarium udum, F. solani, Sclerotium rolfsii* and *Macrophomina phaseolina*. The percent inhibitory effect among *T. harzianum* isolates was between 70 to 90% and *T. virens* isolates ranged from 50 to 80%. However, Vn09 and Th-12 were distinguishable in exhibiting higher degree of antagonism.

Key words: Biocontrol, ITS, *tef1*, *Trichoderma*.

INTRODUCTION

The anamorphic fungal genus *Trichoderma* (*Hypocreales, Ascomycota*) is frequently found on decaying wood and in soil (Samuels, 1996) where its individual species can comprise a major portion of the total fungal biomass (Nelson, 1982; Widden and Abitbol, 1980). The genus *Trichoderma* comprises species that are economically important. Some of the species are producers of Industrial enzymes (*T. reesei*, *Hypocrea jecorina*) (Kubicek and Penttilä, 1998) and antibiotics (Sivasithamparam and Ghisalberti, 1998). Some are potential biocontrol agents against plant pathogens (for example, *T. harzianum*, *H. lixii*, *T. atroviride*, *H. atroviridis*, *T. asperellum,* and *T. virens*) (Hjeljord and Tronsmo, 1998; Jeger et al., 2009). The diverse implications of genus *Trichoderma* renders accurate identification of the species. However, due to the homoplasy of the characters used, morphological determination of taxa is difficult even for experts. This has frequently resulted in the incorrect use of species names in *Trichoderma* for strains associated with enzyme production (Kovacs et al., 2004; Wey et al., 1994), biocontrol (Kullnig et al., 2001), human infection (Gautheret et al., 1995), and formation of secondary metabolites (Cutler et al.,1999; Humphris et al., 2002). Consequently, most recent studies have used molecular data to characterize and identify *Trichoderma* species (Kubicek et al., 2003; Kraus et al., 2004; Jaklitsch et al., 2005).

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Characterization of species of *Trichoderma* and *Hypocrea* based on a single gene is not sufficient (Bissett et al., 2003; Chaverri et al., 2004). Although generally the ITS region is used for identification but it must be used with caution because sometimes closely related species cannot be distinguished using this region. This is especially true of *Trichoderma* sect. *Trichoderma*, which includes *T. viride*, where more than one species can share the same ITS sequence (Lieckfeldt et al., 1999). Thus, *tef1* can be the alternative choice because it is more variable than the ITS rDNA and could be better able to reflect species differences within and among groups of closely related species (Samuels, 2006). The different *Trichoderma* species are able to antagonize phytopathogenic fungi by using substrate colonization, antibiosis and mycoparasitism as the main mechanisms (Hjeljord and Tronsmo, 1998). This antagonistic potential is the base for effective applications of *Trichoderma* strains as alternatives to the chemical control of a wide set of phytopathogenic fungi (Harman and Björkman, 1998). Biological control that is, the antagonism and eventual killing of plant pathogens by other living organisms, which are themselves not harmful to the plants could present an attractive alternative for combating plant diseases. Species of the anamorphic genus *Trichoderma* (teleomorph: *Hypocrea*, Ascomycota) have been proven as effective biocontrol agents of different plant diseases (Harman et al., 2004; Jensen and Wolffhechel, 1993).

The current work is an integrated study for the authentic identification of different *Trichoderma* isolates which combines phylogenetic analysis based on sequences of Internal transcribed spacer (ITS 1 and ITS 2) and Translation elongation factor alpha1 (*tef1*) and their bioefficacy against plant pathogens.

MATERIALS AND METHODS

The *Trichoderma* fungi were isolated from soil (Askew and Laing, 1993) from different geographical zones of India (Table 1 and Figure 1). Single spore isolations were made, and the morphological characters viz., cultural characters (colony - color, growth and texture), and microscopic characters (conidiophore - branching (tube, slide), phialide disposition, conidia - color, size and shape) of all the isolates were recorded. Considering all the morphological characters, these isolates of *Trichoderma* were placed under different groups (Rifai, 1969; Bissett 1984, 1991a, b, c).

