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ORIGINAL PAPER

Trichoderma songyi sp. nov., a new species associated with the pine mushroom (*Tricholoma matsutake*)

Myung Soo Park · Seung-Yoon Oh · Hae Jin Cho · Jonathan J. Fong · Woo-Jae Cheon · Young Woon Lim

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Abstract A new species, *Trichoderma songyi*, was found to be associated with the pine mushroom (*Tricholoma matsutake*) in Korea. This species was isolated from three different substrates: *Tricholoma matsutake* basidiomata, as well as roots of *Pinus densiflora* and soil in the fairy ring. Based on its molecular and phenotypic characteristics, we demonstrate that *Trichoderma songyi* is unique and distinguishable from closely related species. We performed phylogenetic analyses based on two molecular markers, the genes for both translation elongation factor 1-alpha and the second largest subunit of RNA polymerase II. Phylogenetic analyses showed that *Trichoderma songyi* is closely related to *Trichoderma koningii* aggregate and *Trichoderma caerulescens*. Morphologically, *Trichoderma songyi* can be distinguished from these closely related taxa by its growth rates, colony morphology on PDA in darkness, and coconut-like odour. Due to the economic importance of the pine mushroom, the relationship between *Trichoderma songyi* and *Tricholoma matsutake* should be studied further.

Keywords New species · Pine mushroom · *Trichoderma koningii* aggregate · Translation elongation factor 1-alpha · Second largest subunit of RNA polymerase II

Introduction

Species of *Trichoderma* Pers. are frequently found in soil, decaying wood, and vegetable matter, as well as on other fungi (Druzhinina et al. 2011; Samuels 2006). *Trichoderma* is noteworthy because numerous bioactive compounds, such as polysaccharases, toxins, and antibiotics, have been isolated from this genus (Sivasithamparam and Ghisalberti 1998; Reino et al. 2007; Klein and Eveleigh 1998). In particular, *Trichoderma* species are known as biocontrol agents of plant pathogens (Harman et al. 2004).

Traditional taxonomy of *Trichoderma* was based on a combination of morphological characters. However, these morphological characters can be insufficient to differentiate species (Samuels et al. 2002) and/or unreliable because they can change based on environmental conditions (Ospina-Giraldo et al. 1998). In recent years, sequence analysis of the nuclear ribosomal internal transcribed spacers region, the translation elongation factor 1-alpha (*tef1*) gene, and the second largest subunit of RNA polymerase II gene (*rpb2*) has been used

Myung Soo Park and Seung-Yoon Oh contributed equally to this work.

M. S. Park · S.-Y. Oh · H. J. Cho · J. J. Fong · Y. W. Lim (✉)
School of Biological Sciences, Seoul National University, Seoul 151-747, South Korea
e-mail: ywlim@snu.ac.kr

W.-J. Cheon
Gyeongsanbuk-do Forest & Environment Research Institute, Gyeongju 780-936, South Korea

to study the phylogenetic relationships within *Trichoderma* (Chaverri et al. 2003; Druzhinina et al. 2012; Samuels et al. 2006). Currently, based on a combination of genetic and morphological data, approximately 200 *Trichoderma* species have been recognized (Jaklitsch 2009, 2011; Jaklitsch and Voglmayr 2013).

The pine mushroom (*Tricholoma matsutake* Sing.) is a highly prized, edible wild mushroom found in the Northern Hemisphere (Yun et al. 1997; Bergius and Danell 2000; Hall et al. 2003). To date, it has not been successfully cultivated, and it is only found in ectomycorrhizal associations with trees in the families Pinaceae and Fagaceae (Yamada et al. 2010, 2014; Gill et al. 2000). *Trichoderma* species can negatively impact on commercial mushrooms (Samuels et al. 2002; Park et al. 2005; 2006; Kim et al. 2012), but it has been suggested that they may participate in a mutualistic relationship with *Tricholoma matsutake* due to positive correlation of existence (Vaario et al. 2011). We surveyed *Trichoderma* species from substrates associated with *Tricholoma matsutake* basidiomata, as well as roots of *Pinus densiflora* and soil from the fairy ring. Upon sequencing and comparing these data with GenBank using BLAST, we found seven unique *Trichoderma* strains whose DNA had low similarity to the available sequences, raising the possibility that they represent a new species. In the current study, we used the genealogical concordance phylogenetic species recognition (GCPSR; Taylor et al. 2000) approach to test whether these strains represent a new species. We compared the genealogies of two loci (*tef1* and *rpb2*) that have shown high resolution for *Trichoderma* and confirm that these strains are distinct. Additionally, we provide detailed descriptions of macro- and micromorphological characteristics of the potential new species and compared them with closely related species to demonstrate that the new species is also morphologically distinct. Because these seven strains are molecularly and morphologically distinct from known species of *Trichoderma*, herein we describe it as a new species.

