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Reclassification of *Trichoderma Viride* (TNAU), the Most Widely Used Commercial Biofungicide in India, as *Trichoderma Asperelloides*

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Abstract: *Trichoderma* "*viride*" TNAU is the most widely used biofungicide in India with more than 250 registered commercial formulations being available. We have studied the phylogenetic position of this strain using a molecular marker and determined that this commercial strain is, in fact, a strain of *Trichoderma asperelloides*, and not *T. viride*. The implications of these findings on the registration and commercial distribution of biofungicides based on this strain has been discussed.

Keywords: *Trichoderma viride, T. asperelloides,* biofungicides, formulations.

INTRODUCTION

Trichoderma spp. are widely used as commercial biofungicides the world over [1]. Correct identification is important for successful and safe use of these fungi, comprising of more than 200 defined species. This is especially important since many beneficial and harmful traits are species-, and often strain-specific. For example, *Trichoderma brevicompactum* produces trichothecins (mycotoxins), and some species like *T. longibrachiatum* and *T. citrinoviride* are reported as human pathogens [2-4]. Unfortunately, *Trichoderma* taxonomy, relied earlier on morphology, has been confusing. This situation prevails till date for many isolates that are widely used, including many that are deposited in type culture collections [5]. A well known example is *Trichoderma viride*, the type species of this genus. Back in 1939, Bisby [6] merged all *Trichoderma* species into *T. viride* with perfect stage in *Hypocrea rufa*. In 1969, Rifai [7] proposed *T. viride* as one of the 9 species aggregates, and since then all *Trichoderma* strains having globose, subglobose, or ellipsoidal warted conidia were identified as *T. viride*. Lieckfeldt *et al*. [8], based on morphological, physiological and molecular data, proposed a new species *T. asperellum* within *T. viride* aggregate, and recently, Samuels *et al*. [9], based on multilocus genealogies, along with morphological and proteome data proposed a new species *T. asperelloides* that is distinctly different from *T. asperellum*. Therefore, the original *T. viride* isolates now comprise of three distinct species- *T. viride*, *T. asperellum* and *T. asperelloides*. The species could be differentiated based on sequences of *tef1* (translation elongation factor 1-alpha) or *rpb2* (RNA polymerase B, subunit 2) genes. In their studies, Samuels *et al*. [9] reclassified many widely used "*T. viride*" strains as *T. asperelloides*. Some of the extensively used strains that have been reclassified are *T. harzianum* T103 [10], *T. asperellum* T44 [11], *T. viride* ATCC 52439 and TR 31 [12]. *T. viride* strain, isolated and developed at the Tamil Nadu Agricultural University, Coimbatore, India [13] is the most popular biofungicides approved by the Central Insecticides Board (CIB), Govt. of India, and is extensively marketed in India in the form of more than 250 commercial formulation products [14].

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MATERIALS AND METHODS

For checking if this commercial strain is indeed *T. viride*, we amplified and sequenced the large intron of *tef1* gene using the primer pair EF1-728F and EF1-986R (one of the most reliable phylogenetic markers for identification of *Trichoderma* spp.- http://www.isth.info/tools/blast/markers.-php). The sequences were compared with those of authentic sequences of *T. viride, T. asperellum* and *T. asperelloides* from NCBI GenBank. The phylogenetic analysis was performed on www.phylogeny.fr.

RESULTS AND DISCUSSION

Trichoderma taxonomy has been very dynamic and has seen a great deal of changes after the switch from sole morphological keys to a combination of morphology and molecular phylogeny. Since the genus represents species ranging from saprophytes to plant symbionts and mycotoxin-producers to human pathogens, correct identification, especially of the commercial strains, are of utmost importance. Moreover, many traits are species specific and hence correct identification is necessary to exploit these fungi in a commercial setting. In the present study, we have analysed a strain of *Trichoderma* that is sold widely in India as *T. viride* and a DNA-sequencing (*tef1* gene)- based phylogeny revealed that this isolate is not *T. viride* but *T. asperelloides* (Fig. **1**). We also performed this analysis on a commercial formulation based on *T. viride* TNAU isolate (Phytoguard 1% WP, Central Biotech, Nagpur, India) and the sequence was identical with that of the original TNAU isolate, thus confirming the identification. The *tef1* large (4th) intron se-

