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Two new species of *Trichoderma* from Yunnan, China

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Abstract Two new species of the fungal genus *Trichoderma*, *Trichoderma compactum* and *Trichoderma yunnanense*, isolated from rhizosphere of tobacco in Yunnan Province, China are described based on morphological characters and phylogenetic analyses of nucleotide sequences. Our DNA sequences included the internal transcribed spacer (ITS) regions of the rDNA cluster (ITS1 and ITS2), and partial sequences of the translation elongation factor 1-alpha (*tef1*) and a fragment of the gene coding for endochitinase 42 (*ech42*). The analyses show that *T. compactum* belongs to the Harzianum clade, and *T. yunnanense* belongs to the Hamatum clade.

Keywords New species · Phylogenetic analysis · *Trichoderma compactum* · *Trichoderma yunnanense*

Introduction

Species in the genus *Trichoderma* are well known for their production of a spectacular array of secondary metabolites including polysaccharases, toxins, and antibiotics (Gams and Bissett 1998). In addition, strains in several species of this genus are widely used in the biocontrol of soilborne plant pathogenic fungi (Samuels 1996).

Because of their economical significance, the taxonomy of this genus and correct identification of the species is necessary. However, because of abundant homoplasy, morphological characters have been proven to be inadequate for species identification (Bissett et al. 2003). With the availability of techniques in analyzing DNA sequence polymorphisms and their increasing use in fungal systematics, the evolutionary relationships among species in *Trichoderma* are beginning to emerge. The molecular techniques include restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) sequence analysis using a single gene (Kindermann et al. 1998) to multiple genes (Kullnig-Gradinger et al. 2002; Samuels et al. 2006; Chaverri et al. 2003a, b). Genes that have been used to study the phylogenetic relationships among species of *Trichoderma* include the nuclear ribosomal internal transcribed spacers (ITS1 and 2) and partial or complete sequences of several genes [the 28S rRNA gene, the translation elongation factor (*tef1*) gene, the gene coding for endochitinase 42 (*ech42*), the calmodulin gene (*cal*), the actin gene (*act*), and the RNA polymerase subunit II (RPB2) gene].

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For the identification of new species in the genus *Trichoderma*, most authors have used the combination of ITS and *tef1* (Kubicek et al. 2003; Bissett et al. 2003; Kraus et al. 2004; Zhang et al. 2005; Lu et al. 2004). Up to now, the number of phylogenetically distinct species of the *Hypocrea* / *Trichoderma* has increased to 100 from 27 morphological species (Bissett 1991a–c; Druzhinina et al. 2006). This increase was mainly due to the introduction of molecular data and the recent extensive sampling in previously under-sampled geographic areas and new ecological niches(Samuels et al. 2006b). Indeed, molecular data have now become essential for the delineation of new species in *Trichoderma* / *Hypocrea* and for determining their connections to teleomorphic species (Bissett et al. 2003; Chaverri et al. 2001; Dodd et al. 2003; Lu et al. 2004; Kraus et al. 2004).

In our study of the occurrence of *Trichoderma* spp. from tobacco rhizosphere soils in Yunnan, China, two new species were identified from a collection of 424 strains that included 10 species of *Trichoderma*. We provide their morphological characteristics and compare their morphological features with those of known sibling species. The phylogenetic affiliations of the two putative new species were determined by the combined analyses of sequences from the ITS, *tef1* and *ech42* gene fragments. Taxonomic descriptions and name validations for the two new species are provided below.

Materials and methods

Trichoderma strains

Soil samples were collected from the rhizosphere of tobacco plants in Yunnan, China. *Trichoderma* strains were isolated from soil samples by first washing the soil, then followed by plating on a selective medium (Elad et al. 1981) and incubating the plates for 6 days at 25°C. Representative colonies were transferred to malt extract agar (MA, Oxoid Ltd., Basingstoke, U.K.) for strain purification and species identification.