Materials and methods

Sampling and isolation

For sampling, three *Tricholoma matsutake* harvesting sites in South Korea were selected: Hongcheon (N37°41′35″ E127°58′51″), Uljin (N37°02′09″ E129°17′62″), and Pohang (N36°06′21″ E129°07′24″). We screened for *Trichoderma* species from pine mushroom basidiomata, as well as roots of *Pinus densiflora* and soil from the fairy ring. Basidiomata were collected only in Hongcheon, whereas roots and soil were collected from all three sampling sites. All samples were collected from September to October of 2013 and stored at 4 °C until use. For isolation, basidiomata and roots were rinsed with distilled water to remove soil and organic debris; then, 5-mm pieces were placed on either potato dextrose agar (PDA; Difco, USA) or dichloran rose bengal chloramphenicol agar (DRBC; Difco, USA). For 5 g of soil, three serial dilutions (1/10, 1/100, 1/1,000) were made using deionized water, and 0.1 mL of each serial dilution was transferred to the surface of PDA and DRBC plates. After plates were incubated at 25 °C for 2–7 days, individual isolates were transferred to new PDA plates and incubated at 25 °C. The strains isolated in this study were stored in 20 % glycerol at −80 °C in the Seoul National University Fungus Collection (SFC), Seoul, Korea, and they were deposited at the Korean Collection for Type Culture (KCTC), Daejeon, Korea. The ex-type was also deposited in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands (Table 1).

DNA extraction and PCR amplification

Approximately 50 mg of fungal material from each culture was placed in 500 μL of 2X CTAB buffer and ground with a plastic pestle. Genomic DNA was extracted using a modified CTAB extraction protocol (Rogers and Bendich 1994). We collected data from two genetic markers, *tef1* and *rpb2*, which have been shown to have high resolution for *Trichoderma* species (Jaklitsch et al. 2013). The amplification of *tef1* was performed using EF1-728F (Carbone and Kohn 1999) and TEF1rev (Samuels et al. 2002), whereas *rpb2* was amplified using fRPB2-5F and fRPB2-7CR (Liu et al. 1999). Each PCR amplification was performed on a C1000^{TM} thermal cycler (Bio-Rad, CA, USA) using Maxime PCR PreMix i-StarTaq (Intron Biotechnology Inc., Seoul, Korea) in a final volume of 20 μL containing 10 pmol of each primer and 1 μL of DNA (10 ng/μL). PCR amplification of each gene was performed as described by Park et al. (2013). PCR products were electrophoresed through a 1 % agarose gel stained with loading STAR (Dyne Bio, Seoul, Korea) and purified using the Expin^{TM}

Table 1 Strains and accession numbers of *Trichoderma songyi*. "T" superscript denotes the type strain

Strain	Other collection number	Habitat	Collection date	Locality	GenBank accession numbers	
					tef1	*rpb2*
SFC20130926-S001^{T}	KCTC 46205, CBS 138099	Basidioma of *Tricholoma matsutake*	26 Sep 2013	Hongcheon, South Korea	KJ636511	KJ636525
SFC20130926-S002	KCTC 46206	Basidioma of *Tricholoma matsutake*	26 Sep 2013	Hongcheon, South Korea	KJ636512	KJ636526
SFC20130926-S003	KCTC 46207	Basidioma of *Tricholoma matsutake*	26 Sep 2013	Hongcheon, South Korea	KJ636513	KJ636527
SFC20130926-S004	KCTC 46208	Basidioma of *Tricholoma matsutake*	26 Sep 2013	Hongcheon, South Korea	KJ636514	KJ636528
SFC20131005-S042	KCTC 46209	Roots of *Pinus densiflora* from fairy ring of *Tricholoma matsutake*	04 Oct 2013	Hongcheon, South Korea	KJ636515	KJ636529
SFC20131005-S074	KCTC 46210	Roots of *Pinus densiflora* from fairy ring of *Tricholoma matsutake*	04 Oct 2013	Uljin, South Korea	KJ636516	KJ636530
SFC20131005-S109	KCTC 46211	Soil from fairy ring of *Tricholoma matsutake*	04 Oct 2013	Pohang, South Korea	KJ636517	KJ636531

PCR Purification Kit (GeneAll Biotechnology, Korea) according to the manufacturer's instructions.