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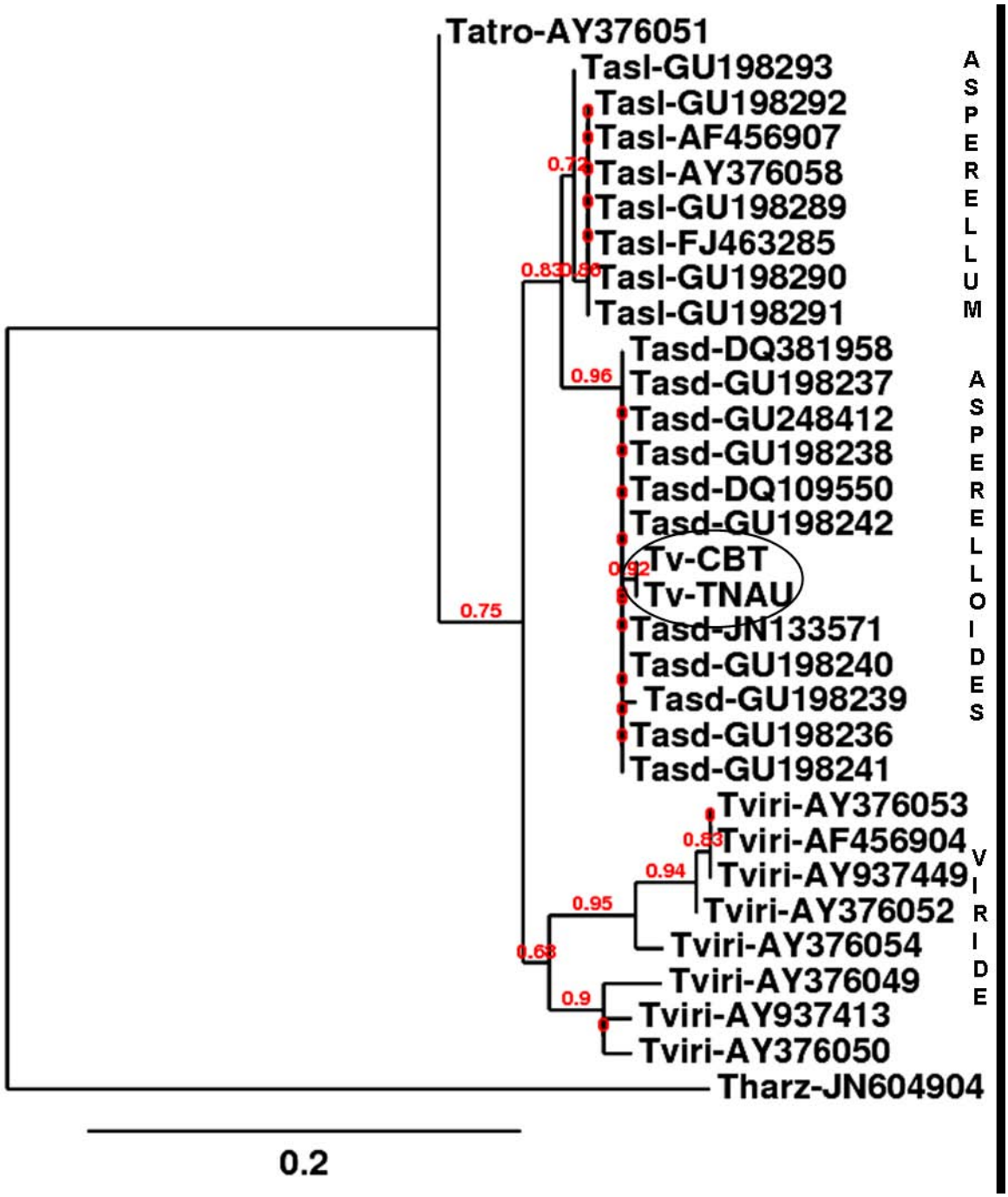

Fig. (1). Phylogenetic position of *Trichoderma viride* TNAU isolate (pure culture and commercial formulation, designated as Tv-TNAU and Tv-CBT, respectively), relative to authentic *T. viride*, *T. asperellum* and *T. asperelloides tef1* sequences. *T. atroviride* and *T. harzianum* sequences were used for comparison.

quence has been deposited with NCBI GenBank (accession no. KC679856). This finding necessitates the re-designation of more than 250 registered commercial formulations based on *T. viride* TNAU isolate as *T. asperelloides*.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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