Morphological examination

All isolates were grown in Petri dishes containing 2% malt extract agar, incubated for two days at 21°C in the dark, and then exposed to artificial light to stimulate conidium formation. Colony morphological features were based on observations on MA under the above growth conditions and on potato-dextrose agar (PDA) without light. Growth rates were determined at 21, 25, 30 and 35°C on PDA and CMD (20 g cornmeal, 20 g dextrose, 20 g agar, 1,000 ml distilled water). Microscopic observations and measurements were made from preparations mounted in water. The structure and morphology of conidiophores were described from macronematous conidiophores taken from the edge of conidiogenous pustules or fascicles when conidia turned green. The conidia morphological features were recorded after 14 days of incubation.

DNA sequencing and phylogenetic analyses

Total DNA was isolated from fresh mycelium as described by Turner (1997).

A region of the nuclear rRNA gene containing the ITS regions 1 and 2 and the 5.8s rRNA gene was amplified by PCR using the primer combinations SR6R and LR1 in a total volume of 50 µl (White et al. 1990) using an automated temperature-cycling device (Biotron, Biometra, Gottingen). The parameters for PCR amplifications are as follows: 1 min initial denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 50°C, 90 s extension at 74°C, and a final extension period of 7 min at 74°C.

A 0.4 kb fragment of *ech42* was amplified by the primer pair Chit42–1a (5′-GCTYTCCATC GGTGGCTGGAC-3′) and Chit42–2a (5′-GGAG TTGGGGTAGCTCAGC-3′). The amplification protocol was follows: 1 min initial denaturation at 94°C, 30 cycles each of 1 min at 94°C, 1 min at 62°C, and 1 min at 74°C, and a final extension period of 7 min at 74°C.

An approximately 0.2-kb fragment of *tef1* was amplified by the primer pair tef1afw (5′-GTG-AGCGTGGTATCACCATCG-3′) and tef1arew (5′-GCCATCCTTGGAGACCAGC-3′) with the

following amplification protocol:1 min initial denaturation at 94°C, 30 cycles each of 1 min at 94°C, 1 min at 59°C, and 50 s at 74°C, and a final extension period of 7 min at 74°C.

Each PCR reaction product was electrophoresed on 1.5% agarose minigels containing 0.5 mg l^{-1} ethidium bromide for 1 h in 1 × Tris–acetate buffer. The PCR products were revealed under UV light. The PCR products were purified by applying a commercial Kit (TaKaRa Biotechnology Co., Ltd.), and sequenced with the aid of a LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase-kit as described by Kindermann et al. (1998).

DNA sequences were aligned using ClustalX 1.83. Parsimony analysis was run in PAUP* 4.0b10 (Swofford 2002). Gaps were treated as missing data, all characters equally weighted, initial 'MaxTrees' setting at 100. All of the trees were obtained by running the heuristic searches with tree-bisection-reconnection (TBR) as branch-swapping algorithm and up to 1000 random-addition sequence replications. To assess the relative support for each clade, bootstrap values were calculated from 1000 replicate analyses with the heuristic search strategy and random addition sequence of the taxa.

Results

Molecular phylogeny

To determine the phylogenetic placements of the two new taxa, we performed a parsimony analysis using sequences of allied species in the genus *Trichoderma* deposited in the GenBank (Table 1). Our initial BLAST searches identified that sequences from these two new taxa were most closely related to those in the Harzianum and the Hamatum clades. Therefore, part taxa (ITS regions, *tef1* gene and *ech42* gene available from GenBank) within these two clades as well as two adjacent clades (the Semiorbis clade and Viride Clade, Samuels 2006b) were downloaded and aligned for further phylogenetic analysis. Sequences from the three genes (the ITS region (excluding the 5.8S region), the *ech42* gene and the *tef1* gene) were combined with a total of 726 aligned nucleotides. 424 characters were constant, 58 variable characters were parsimony-uninformative, the number of parsimony-informative characters was 244. No outgroup was set specially because there is no an appropriate taxon for which three genes were available from GenBank. Using a default outgroup for rooting, our results show that *Trichoderma*