DNA extraction, polymerase chain reaction (PCR) amplifications and sequencing

DNA was isolated from fresh mycelium using CTAB method (Culling, 1992). A region of nuclear rDNA (ITS1 and ITS2) was amplified by PCR using universal primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') (White et al., 1990) in a thermal cycler (Bioer, Japan), using the following parameters: 1 min initial denaturation at 94°C, followed by 30 cycles of 30 s denaturation at94°C, 1 min primer annealing at 57°C, 90 s extension at 72°C, and a final extension period of 10 min at 72°C. A 1 kb fragment of *tef1* was amplified by using EF 1 (5'-ATGGGTAAGGAGGACAAGAC-3') (O'Donnell et al., 1998) and TEF1 rev (5'-GCCATCCTTGGAGATACCAGC-3') (Samules et al., 2002) using the following protocol: 3 min initial denaturation (95°C), 35 cycles each of 1 min at 95°C, 1 min at 60°C, and 3 min at 72°C, and a final extension period of 5 min at 72°C. Each PCR reaction was electrophoresed on 1.5% agarose minigels (containing 0.5 mg/µl ethidium bromide) for 1 h in Tris-acetate buffer (Sambrook et al., 1989). The PCR products were revealed under UV light. Direct sequencing of the PCR amplicons corresponding to the ITS region of ribosomal DNA (rDNA) and *tef* 1 were performed throughout sourcing (Banglore genei, Banglore). Among 72 isolates which were morphologically characterized, 70 ITS sequences and 65 *tef1* sequences were used for phylogenetic analysis. The sequences of the isolates were submitted to GenBank, as indicated in Table 1.

Molecular phylogenetic data analysis

ITS region and *tef1* sequences were submitted to the BLAST in interface in NCBI data base (http://blast.ncbi.nlm.nih.gov) for the species identification of *Trichoderma.* ITS and *tef1* sequences were aligned using CLUSTALX 1.81 (Thompson et al., 1994). Phylogenetic analysis was carried out using MEGA 5 program (Tamura et al., 2011) and a neighbour-joining tree was constructed using the Kimura-2-parameter distance model (Kimura, 1980). Confidence values were assessed from 1000 bootstrap replicates of the original data.

Confrontation assays *in vitro*

In vitro confrontations were studied by performing dual culture technique to test the antagonistic ability of *Trichoderma* isolates against plant pathogenic fungi*, R. solani, P. aphanidermatum, F. udum, S. rolfsii, F. solani and M. phaseolina* (Skidmore and Dickinson, 1976). The culture plates were incubated for six days at 25°C, colony growth of both biocontrol agents and pathogen were observed constantly, and the radial growth of the pathogen recorded daily up to day four of inoculation, the percent inhibition was worked out as follows:

$$PI = (C - T) \times 100 / C$$

Where, PI is the percent inhibition of mycelial growth; C is the radial growth of pathogen in control plates (cm) and T is the radial growth of pathogen in dual culture (cm). Ten replicates were taken for each treatment. Deviation of mean was calculated as standard deviation.

RESULTS

Identification of *Trichoderma*

A total of 72 isolates of *Trichoderma*, obtained from different places of India were studied at the species level by using the morphological characteristics and the analyses of their ITS region and *tef1* nucleotide sequences. All these isolates were identified as *T. virens* (J. H. Mill., Giddens and A. A. Foster) Arx (11 isolates), *T. asperellum* (Samules Lieckfeldt and Nirenberg) (15 isolates), *T. harzianum* Rifai (14 isolates), *T. longibrachiatum* Rifai (32 isolates) (Table 2). Morphological grouping and speciation matched with molecular speciation based on above targeted regions. The identification, origin, and NCBI GeneBank accession numbers of all the isolates were given in Table 1.

Table 1. List of *Trichoderma* isolates included in this study.