Sequencing and phylogenetic analysis

DNA sequencing was performed at Macrogen (Seoul, Korea) using an ABI3700 automated DNA sequencer with the indicated PCR primers. Seven new *Trichoderma* strains were sequenced for both markers. After proofreading and editing the sequences using MEGA 5 (Tamura et al. 2011), all of the sequences were deposited in GenBank (see Table 1 for the accession numbers). Phylogenetic analyses were performed in two steps. First, the new sequences were analyzed with a *tef1* dataset from GenBank spanning the diversity of *Trichoderma* to identify the closely related species. *Protocrea pallida* (EU703900) and *Protocrea farinosa* (EU703892) were selected as the outgroups for these analyses based on previous studies (Jaklitsch 2009, 2011). Next, *rpb2* data were downloaded from GenBank for a reduced species set, focusing on the closely related species identified in the *tef1* analyses. The second set of phylogenetic analyses was performed on a combined *tef1* + *rpb2* dataset. The outgroups selected for these analyses were one *Trichoderma neorufoides* (strain CPK1904) and two *Trichoderma neorufum* (strains CBS119498 and GJS96-132), based on previous studies (Jaklitsch 2009, 2011).

For all analyses, multiple sequence alignments were conducted using MAFFT v7 (Katoh and Standley 2013) with the default settings. Sequence alignments were checked by eye and adjusted manually. Maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses were performed on *tef1* and a combined dataset (*tef1* + *rpb2*). ML analyses were performed using RAxML (Stamatakis 2006) with the GTRGAMMA model of nucleotide substitution and 1,000 bootstrap replicates. BI analyses were conducted using MrBayes v. 3.2.1 (Ronquist et al. 2012) with the best model of nucleotide substitution selected for each marker using the Bayesian information criteria in jModeltest v. 2.1.2 (Darriba et al. 2012). Two independent searches with random starting trees were run for each dataset for 10 million generations, with sampling every 100th generation. Data from independent runs were combined after removing a 25 % burn-in estimated using Tracer v. 1.5 (Rambaut and Drummond 2009). Final consensus trees were

constructed using the 50 % majority rule, with posterior probabilities for each node.

Phenotype analysis

The cultures were incubated on cornmeal dextrose agar with 2 % dextrose (CMD, Difco cornmeal agar+ 2 % (w/v) dextrose), synthetic low-nutrient agar (SNA; Nirenberg 1976), and PDA at 25 °C for 7–10 days under alternating 12 h of cool, white fluorescent light and 12 h of darkness. Culture color names and codes were based on the Methuen Handbook of Colour (Kornerup and Wanscher 1963). All microscopic characters were taken from cultures grown on CMD and SNA for 7 days at 25 °C. The examination and measurement of conidiophores and conidia were conducted in 3 % KOH. When possible, 30 individuals were measured for each microscopic parameter. Microscopy was performed using a light microscope (Nikon 80i). To obtain growth rate data for both the PDA and SNA media, we used the procedure described by Samuels et al. (2002). When the colony growth was visible on PDA, a plug (5 mm in diameter) was taken from the actively growing edge of the colony and incubated on two new 90-mm Petri dishes, one containing PDA and one containing SNA medium. Plates were incubated in darkness at 15, 20, 25, 30, and 35 °C, and the colony radius was measured after 72 h. Each experiment was conducted in triplicate.

Results

Phylogenetic analysis

The sequencing of *tef1* and *rpb2* from all seven strains was successful. Additional taxon sampling for each of the two genes depended on the purpose of the analysis and the availability of sequences in GenBank. For broad taxonomic sampling across *Trichoderma* to understand the overall placement of the seven strains, we included 270 *tef1* sequences from GenBank. The HKY+I+G model of sequence evolution was selected for BI analysis. For both the ML and BI analyses of the *tef1* dataset, the seven strains were shown to be a new taxon because they were monophyletic and distinct from all known, sequenced species. This new taxon was placed in the section *Trichoderma*, showing affinity to the *Trichoderma koningii* aggregate (data not shown).

For the combined dataset (*tef1* + *rpb2*), the sampling focused on the section *Trichoderma* to determine the sister taxon or taxa of the new species. Complete data for 91 taxa from GenBank were included in these analyses. ML and BI analyses partitioned by gene were performed. For the BI analyses, the model of nucleotide substitution selected was HKY+I+G for *tef1* and SYM+G for *rpb2*. The ML and BI topologies were similar, with a slight variation in the nodal support for each marker (Fig. 1). As in the *tef1* analyses, all seven new *Trichoderma* strains formed a monophyletic clade with strong support (ML = 100 %, BI = 1.0; Fig. 1).