Table 1 List of *Trichoderma* strains and Genbank accession number used in this study

Species	Collection and number	GenBank Accession Number		
		ITS1 and ITS2	tef1a	ech42
Hypocrea hunua	CBS 238.63	AF400257	AF401011	AF400745
H. tawa	CBS 246.63	AF400258	AF401006	AF399258
T. agg.f.aggressivum	DAOM 222156	AF456924	AF348098	AF399282
T. asperellum	CBS 433.97	AJ230668	AF401000	AF276652
T. atroviride	DAOM 165779	Z48817	AF400997	AF276650
T. cerinum	DAOM 230012	AF149869	AY605802	AY605863
T. compactum	YMF 1.01693	AY941822	AY941824	DQ531050
T. fertile	DAOM 167161	AF400260	AF401025	AF399262
T. hamatum	DAOM 167057	Z48816	AF401019	AY665690
T. harzianum	CBS 226.95	AF057606	AF401016	AF276646
T. inhamatum	CBS 273.78	Z68187	AF401015	AF399271
T. koningii	CBS 979.70	Z79628	AF400990	AF188918
T. oblongisporum	CBS343.93	AF011976	AF401022	AF400746
T. pubescens	DAOM 166162	AF011978	AY750887	AY665712
T. tomentosum	CBS 349.93	AF011984	AF401024	AF399277
T. velutinum	DAOM 230013	AF149873	AY937415	AY605859
T. virens	CBS 249.59	AF222865	AF400998	AF276654
T. viride	ATCC 28020	AJ230678	AF400992	AF188928
T. yunnanense	YMF 1.01694	AY941823	AY941825	DQ531051

compactum belongs to the Harzianum clade, and *Trichoderma yunnanense* is a member of the Hamatum clade. Most of the branches in the phylogenetic tree from the combined dataset had high bootstrap support (Fig. 1). Trees inferred from individual genes (ITS and *ech42*) all showed the same topology (not shown here).

Taxonomy

Trichoderma compactum Z. F. Yu & K. Q. Zhang, sp. nov. (Fig. 2)

Coloniae 6–6.5 cm diametro post 3 dies in agaro PDA 21°C, partes conidiiferae cinereovirides. Conidiophora fasciculata vel pustuleas compactas usque ad 24–35 μm diametro formantia. Phialides ampulliformes vel lageniformes, 3–5 verticillatae, plerumque 4.0–6.8 × 2.6–3.5 μm. Conidia subglobosa vel obovata, levia, 3.0–3.5 × 2.8–3.2 μm.

Species nova *Trichodermati harziano (T. inhamato)* similis, sed crescentia lentiore, et sequentia ''ITS1–2'' distinguenda..

Etymology: in reference to the characteristically very compact conidiation.

Colonies 62–64 mm diam. after 72 h at 21°C, 50–52 mm diam. at 25°C, 52 mm diam. at 30°C, no growth at 35°C on PDA. Colonies 18–20 mm diam. after 72 h at 21°C, 21–22 mm at 25°C, 30–36 mm at 30°C, no growth 35°C on CMD. Aerial mycelium usually lacking or sparse on MA and PDA. Conidiation mostly aggregated in small, compact, irregular pustules up to 24–35 μm in diameter on MA, frequently concrescent to form irregular or concentric masses; white at first, then soon turning gray-green; surface of pustules appearing granular or powdery owing to dense conidiation, often fringed by sterile white mycelium. Reverse colorless. Exudate lacking. Odour indistinct. Submerged hyphae hyaline, smooth-walled, 2–6 μm wide. Chlamydospores not abundant, developing in old cultures (after two weeks)on PDA, mostly in the submerged mycelium, terminal or intercalary, solitary, subglobose, ellipsoidal, 6–10 μm in diameter, with contents appearing granular. Conidiophores hyaline, smooth-walled. Macronematous conidiophores in pustules straight to slightly flexuous, 3–4 μm wide for the most part, but up to 7 μm wide near the base, primary branches arising at almost right angles, or bent slightly toward the apex; primary branches usually arising in pairs, or single, less often in whorls of 3, slightly increasing in length toward the base of the conidiophores; primary