Culture code	Species	Source/Place of collection	NCBI GeneBank accession numbers	
			ITS and ITS 2	*tef1*
Vn01	*T. virens*	Soil/New Delhi, India	HM046562	JN039090
Vn02	*T. virens*	Tomato Soil / New Delhi, India	HM046563	JN039091
Vn03	*T. virens*	Soil/New Delhi, India	HM046564	JN039092
Vn04	*T. virens*	Soil/Pusa, Samastipur, India	HM046559	JN039093
Vn05	*T. virens*	Soil/Barrackpore, India	HM046560	JN039094
Vn06	*T. virens*	Soil/Barrackpore, India	HM046561	JN039095
Vn07	*T. virens*	Soil/Cuttack, India	JN039040	JN039096
Vn08	*T. virens*	Soil/Cuttack, India	JN039041	JN039097
Vn09	*T. virens*	Soil/Hyderabad, India	JN039042	JN039098
Vn10	*T. virens*	Soil/New Delhi, India	JN039043	-
Vn11	*T. virens*	Soil/Barapani, Mehalaya, India	JN039044	-
Ta01	*T. asperellum*	Compost/Lucknow, India	JN JN104481	JN104496
Ta02	*T. asperellum*	Compost/Lucknow, India	J JN104482	JN104497
Ta03	*T. asperellum*	Compost/New Delhi, India	JN1 JN104483	JN104498
Ta04	*T. asperellum*	Soil/Barrackpore, India	JN104484	JN104499
Ta05	*T. asperellum*	Soil/Cuttack, India	JN104485	JN104500
Ta06	*T. asperellum*	Soil/Varanasi, India	JN104486	JN104501
Ta07	*T. asperellum*	Soil/ Hyderabad, India	JN104487	JN104502
Ta08	*T. asperellum*	Soil/Guntur, India	JN104488	JN104503
Ta09	*T. asperellum*	Soil/Navsari, India	JN104489	JN104504
Ta10	*T. asperellum*	Soil/Navsari, India	JN104490	JN104505
Ta11	*T. asperellum*	Soil/Navsari, India	JN104491	JN104506
Ta12	*T. asperellum*	Soil/Navsari, India	JN104492	JN104507
Ta13	*T. asperellum*	Soil/Iddukki, India	JN104493	JN104508
Ta14	*T. asperellum*	Soil/Iddukki, India	JN104494	JN104509
Ta15	*T. asperellum*	Soil/Iddukki, India	JN104495	JN104510
Th01	*T. harzianum*	Coconut/ Kasargod, India	JN039045	JN039099
Th02	*T. harzianum*	Coconut/ Kasargod, India	JN039046	JN0390100
Th03	*T. harzianum*	Soil/New Delhi	JN039047	JN0390101
Th04	*T. harzianum*	Mushroom compost/Solan, India	JN039048	JN0390102
Th05	*T. harzianum*	Soil/Kasargod, India	-	JN0390103
Th06	*T. harzianum*	Soil/Palampur, India	JN039049	JN0390104
Th07	*T. harzianum*	Compost/New Delhi, India	JN039050	JN0390105
Th08	*T. harzianum*	Soil/Kasargod, India	JN039051	JN0390106
Th09	*T. harzianum*	Soil/Kasargod, India	JN039052	JN0390107
Th10	*T. harzianum*	Compost/New Delhi, India	JN039053	JN0390108
Th11	*T. harzianum*	Soil/Navasari, India	JN039054	JN0390109
Th12	*T. harzianum*	Soil/Shimla, India	JN039055	JN0390110
Th13	*T. harzianum*	Compost/Siliguri, India	JN039056	JN0390111
Th14	*T. harzianum*	Cucumber/Jammu, India	JN039057	JN0390112
T101	*T. longibrachiatum*	Onion/Pune, India	JN039058	JN0390113
Tl02	*T. longibrachiatum*	Chick pea/New Delhi, India	JN039059	JN0390114
Tl03	*T. longibrachiatum*	Chick pea/New Delhi, India	JN039060	JN0390115
Tl04	*T. longibrachiatum*	Paddy soil/ Tezpur, India	JN039061	JN0390116
Tl05	*T. longibrachiatum*	Paddy soil/ Tezpur, India	JN039062	JN0390117
Tl06	*T. longibrachiatum*	Paddy soil/ Tezpur, India	JN039063	JN0390118
Tl07	*T. longibrachiatum*	Soil/Shimla, India	JN039064	JN0390119
T108	*T. longibrachiatum*	Soil/Shimla, India	JN039065	JN0390120
Tl09	*T. longibrachiatum*	Soil/Shimla, India	JN039066	JN0390121
Tl10	*T. longibrachiatum*	Soil/ Tezpur, India	JN039067	JN0390122

Table 1. Contd.