Based on the combined dataset, the *Trichoderma koningii* aggregate was inferred to be the sister group to the new species, with strong support (ML = 82, BI = 0.99; Fig. 1). Another relatively closely related species was *Trichoderma caerulescens*, being the sister species to the clade containing the *Trichoderma koningii* aggregate and the new species (ML = 100, BI = 1.0; Fig. 1). Comparing sequences among these closely related groups, for *tef1*, the new species showed sequence similarity of 88.2–89.8 % to *Trichoderma caerulescens* and 85.9–90.6 % to the *Trichoderma koningii* aggregate, with *Trichoderma koningii* being the most similar (89.5–90.6 %). For *rpb2*, the new species was 94.6–94.8 % similar to *Trichoderma caerulescens* and 94.7–96.6 % similar to the *Trichoderma koningii* aggregate, with *Trichoderma ovalisporum* showing the highest similarity at 96.2–96.6 %.

Morphological comparison

We compared the morphology of *Trichoderma songyi* and the closely related species identified in the molecular analyses: *Trichoderma koningii* aggregate and *Trichoderma caerulescens* (see Table 2 for selected comparisons). The *Trichoderma koningii* aggregate comprises 13 taxa (11 species, 2 varieties) that are phylogenetically and morphologically distinct (Samuels et al. 2006). We compared the morphology of *Trichoderma songyi* with those of all 13 taxa in the *Trichoderma koningii* aggregate, with three highlighted in this paper: *Trichoderma koningii* (highest sequence similarity of *tef1*), *Trichoderma ovalisporum* (highest sequence similarity of *rpb2*), and *Trichoderma caribbaeum* var. *caribbaeum* (highest morphological similarity). *Trichoderma songyi* can be distinguished from *Trichoderma koningii, Trichoderma*