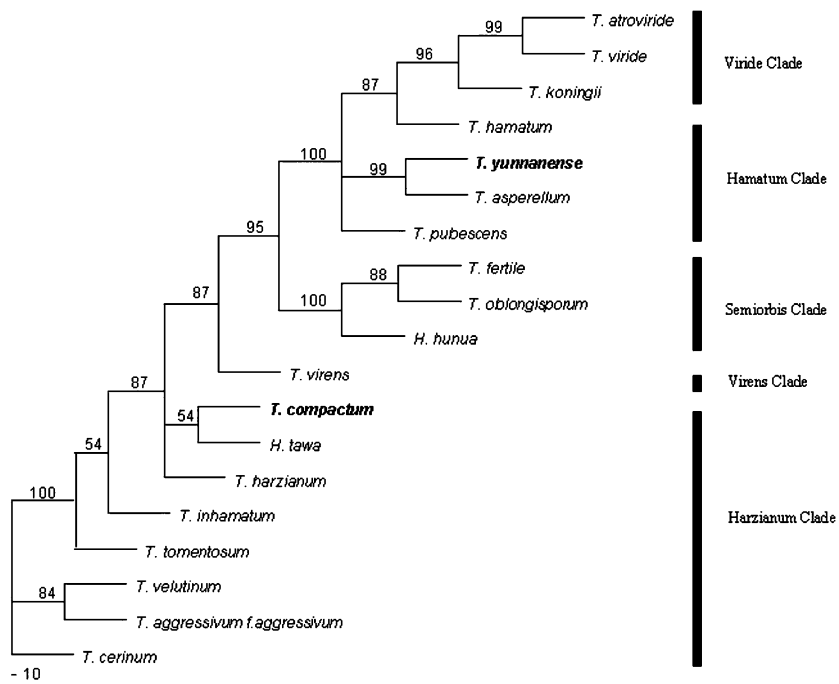

Fig. 1 Cladogram showing the single most parsimonious tree, inferred by combined sequence of ITS1 and ITS2, *tef1* and *ech42* sequences. Numbers above lines represent bootstrap values from 1,000 replicates on all parsimony-informative characters, only bootstrap values >50% shown. Tree length 613, CI is 0.6803, HI is 0.3197, RI is 0.8207. Vertical bars indicate the clades previously identified by Druzhinina (2006)