Tl11	*T. longibrachiatum*	Soil/Bilaspur, India	JN039068	JN0390123
Tl12	*T. longibrachiatum*	Tomato soil/Srinagar, India	JN039069	-
Tl13	*T. longibrachiatum*	Brinjal/Srinagar, India	JN039070	JN0390124
Tl14	*T. longibrachiatum*	Tomato soil/Srinagar, India	JN039071	-
Tl15	*T. longibrachiatum*	Soil/Tirupathi, India	JN039072	JN0390125
Tl16	*T. longibrachiatum*	Soil/Tirupathi, India	JN039073	JN0390126
Tl17	*T. longibrachiatum*	Soil/Bikaner, India	JN039074	JN0390127
Tl18	*T. longibrachiatum*	Tomato soil/Ludhiana, India	JN039075	JN0390128
Tl19	*T. longibrachiatum*	Soil/Jammu, India	JN039076	-
Tl20	*T. longibrachiatum*	Soil/New Delhi, India	JN039077	-
Tl21	*T. longibrachiatum*	Soil/New Delhi, India	JN039078	
Tl22	*T. longibrachiatum*	Soil/Tirupathi, India	JN039079	JN0390129
Tl23	*T. longibrachiatum*	Soil/Siliguri, India	JN039080	-
Tl24	*T. longibrachiatum*	Soil/Siliguri, India	JN039081	-
Tl25	*T. longibrachiatum*	Soil/Siliguri, India	JN039082	JN0390130
Tl26	*T. longibrachiatum*	Soil/Siliguri, India	JN039083	JN0390131
Tl27	*T. longibrachiatum*	Sewage/Karnal, India	JN039084	JN0390132
Tl28	*T. longibrachiatum*	Soil/Shimla, India	JN039085	JN0390133
Tl29	*T. longibrachiatum*	Chickpea/Bikaner, India	JN039086	-
Tl30	*T. longibrachiatum*	Soil/Kanpur, India	JN039087	JN0390134
Tl31	*T. longibrachiatum*	Soil/Kanpur, India	JN039088	-
Tl32	*T. longibrachiatum*	Soil/Kanpur, India	JN039089	-

Molecular phylogenetic analysis

The phylogenetic trees were obtained by sequence analysis of ITS1 and 2 and *tef1* gene of *Trichoderma* isolates (Figures 2 and 3). The Phylogenetic tree based on ITS revealed that the isolates of different *Trichoderma* species were not clearly separated; this may be due to less variation among the isolates (figure 2). This was well explained by less transition/transversion ratio (0.50) and evolutionary divergence (0.510). Thus, *tef1* became the marker choice for the present study because it is usually more variable than ITS1-ITS2 as it has greater transition/ transversion ratio (1.32) and evolutionary divergence (1.965). The phylogenetic tree based on *tef1* sequences clearly divided the *Trichoderma* isolates into four groups (Figure 3). Group 1 (*T. longibrachiatum*, 32 isolates), is supported with a bootstrap value of 97% and contains three subgroups supported by bootstrap values higher than 50%. Group 2 includes eleven strains representing *T. virens* and is supported by a bootstrap value of 99%. Two distinct subgroups corresponding to *T. virens* were also observed. Group 3 includes *T. harzianum* (14 isolates), with a bootstrap stability of 99%. This grouping is well supported with a bootstrap value of 98%. Group 4 comprises *T. asperellum* (15 isolates) which is supported by a bootstrap value of 77%, and is divided into two subgroups supported by bootstrap values of 98 and 99%.

Antagonism of *Trichoderma* isolates against soil borne pathogens

The isolates of *Trichoderma* spp., Th01 to Th15 of *T. harzianum* and Vn01 to Vn11 of *T. virens* were evaluated for their antagonistic ability against *R. solani, P. aphanidermatum, F. udum, F. solani, S. rolfsii, and M. phaseolina* based on percent inhibition of mycelial growth (Tables 3 and 4). The isolate Th12 of *T. harzianum* was found to have the maximum inhibiting effect on the growth of *F. solani* (91%) and *S. rolfsii* (87%). Among the *T. virens* isolates, Vn09 was more effective on *S. rolfsii* (with 88% inhibition) whereas Vn03 had 87% impact on *F. udum*.

DISCUSSION

Isolates of *Trichoderma* spp. have been demonstrated to be antagonistic toward a number of fungi (Howell, 2003). In pursuit for a probability of corroborating the myco-parasitic antagonism ability of *Trichoderma* with respect to their morphological and molecular characters, the current study was undertaken. Such corroboration will certainly be a needful aspect in elucidating the molecular mechanism involved in mycoparasitic antagonism. The source isolates were subjected to morpho-taxonomic

Figure 1. Map of India showing sampling regions of *Trichoderma* isolates during 2009 and 2010.

observations which are the pedestal for any taxonomic work. The quantitative and qualitative characters taken for morphological characterization were able to differentiate isolates into four groups, *T. virens*, *T. asperellum*, *T. harzianum* and *T. longibrachiatum.* Though the morphological characterization had resulted in classifying the *Trichoderma* isolates, it can only give a broader picture. Waalwijk et al. (1996) indicated the

Table 2. Speciation of *Trichoderma* isolates.