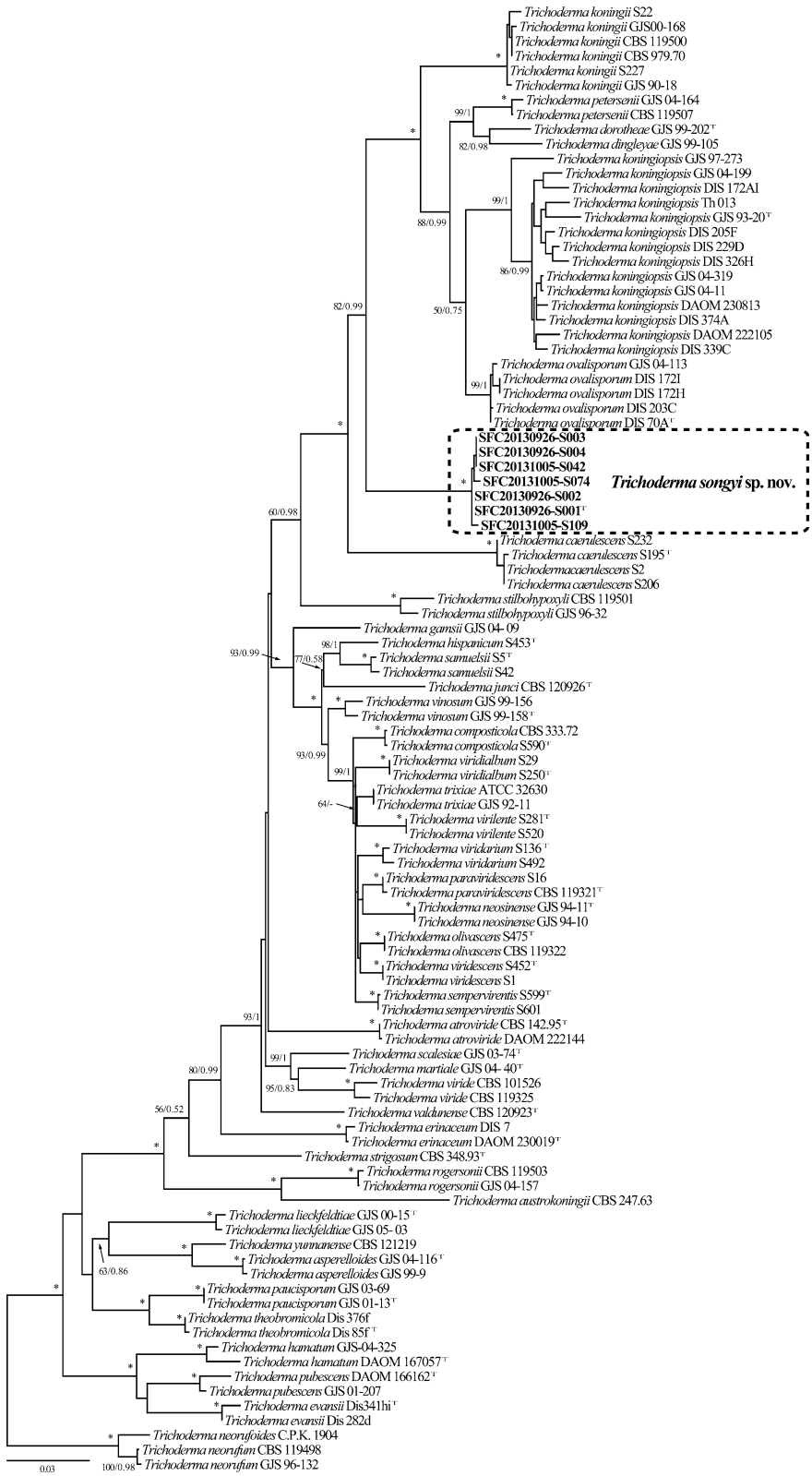

Fig. 1 Phylogenetic tree based on the maximum likelihood (ML) analysis of the combined dataset (*tef1* + *rpb2*). Branch support values are given as ML bootstrap values and Bayesian posterior probabilities. The *scale bar* indicates the number of nucleotide substitutions per site. *Asterisks* on branches indicate high support (100 % ML bootstrap and 1.00 posterior probability)

Table 2 Morphological comparisons of *Trichoderma songyi* and closely related species

Character	*T. songyi* (This study)	*T. caerulescens* (Jaklitsch et al. 2012)	*T. caribbaeum* var. *caribbaeum* (Samuels et al. 2006)	*T. koningii* (Samuels et al. 2006)	*T. ovalisporum* (Samuels et al. 2006)
Geographic origin	Republic of Korea	Southern Europe	Puerto Rico, Guadeloupe	Eastern N. America, Europe	Brazil (Amazonia), Ecuador
Phialides					
Shape	Narrowly lageniform, somewhat swollen in the middle, straight	Narrowly lageniform, less commonly ampulliform and then often with long neck, straight or curved or sometimes sigmoid	Straight, lageniform, somewhat swollen in the middle; intercalary phialides present but not common	Lageniform, somewhat swollen in the middle, straight	Lageniform and more or less swollen below the tip to (less frequently) cylindrical
L (μm)	8.0–14.9 (–18.0)	(4.5–) 6.2–10.8 (–16.0)	3.5–9.0(–15.0)	(4.2–) 6.2–10.0 (–15.5)	(4.0–) 5.7–9.2 (–13.5)
95 % CI	10.6–11.6	n/a	5.2–6.2	7.9–8.3	7.2–7.8
Widest point (μm)	(1.9–) 2.6–3.4 (–3.8)	(2.0–) 2.7–3.7 (–4.5)	(2.0–) 2.5–3.2 (–4.0)	(2.0–) 2.7–3.5 (–4.2)	(2.0–) 2.5–3.5 (–4.2)
95 % CI	2.9–3.2	n/a	2.8–3.0	3.0–3.1	3.0–3.2
Base (μm)	(1.0–) 1.3–1.5 (–2.0)	(1.2–) 1.5–2.3 (–3.2)	(1.2–) 1.5–2.2 (–2.5)	(1.0–) 1.5–2.2 (–3.0)	(1.0–) 1.5–2.5 (–3.2)
95 % CI	1.3–1.5	n/a	n/a	1.9–2.0	1.9–2.1
L/W	(2.4–) 2.8–4.3 (–6.1)	(1.2–) 1.6–3.9 (–7.1)	1.2–3.1 (–6.6)	(1.2–) 1.8–3.6 (–6.6)	(1.2–) 1.7–3.3 (–6.8)
95 % CI	3.5–4.0	n/a	1.8–2.4	2.6–2.8	1.6–3.4
Conidia					
Colour	Yellowish green (M. 30A7) to deep green (M. 28E8)	Green	Green	Green	Green
Shape	Broadly ellipsoidal to ellipsoidal or subglobose, smooth	Ellipsoid, oval or subglobose, green, distinctly warted when young	Ellipsoidal to nearly oblong, smooth	Oblong, smooth	Ovoidal to broadly ellipsoidal or subglobose
L (μm)	(2.7–) 3.0–3.7 (–4.0)	(3.0–) 3.7–4.5 (–5.5)	(3.5–) 3.7–4.5 (–4.7)	(3.0–) 3.7–4.5 (–4.7)	(2.7–) 3.2–4.0 (–4.2)
95 % CI	3.3–3.6	n/a	4.0–4.1	4.1–4.2	3.5–3.6
W (μm)	(2.3–) 2.5–3.0 (–3.2)	(2.7–) 3.0–3.5 (–3.8)	(2.2–) 2.5–3.2 (–3.5)	(2.0–) 2.5–3.0 (–3.5)	(2.5–) 3.0–3.2 (–3.7)
95 % CI	2.6–2.8	n/a	2.8–3.0	2.6–2.7	3.0–3.1
L/W	(1.05–) 1.2–1.4 (–1.5)	(1.0–) 1.1–1.4 (–1.9)	(1.0–) 1.2–1.6 (–1.9)	(1.3–) 1.5–1.7 (–2.0)	1.1–1.3 (–1.6)
95 % CI	1.2–1.4		1.4–1.5	1.5–1.6	1.15–1.19
Odour	Coconut-like odour	Coconut-like odour	No distinctive odour	No distinctive odour	No distinctive odour