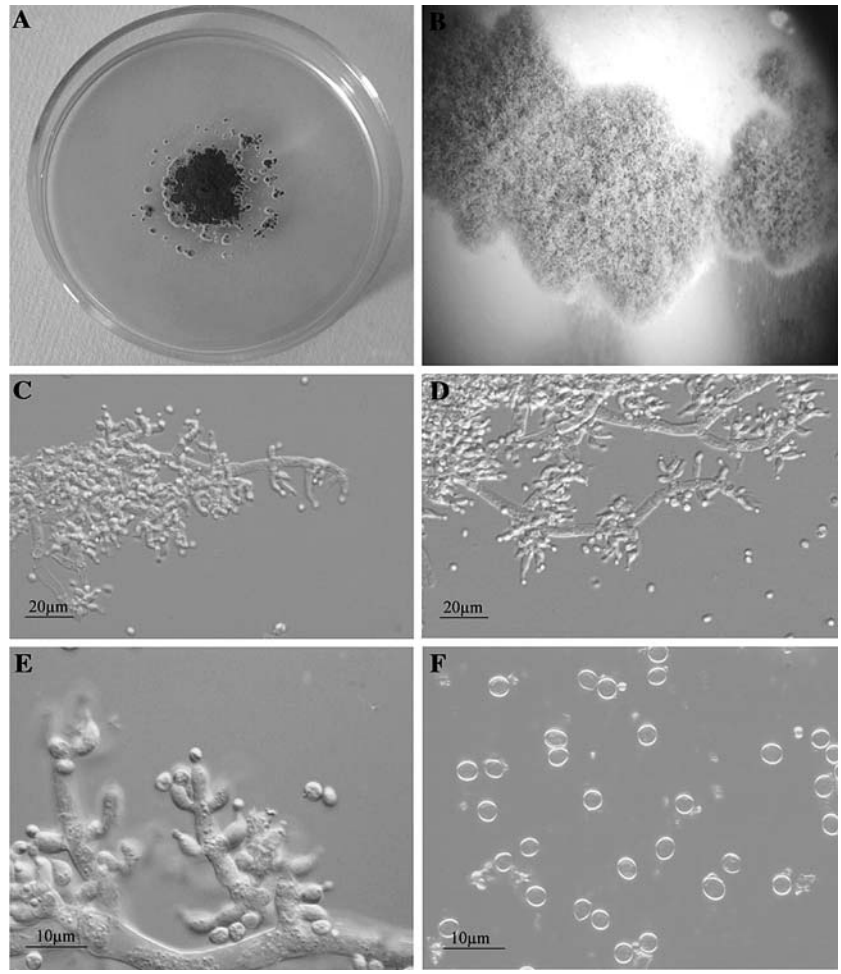

Fig. 2 *Trichoderma compactum* (YMF 1.01693). A.Colony on 2% malt agar after 6 days. B. Compact irregular conidiogenous pustules. C and D. Conidiophore with short side branches arising in pairs, or solitary. E. Small, lageniform to ampulliform phialides, only slightly constricted at the base. F. Small, subglobose or obovoid conidia

branches mostly 1- or 2-celled, less frequently rebranched. Phialides arising in whorls of 2–5, but mostly in uncrowded verticils of 2 or 3 or solitary, lageniform to ampulliform, mostly 4.0–6.8 (–8) × 2.6–3.5 (–4) μm, not or only slightly constricted at the base, often bent toward apex of conidiophore, gradually narrowing to a short conidium-bearing tube about 0.8 μm wide;terminal phialides narrower, 9–10 × 2–3 μm. Whole branches appearing crowded due to branches arising at close intervals (about 7 μm). Conidia subglobose or obovoid, both ends broadly rounded or the base slightly narrower, 3.0–3.5 (–4) × 2.8–3.2 (–3.3) μm (av. 3.3 × 3 μm); smooth-walled, appearing thin-walled, pale green viewed singly, usually greenish in mass.

Holotype: China, isolated from tobacco rhizosphere soil near Yuxi county, Yunnan Province, June 2002, Z. F. Yu, YMF 1.01693 (dried culture YMF 1.01693, YMF is a shortened form for Key laboratory of Yunnan Microbiology Fermentation).

Trichoderma yunnanense Z. F. Yu & K. Q. Zhang, sp. nov. (Fig. 3)

Coloniae 4–4.5 cm diametro post 3 dies (21°C), partes conidiiferae flavovirentes vel atrovirentes, reverso concolori vel luteo. Conidiophora fasciculata vel pustulas compactas usque ad 24–75 μm diametro formantia. Phialides ampulliformes vel lageniformes, solitariae, oppositae, raro ternae verticillatae, plerumque 7–11 × 4–5 μm. Conidia obovata, levia, 4.0–5 × 3.5–4 μm.

Species nova *Trichodermati virenti* similis, ITS sequentea ad *T. asperellum*spectat, sed sequentia