Group	Colony	Conidio-phore	Phialide	Conidia	References
T. asperellum	Cottony and compact, and dark green	Regularly branched, branches typically paired	Straight, ampulliform to laginiform	Globose, to sub-globose, inconspicuous ornamentation,3.7-6.6 × 3.0-5.0 μm, light green Chlamydospores abundant	Samuels et al. (1999)
T. harzianum	Whitish green to pale green, reverse- colorless to dull yellow, floccose, flat pustules, conidiation effuse, covering the entire surface of the plate,	Flexuous, branches almost right angled, less extensively branched, irregular and narrower	Whorls of 2-6, ampulliform to laginiform,, subulate, short, skittle-shaped, narrower at the base	Globose to sub-globose to short obovoid, pale green Size- 1.7-3.2 × 1.3 -2.5μm, Chlamydospores abundant	Rifai (1969) and Bisset (1991 b)
T. longibrachiatum	Dark olive green with yellow tinge, reverse- colorless to yellowish green, effuse, compact tufts	Long main branches produce only a few side short branches stand at right angles	Solitary, alternately, or irregularly,, directly along the long main branches, laginiform or bottle shaped	Sub-cylindrical with distinct truncate base, pale green Size- 3.4-5.2 × 2.3-3.5μm, chlamydospores frequent	Rifai (1969) and Bisset (1984)
T. virens	Dull blackish green, Reverse-colourless, effuse conidiation, flat pustules concentrated near the margin of the plate	Branching irregular, and uncrowded, C. phore broad, terminated by a cluster of 3-6 closely ad pressed phialides, conidia from adjacent phialides coalesce into single large conidial ball	Whorls of 2-5, nine pin shaped, laginiform to ampulliform,	Broadly ellipsoid to obvoid, dark green Size- 3.5-6.0 × 2.8-4.1μm, chlamydospores abundant	Bisset (1991 b)

difficulties to distinguish species based on morphology alone. Seaby (1996) was also of the same opinion where he reported that the morphological traits are subjected to environmental influence, and can vary substantially from culture to culture. The taxonomic consideration based solely on phenotype may be subjected to ambiguities induced by environmental conditions. Thus, to improve the reliability of morphological characters and to resolve the ambiguities, the characterization should be complemented with molecular data.

Despite the suggestion by other authors (Chaverri et al., 2003; Dodd et al., 2000) of occurrence of two or more haplotypes of ITS1 and 2, these differed in most cases only in 1 to 3 nucleotides, and clustered together in phylogenetic analyses. The phylogenetic analysis of ITS1 and 2 sequences did not clearly separate different species of *Trichoderma*. Hoyos-Carvajal et al. (2009) also stated that sequencing of the internal transcribed spacer regions (ITS) of the ribosomal repeat could not differentiate some species, and taken alone, gave several misidentifications due to the presence of non orthologous copies of the ITS in some isolates. Therefore, *tef1* region was also explored to distinguish *Trichoderma* species because *tef1* is more variable than the ITS rDNA, and it is better able to reflect species differences within and among groups of closely related species (Samuels, 2006). Isolates were mostly placed in their 'correct' clade and, in most cases, isolates identified by DNA sequence analysis clustered together by *tef1* region. This results confirmed several molecular phylogenetic studies (Druzhinina et al., 2005; Hermosa et al., 2004; Kullnig-Gradinger et al., 2002) in the separation of the morphologically defined *Trichoderma.* Bissett