Table 2 continued

Character	*T. songyi* (This study)	*T. caerulescens* (Jaklitsch et al. 2012)	*T. caribbaeum* var. *caribbaeum* (Samuels et al. 2006)	*T. koningii* (Samuels et al. 2006)	*T. ovalisporum* (Samuels et al. 2006)
Formation of chlamydospores	+	+	+	+	+
Colony radius (mm) on PDA after 72 h at					
15 °C	22–28	19–24	13–15	(12–) 15–19	(5–) 6–13
20 °C	41–51	n/a	36	(26–) 31–38	(31–) 31–37
25 °C	(48–)56–60	27–36	53–56	(41–) 49–58	(38–) 43–66
30 °C	32–39	0.5–2	54–58	(35–) 45–61	48–70
35 °C	0–3	0	1–2	(0–) 1–2 (–3)	5–14
Colony radius (mm) on SNA after 72 h at					
15 °C	10–15	17–22	8–9	(5–) 7–13	(5.0–) 5.0–10.0
20 °C	22–28	n/a	22–24	(14–) 18–23	(25–) 19–27
25 °C	27–35	24–30	41–42	(25–) 29–35	40–48
30 °C	24–36	0.7–3.5	36–46	(20–) 21–27 (–28)	39–41 (–51)
35 °C	0	0	0.5–1.0	(0.5–) 1.0–1.8 (–2.0)	4–7
Colony morphology on PDA					
Colony type after 96 h in light	Mycelium abundant; sterile	Mycelium abundant, forming strands and a whitish hairy or floccose mat	Faint concentric rings; few conidia	Faint to pronounced concentric rings, conidia typically abundant	Faint to pronounced concentric rings, conidia typically abundant
Time of appearance of green conidia on PDA in darkness (h)	>168	72–120	72–96	72	48