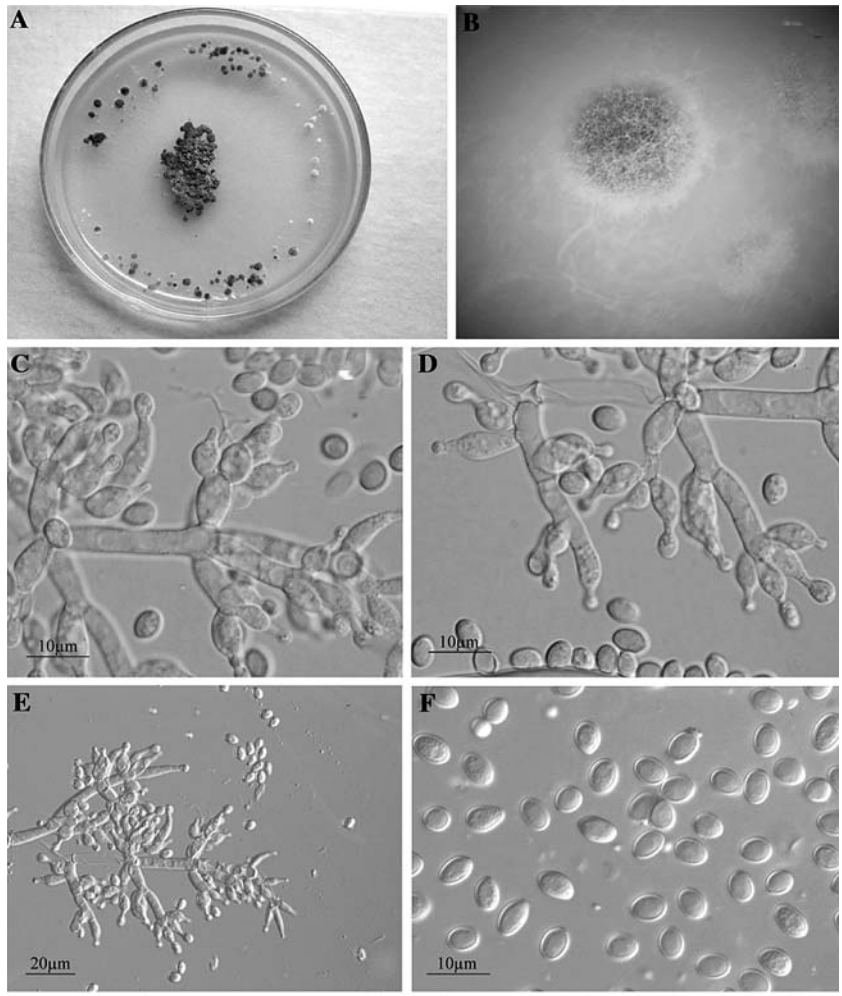

Fig. 3 *Trichoderma yunnanense* (YMF 1.01694) A.Colony on 2% malt agar after 6 days. B. Hemispherical conidiogenous pustule on MA after 6 days. C and D. Lageniform to ampulliform, slightly convergent phialides. E. Conidiophore showing irregular branching. F. Large, obovoid conidia

''*tef1*'' distinguenda, et conidiophoris magis intricatis aggregatis differt.

Etymology: in reference to the site where sample collected.

Colonies growing moderately rapidly, reaching 42–43 mm diam. at 21°C after 72 h, 64 mm at 25°C, 66 mm at 30°C, 12 mm at 35°C on PDA. Colonies on CMD reaching 50–60 mm diam. after 72 h at 21°C, 85 mm at 25°C, 57–60 mm at 30°C, 42–46 mm 35°C. Aerial mycelium usually limited on MA, on PDA forming white or grayish woolly mycelium. Conidiation typically in numerous irregular cushion-shaped to hemisphaerical pustules, 24–75 μm diam., evenly distributed, not concrescent and forming masses on MA, but sometimes concrescent on PDA; white at first, slowly turning yellow-greenish, white green to dark green. Reverse colorless. Exudate lacking. Emitting a distinct coconut odour. Aerial mycelium hyaline, smooth-walled, 2–3.5 μm in diameter. Submerged mycelium rarely up to 5 μm wide. Chlamydospores fairly abundant, developing in old cultures mostly in the submerged mycelium, mostly terminal on short branches of the mycelium, occasionally intercalary, solitary, subhyaline, subglobose, ellipsoidal or pyriform, 7–11 μm in diameter, smooth- and thin-walled, with contents appearing granular. Conidiophores hyaline, smooth-walled; macronematous conidiophores in pustules flexuous, 3 μm wide in the apex part but up to 5 μm wide near the base. Branching irregularly, primary branches arising at acute angles, or bent slightly toward the apex of conidiophores, solitary or paired, rarely in whorls of 3, increasing in length toward the base of the conidiophores, entire structure more or less