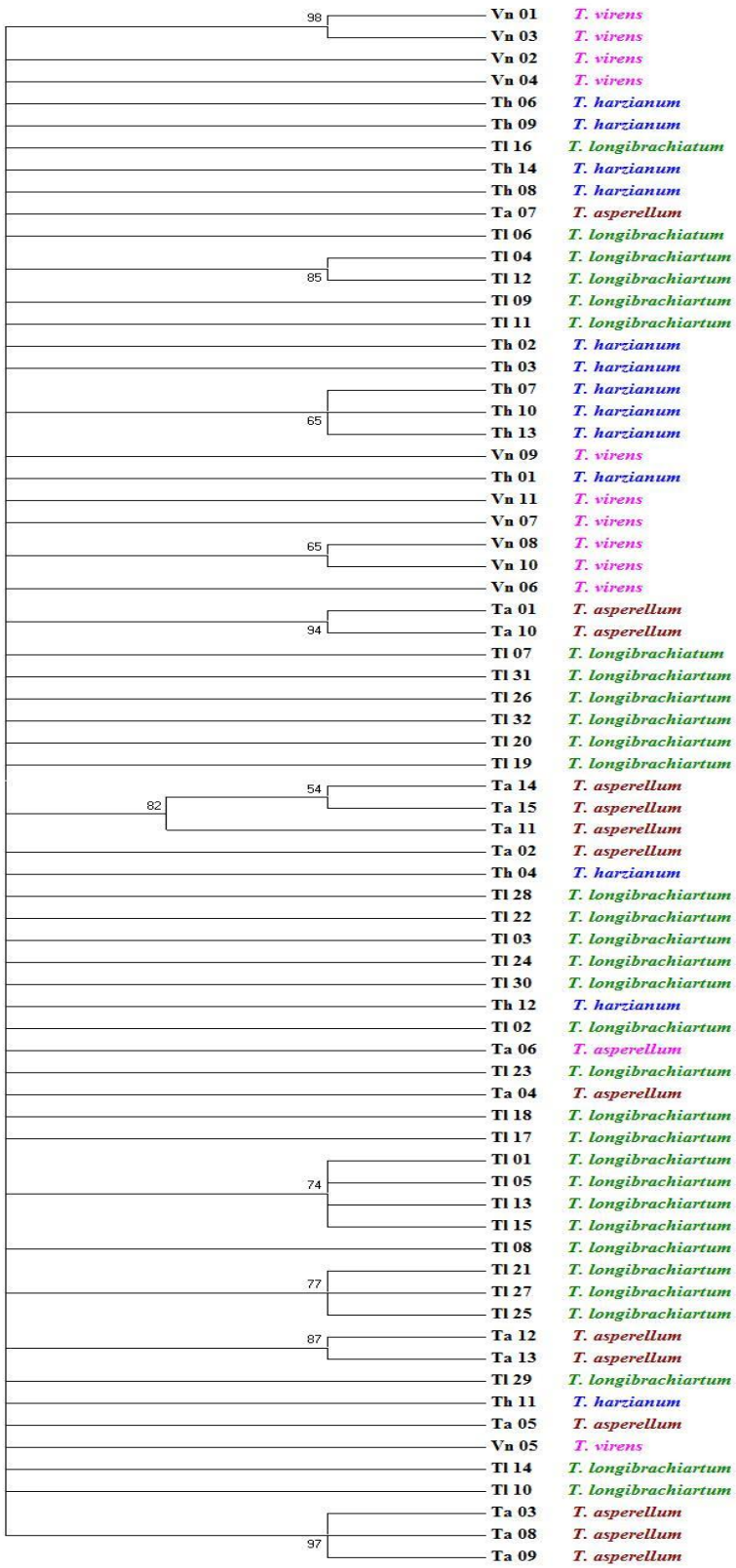

Figure 2. Phylogenetic relationships of 70 isolates of *Trichoderma* spp. inferred by analysis of ITS 1 and b2 sequences. The tree was obtained from analysis by the NJ method using the Kimura (1980) two-parameter technique of the MEGA 5 program. Vn: *T. virens,* Ta: *T. asperellum,* Th: *T. harzianum,* Tl: *T. lonibrachiatum.*