L length, *W* width, *CI* confidence interval

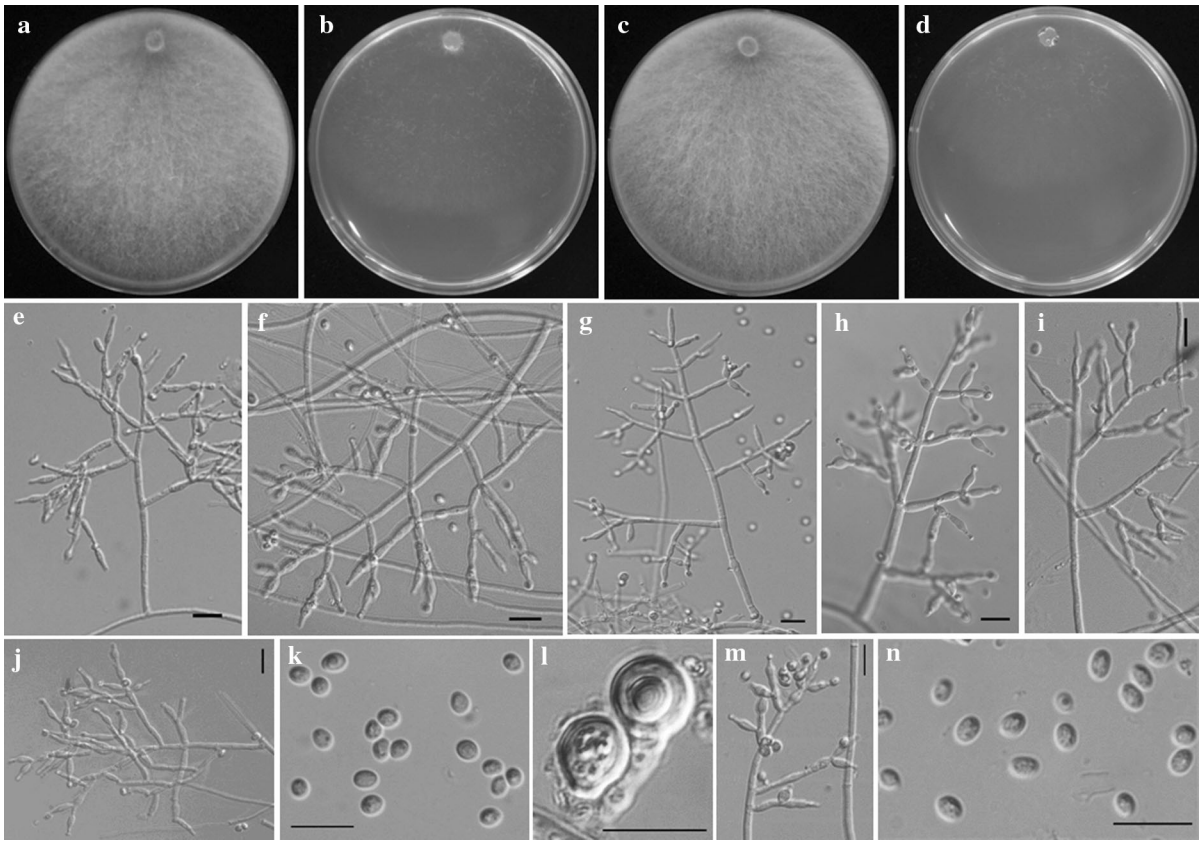

Fig. 2 Macro- and microscopic features of *Trichoderma songyi*. **a–d** Cultures after 72 h (**a**, **c** on PDA; **b**, **d** on SNA). **e–j** and **m** Conidiophores; **l** Chlamydospores; **k**, **n** Conidia. **a**, **b**, **e**, **f**, **j–l** from SFC20130926-S001^{T}; **c**, **d**, **g–i**, **m**, **n** from SFC20130926-S003

ovalisporum, and *Trichoderma caribbaeum* var. *caribbaeum* by the odor of the culture, growth rate on PDA at 15 and 20 °C, and colony morphology on PDA; *Trichoderma songyi* produced a coconut-like odor and had a faster growth rate, whereas the other three species produced no distinctive odour and had slower growth rates. On PDA in darkness, *Trichoderma songyi* did not form green conidia but instead produced white, sterile mycelium after 1 week, whereas the other three species formed white mycelia with green conidia after 2–4 days (Table 2).

For the comparison of *Trichoderma songyi* and *Trichoderma caerulescens*, we found clear differences in the growth rate on SNA. *Trichoderma songyi* had a slower growth rate than did *Trichoderma caerulescens* on SNA at 15 °C but a faster growth rate on SNA at 30 °C.

Taxonomy

Trichoderma songyi M.S. Park, S.-Y. Oh & Y.W. Lim, sp. nov. (Fig. 2), Mycobank (MB808394).

Etymology Named after the Korean common name of *Tricholoma matsutake*—songyi. The type strain was isolated from the basidioma of *Tricholoma matsutake*.

Diagnostic characters Growth rate on PDA and SNA; Colony morphology on PDA in darkness; coconut-like odour.

Similar species Trichoderma songyi is phylogenetically closely related to the *Trichoderma koningii* aggregate and to *Trichoderma caerulescens*. The distinguishing culture characteristics of *Trichoderma songyi* are growth rate, colony morphology, and coconut-like odour (Table 2).

Colony radius on CMD after 72 h: 21–24 mm at 15 °C, 45–47 mm at 20 °C, 54–55 mm at 25 °C,

42–52 mm at 30 °C, 0–1 mm at 35 °C; colonies on CMD fill the Petri dish within 5 days at 20–30 °C; mycelium loose; deep green (M. 27E8) or yellowish green (M. 30B8) conidia forming around the margin of the colony, sometimes forming cottony pustules; odour coconut-like; agar not pigmented. Conidiophores tending to be regularly verticillium-like; phialides narrowly lageniform, somewhat swollen in the middle, straight, 8.0–14.9 (–18.0) × (1.9–) 2.6–3.4 (–3.8) µm, L/W (2.4–) 2.8–4.3 (–6.1) ($n = 30$). Conidia yellowish green (M. 30A7) to deep green (M. 28E8), smooth, mostly broadly ellipsoidal to ellipsoidal, (2.7–) 3.0–3.7 (–4.0) × (2.3–) 2.5–3.0 (–3.2) µm, L/W (1.05–) 1.2–1.4 (–1.5) ($n = 30$). Chlamydospores, globose to subglobose, terminal in hyphae, (6.1–) 7.0–8.6 × (5.0–) 6–7.7 (–8.2) µm, L/W 1–1.3 ($n = 30$).

Colony radius on PDA after 72 h: 22–28 mm at 15 °C, 41–53 mm at 20 °C, 48–63 mm at 25 °C, 46–58 mm at 30 °C, 0–3 mm at 35 °C; colonies on PDA fill the Petri dish within 5 days at 20–30 °C; white aerial mycelium, sterile after 1 week; odour coconut-like; agar not pigmented.