broadly pyramidal. Phialides from macronematous conidiophores lageniform to ampulliform, mostly 7–11 × 4–5 μm, conspicuously constricted at the base, abruptly narrowed to a short conidium bearing tube about 1 μm wide; arising seperately, or more often paried with branches, rarely in whorls of 3, usually reflexed toward the apex of the conidiophore and appressed rather than divergent. Conidia obovoid, 4–5 × 3.5–4 μm (avg. 4.5–4 μm), both ends broadly rounded or the base slightly narrower, smooth-walled, pale green viewed singly, dark green in mass.

Holotype: China, isolated from tobacco rhizosphere soil near Yuxi county, Yunnan Province, June 2002, Z. F. Yu, YMF 1.01694 (dried cultureYMF 1.01694).

Discussion

T. compactum is known only from a single isolate from soil. It is readily distinguished by the short and bent phialides, small obovoid to subglobose conidia. From the form of conidia, this species most resembles *Trichoderma harzianum*(in particular its subordinate *Trichoderma inhamatum*). However, *T. compactum* is distinguished from *T. harzianum* in growing more slowly, in having larger conidia and more compact pustules of conidiation. In the branching pattern of the conidiophore, *T. compactum* resembles *Trichoderma croceum* and *Trichoderma hamatum*, but there are large differences in the conidia size. Additionally, *T. croceum* and *T. hamatum* have a sterile apex that is lacking in *T. compactum*.

A BLAST search (NCBI GenBank, http://www.ncbi.nlm.nih.Gov/ BLAST) with the ITS1 and 2 sequences of the haplotype of *T. compactum* revealed that the closest species to *T. compactum* is *Trichoderma aggressivum* f. *aggressivum* (GenBank accession AY605757), and similarity is 96.6%. However, *T. compactum* is distinguished from *T. aggressivum* f. *aggressivum* by branching systems (the former mostly paired), the shape and size of phialides (the former flask-shaped, (4.0–) 5.7–7.8 (–21.0) × (1.3–) 2.7–3.5 (–4.3) μm, the later lageniform to ampulliform, often bent, 4.0–6.8 (–8) × 2.6–3.5 (–4) μm).

Morphologically, *T. yunnanense* is readily distinguished by slightly appressed phialides, and large, obovoid conidia. In addition, conidial areas form distributed pustules on MA, and cultures emit a distinctive coconut odour. This most similar species is *Trichoderma crassum*. Both species have broad, irregularly branched conidiophores, appressed rather than divergent phialides and large dark green conidia. However, *T. crassum* has sterile apical elongations that are lacking in *T. yunnanense*. In addition, *T. yunnanense* is also similar to *Trichoderma virens* in having appressed phialides and large dark conidia; *T. yunnanense* and *T. virens* differ in the conidiophores: *T. yunnanense* shows more complex branching and conidiophores organized in conspicuous pustules, whereas *T. virens* has predominantly effuse conidiation.

T. yunnanense is most similar to species in section *Pachybasium* as defined by Bissett (1991a–1991c), such as conidiation forming hemisphaerical pustules and lageniform to ampulliform phialides. However, it is evolutionarily more similar to species of section *Trichoderma*. This result further shows that sectional definitions based on morphological features have lost their meaning.

Sequences from *T. yunnanense* were very similar to *Trichoderma asperellum* (Samuels et al. 1999) for DNA fragments from both the ITS and *ech42* (the similarity of ITS is 99.6%, *ech42* is 98.1%). However, *T. asperellum* can be easily distinguished from *T. yunnanense* by its faster growth rate, mostly paired branches, fine conidial ornamentation, and slightly smaller conidia. In addition their *tef1* sequences were quite different (similarity 90.3%). These results underline the importance of using multiple genes in inferring phylogenetic relationships among species in *Trichoderma*.