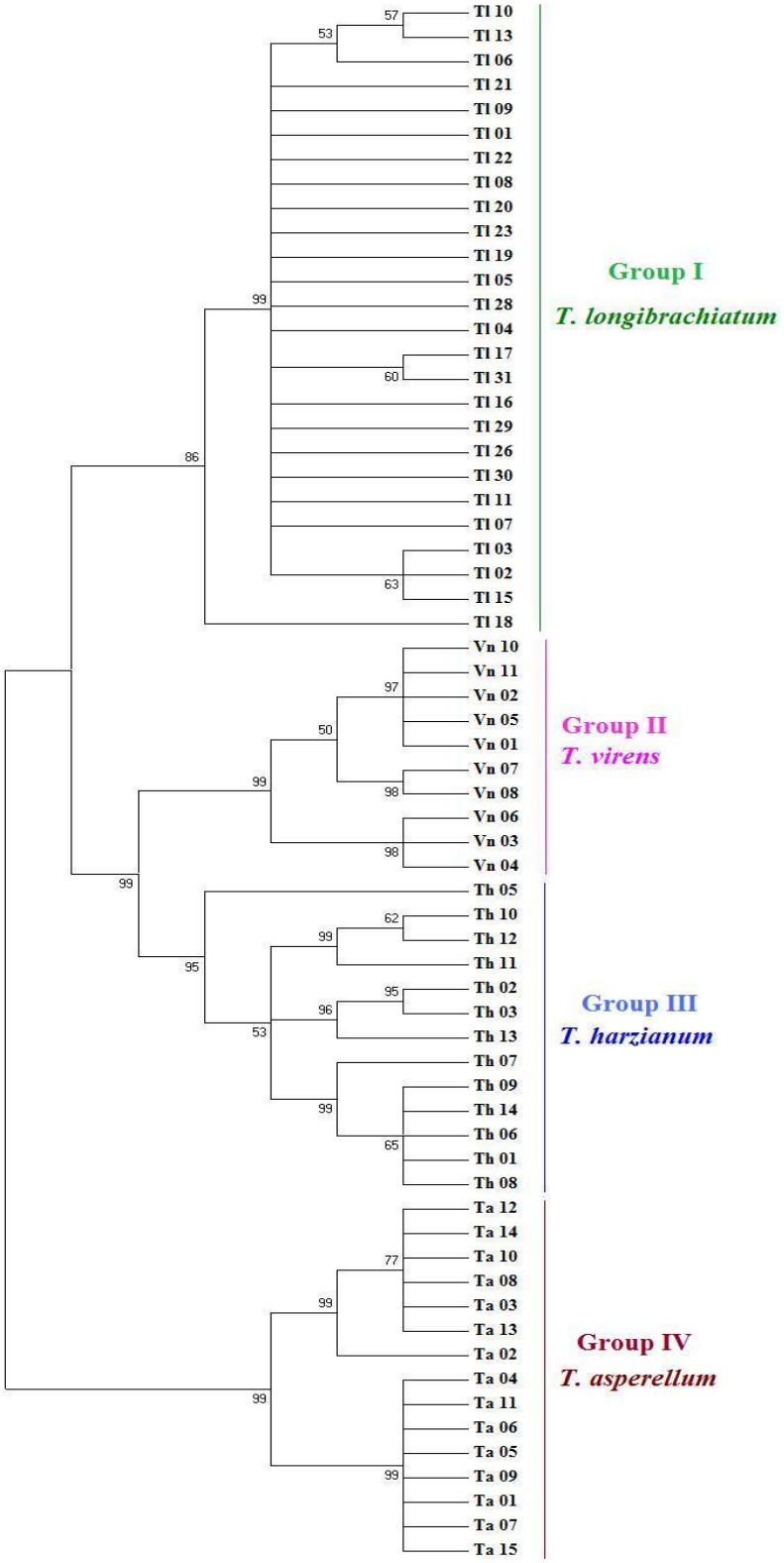

Figure 3. Phylogenetic relationships of 65 isolates of *Trichoderma* spp. inferred by analysis of *tef1* sequences. The tree was obtained from analysis by the NJ method using the Kimura (1980) two-parameter technique of the MEGA 5 program. Group I *T. lonibrachaiatum,* Group II *T. virens*, Group III *T. harzianum*, Group IV *T. aseperellum.* Vn: *T. virens,* Ta: *T. asperellum,* Th: *T. harzianum,* Tl: *T. lonibrachiatum.*

Table 3. Dual-culture assay of *T. virens* after 7 days at 25°C with the plant pathogenic fungi.

Trichoderma isolates/pathogens	Percent inhibition of mycelia growth-PIMG on PDA (scale results±SD[a])					
	Rhicontonia solani	*Pythium phanidermatum*	*Fusarium udum*	*Macrophomia phaseolina*	*Sclerotium rolfsii*	*Fusarium solani*
Vn01	55.00±1.00	71.00±1.00	76.66±2.08	55.00±2.00	84.00±1.00	64.00±3.60
Vn02	80.00±4.35	69.00±6.55	77.66±3.78	55.00±2.00	82.00±2.64	70.00±2.64
Vn03	53.00±2.64	73.00±2.64	86.67±2.08	55.00±3.61	76.00±2.64	79.00±2.64
Vn04	52.00±1.73	68.00±4.35	77.33±2.08	51.00±1.73	81.00±2.64	65.00±3.60
Vn05	56.00±3.60	76.00±5.29	72.33±3.05	51.00±1.00	83.00±1.73	64.00±2.64
Vn06	73.33±1.52	64.00±3.60	79.00±3.60	60.00±2.00	75.00±1.73	72.00±5.29
Vn07	56.00±5.29	62.00±2.00	82.33±2.08	62.00±2.00	80.00±3.60	73.00±2.64
Vn08	52.00±3.60	73.00±2.64	79.00±3.60	53.00±2.64	82.00±2.64	62.00±2.64
Vn09	76.00±3.60	74.00±2.64	81.00±2.64	58.00±2.00	88.00±2.00	72.00±2.64
Vn10	53.00±3.00	71.00±4.58	83.33±1.52	55.00±4.35	82.00±4.58	71.00±2.64
Vn11	85.66±4.00	66.00±2.64	81.00±3.60	52.00±2.64	82.00±2.64	74.00±1.00

[a]Values correspond to the following growth parameters: sporulation on the plate and sporulation on the pathogen colony. The values are mean of ten replications.