Colony radius on SNA after 72 h: 10–15 mm at 15 °C, 22–28 mm at 20 °C, 27–35 mm at 25 °C, 24–36 mm at 30 °C, 0 mm at 35 °C; colonies on SNA fill the Petri dish within 10 days at 25–30 °C; mycelium loose with white aerial mycelium; yellowish green (M. 30B8) conidia forming around the inoculum; odour coconut-like; agar not pigmented.

Holotype: South Korea, Gangwon-do, Hongcheon, on basidioma of *Tricholoma matsutake*, collected 26 September 2013 by S.-Y. Oh, deposited in SFC (SFC20130926-S001) as a culture permanently preserved in a metabolically inactive state (20 % glycerol at −80 °C). Ex-types were deposited at KCTC and CBS (KCTC 46205, CBS 138099).

Additional strains examined: South Korea, Gangwon-do, Hongcheon, on basidioma of *Tricholoma matsutake*, collected 26 September 2013 by S.-Y. Oh (SFC20130926-S002, SFC20130926-S003, SFC20130926-S004); South Korea, Gangwon-do, Hongcheon, on roots of *Pinus densiflora* in the fairy ring of *Tricholoma matsutake*, collected 4 October 2013 by S.-Y. Oh (SFC20131005-S042); South Korea, Gyeongsangbuk-do, Uljin, on roots of *Pinus densiflora* in the fairy ring of *Tricholoma matsutake*, collected 4 October 2013 by S.-Y. Oh (SFC20131005-S074); South Korea, Gyeongsangbuk-do, Pohang, on soil in the fairy ring of *Tricholoma matsutake*, collected 4 October 2013 by S.-Y. Oh (SFC20131005-S109).

Distribution: South Korea

Habitat Basidiomata of *Tricholoma matsutake*, roots of *Pinus densiflora* and soil in the fairy rings of *Tricholoma matsutake*.

Discussion

In this study, we explored *Trichoderma* species associated with *Tricholoma matsutake* in Korea by isolating strains from the basidiomata of *Tricholoma matsutake*, as well as roots of *Pinus densiflora* and soil from the fairy ring. While comparing sequence data of isolates with GenBank using BLAST, we discovered unique strains, raising the possibility of a new species. We tested this hypothesis through molecular (*tef1* and *rpb2*) and morphological comparisons across *Trichoderma*. Based on phylogenetic analyses and comparisons of macro- and micro-morphological characters, we demonstrate this taxon to be a new species. We name this new species *Trichoderma songyi* after the Korean common name of the pine mushroom (i.e., songyi) because all of the substrates from which *Trichoderma songyi* was isolated were associated with the pine mushroom.

In the phylogenetic analyses of the combined *tef1* + *rpb2* dataset, *Trichoderma songyi* was placed within the section *Trichoderma* as the sister species to the *Trichoderma koningii* aggregate, and it was also closely related to *Trichoderma caerulescens*. *Trichoderma songyi* can be distinguished morphologically from species within the *Trichoderma koningii* aggregate and from *Trichoderma caerulescens* by distinctive phenotypic features: growth rate on PDA and SNA, colony morphology on PDA in darkness, and coconut-like odour (Table 2).

Furthermore, the available data show that the species of the *Trichoderma koningii* aggregate have distinct biogeographic patterns (Samuels et al. 2006). The three *Trichoderma koningii* species we highlighted in the morphological comparisons (*Trichoderma caribbaeum* var. *caribbaeum*, *Trichoderma*

koningii, and *Trichoderma ovalisporum*) also have different geographic distributions from *Trichoderma songyi*. Whereas *Trichoderma songyi* is currently known only from the Republic of Korea, *Trichoderma caribbaeum* var. *caribbaeum* is from the islands of Guadeloupe and Puerto Rico, *Trichoderma koningii* is from eastern North America and Europe, and *Trichoderma ovalisporum* is from Ecuador (Samuels et al. 2006). *Trichoderma caerulescens*, another closely related species, is found in southern Europe (Jaklitsch et al. 2012). Additional surveys are needed to verify whether the distributions of these species are geographically restricted.

Trichoderma species have demonstrated positive effects as biocontrol agents of plant pathogens (Harman et al. 2004) and negative effects as diseases of commercial mushroom (Samuels et al. 2002; Park et al. 2005; 2006; Kim et al. 2012). In studies of *Trichoderma* and *Tricholoma matsutake*, a positive correlation was found in fairy rings (Vaario et al. 2011). Higher enzyme activity was detected in the fairy rings, and it has been suggested that litter or wood degradation by these enzymes provides an important carbon source for *Tricholoma matsutake* (Vaario et al. 2011). In this study, we describe a new species, *Trichoderma songyi*, that is associated with the pine mushroom. Further studies are required to determine whether *Trichoderma songyi* has a positive or negative interaction with *Tricholoma matsutake*.

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