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References

Bissett J (1991a) A revision of the genus *Trichoderma*.II. Infrageneric classification. Can J Bot 69:2357–2372

Bissett J (1991b) A revision of the genus *Trichoderma*.III. Sect. *Pachybasium*. Can J Bot 69:2373–2417

Bissett J (1991c) A revision of the genus *Trichoderma*. IV. Additional notes on section *Longibrachiatum*. Can J Bot 69:2418–2420

Bissett J, Szakacs G, Nolan CA et al (2003) New species of *Trichoderma* from Asia. Can J Bot 81:570–586

Chaverri P, Samuels GJ, Stewart EL (2001) *Hypocrea virens* sp. nov., the teleomorph of *Trichoderma virens*. Mycologia 93:1113–1124

Chaverri P, Castlebury LA, Samuels GJ et al (2003a) Multilocus phylogenetic structure within the *Trichoderma harzianum* / *Hypocrea lixii* complex. Mol Phylogenet Evol 27:302–313

Chaverri P, Castlebury LA, Overton BE et al (2003b) *Hypocrea* / *Trichoderma*: species with conidiophore elongations and green conidia. Mycologia 95:1100–1140

Dodd SL, Lieckfeldt E, Samuels GJ (2003) *Hypocrea atroviridis* sp.nov., the teleomorph of *Trichoderma atroviride*. Mycologia 95:27–40

Druzhinina IS, Kopchinskiy A, Kubicek CP et al (2006) The first 100 Trichoderma species characterized by molecular data. Mycoscience 47:55–64

Elad Y, Chet I, Henis Y (1981) An selective medium for improving quantitative isolation of *Trichoderma*spp. from soil . Phytoparasitica 9:59–67

Gams W, Bissett J (1998) Morphology and identification of *Trichoderma*. In: Kubicek CP, Harman GE (eds) *Trichoderma* and *Gliocladium* Basic biology, Taxonomy and Genetics, vol 1. Taylor and Francis, London, pp 3–34

Kindermann J, El-Ayouti Y, Samuels GJ et al (1998) Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genet Biol 24: 298–309

Kraus GF, Druzhinina I, Gams W et al (2004)*Trichoderma brevicompactum* sp. nov. Mycologia 96:1059–1073

Kubicek CP, Bissett J, Druzhinina I et al (2003) Genetic and metabolic diversity of *Trichoderma*: a case study on South–East Asian isolates. Fungal Genet Biol 38: 310–319

Kullnig CM, Szakacs G, Kubicek CP (2002) Phylogeny and evolution of the genus *Trichoderma*: a multigene approach. Mycol Res 106:757–767

Lu BS, Druzhinina IS, Fallah P et al (2004) *Hypocrea/ Trihcoderma* species with *pachybasium*-like conidiophores: teleomorphs for *T. minutisporum* and *T. polysporum* and their newly discovered relatives. Mycologia 96:310–342

Samuels GJ (1996) *Trichoderma*: a review of biology and systematics of the genus. Mycol Res 100:923–935

Samuels GJ, Lieckfeldt E, Nirenberg HI (1999) *Trichoderma asperellum*, a new species with warted conidia, and redescription of *Trihcoderma viride*. Sydowia 51: 71–88

Samuels GJ, Suarez C, Solis K et al (2006) *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. Mycol Res 110: 381–392

Samuels GJ (2006b) Trichoderma: systematics, the sexual state, and ecology. Phytopathology 96:195–206

Swofford DL (2002) PAUP. Phylogenetic analysis using parsimony, Version 4.0. Sinauer Associate, Sunderland, Massachusetts, USA

Turner D, Kovacs W, Kuhls K et al (1997) Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycol Res 101:449–459

White TJ, Bruns T, Lee S et al, (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego CA, pp 315–322

Zhang CL, Druzhinina IS, Kubicek CP et al (2005) *Trichoderma* biodiversity in China: Evidence for a North to South distribution of species in East Asia. FEMS Microbiol Lett 251:251–257