Table 4. Dual-culture assay of *T. harzianum* after 7 days at 25°C with the plant pathogenic fungi.

Trichoderma isolates/pathogens	Percent inhibition of mycelia growth-PIMG on PDA (scale results±SD[a])					
	Rhicontonia solani	*Pythium aphanidermatum*	*Fusarium udum*	*Macrophomia phaseolina*	*Sclerotium rolfsii*	*Fusarium solani*
Th01	86.70±4.20	81.00±1.70	76.70±1.20	77.00±1.00	70.00±2.00	77.00±1.00
Th02	74.30±3.50	85.0 0±2.60	80.30±2.10	77.00±1.00	79.00±2.00	75.00±2.60
Th03	76.00±2.00	75.00±1.00	82.70±2.50	83.00±1.00	87.00±1.00	81.00±1.70
Th04	83.00±1.00	76.00±3.00	79.7 0±2.10	76.00±1.00	84.00±2.00	89.00±1.00
Th05	85.00±1.00	85.00±1.00	72.0 0±2.00	73.00±1.00	72.00±3.50	73.00±3.50
Th06	78.7 0±1.20	75.00±1.00	88.0 0±1.00	74.00±3.50	75.00±2.60	75.00±2.60
Th07	77.70±4.00	80.00±2.60	75.30±0.60	77.00±3.60	85.00±1.00	87.00±1.00
Th08	87.30±3.10	85.00±1.00	73.70±0.60	78.00±4.60	79.00±4.00	84.00±2.00
Th09	77.7 0±1.50	71.00±1.00	78.70±2.30	70.00±2.00	85.00±1.00	73.00±1.00
Th10	75.0 0±1.00	72.00±1.00	72.00±2.00	70.00±2.00	79.00±2.00	79.00±1.00
Th11	72.30±1.50	73.00±1.00	72.00±2.00	72.00±1.00	83.00±1.00	77.00±1.00
Th12	80.30±2.50	82.00±2.00	82.00±2.00	83.00±1.00	87.00±1.00	91.00±1.00
Th13	75.30±1.20	74.00±3.50	76.00±2.00	70.00±2.00	87.00±1.00	75.00±1.00
Th14	79.00±1.70	72.00±2.00	77.30±1.50	78.00±4.60	87.00±1.00	77.00±3.00

[a]Values correspond to the following growth parameters: sporulation on the plate and sporulation on the pathogen colony. The values are mean of ten replications.

(Bissett, 1984, 1991a, b, c) into sections *Trichoderma* (Clade Asperellum), *Pachybasium* (Clade Harzianum and Clade Virens), and *Longibrachiatum* (Clade Longibrachiatum).

Among the four *Trichoderma* species studied, *T. virens* and *T. harzianum* are reported to be most potential biocontrol agents (Hjeljord and Tronsmo, 1998; Jeger et al., 2009). Therefore, 11 isolates of *T. virens* and 14 isolates of *T. harzianum* were subjected to check their biocontrol activity against different plant pathogens. All the isolates of *T. virens* and *T. harzianum* inhibited mycelial growth of the pathogens. Shimla isolate of *T. harzianum* (Th12) inhibited maximum (91%) mycelial growth of *F. solani* and Hyderabad isolate of *T. virens* (Vn09) inhibited 88% of the mycelial growth of *S. rolfsii*. Thus, it was observed that *T. harziamum* (Th12) was more effective against *F. solani and T. virens* (vn09) against *S. rolfsii*. The observation is similar to the findings of Najar et al. (2011), Federico et al. (2007) and Papavizas and Lewis (1989) in their investigation on the effects of *T. harzianum* and *T. virens* on wilt disease of brinjal, peanut brown root rot and damping-off and blight of snapbean, respectively.

Our result explains the significant success in representing *tef1* as good molecular marker to distinguish the Indian native *Trichoderma* isolates. This study establishes the potential use of *T. harzianum* (Th12) and *T. virens* (Vn09) for controlling disease caused by *F. solani* and *S. rolfsii*, respectively. Although further studies are necessary to achieve proper formulations and delivery systems for the efficacy of the biocontrol agents under different management practices.

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