Five New Records of Soil-Derived *Trichoderma* in Korea: *T. albolutescens*, *T. asperelloides*, *T. orientale*, *T. spirale*, and *T. tomentosum*

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**Abstract** Despite the huge worldwide diversity of *Trichoderma* (Hypocreaceae, Ascomycota), only about 22 species have been reported in Korea. Thus, between 2013 and 2015, soil-derived *Trichoderma* spp. were isolated to reveal the diversity of Korean *Trichoderma*. Phylogenetic analysis of translation elongation factor 1 alpha gene was used for identification. Among the soil-derived *Trichoderma*, *Trichoderma albolutescens*, *T. asperelloides*, *T. orientale*, *T. spirale*, and *T. tomentosum* have not been previously reported in Korea. Thus, we report the five *Trichoderma* species as new in Korea with morphological descriptions and images.

**Keywords** EF1α, Phylogeny, Taxonomy, *Trichoderma*

*Trichoderma* Persoon (= *Hypocre a* Fr.) is an ecologically influential genus that contains more than 200 species [1]. These species are basically saprophytes, but have various roles in the ecosystem [2]. They can be easily isolated from soil, wood, air, and even from other fungi as mycoparasites [2].

*Trichoderma* produce a number of secondary metabolites and a variety of enzymes. Their secondary metabolites include antibiotics, which act against fungi and bacteria and can be used as biocontrol agents for pathogens [3, 4]. *Trichoderma* species also produces enzymes that degrade cellulose, hemicellulose, or chitin. The extracellular enzymes from some *Trichoderma* species, such as the cellulases from *T. reesei* E. G. Simmons, have been used for industrial applications [5].

Despite the huge diversity of the genus *Trichoderma*, the reported diversity of *Trichoderma* is limited to about 22 species in Korea [6-11]. Among them, 11 species of *Trichoderma* from the Korea University Culture Collection (KUC; Seoul, Republic of Korea) were identified in a previous study [10]. However, the study was limited to wood-derived *Trichoderma* and was not able to fully explore the diversity of Korean *Trichoderma* spp. Thus, an investigation of soil-derived *Trichoderma* was needed.

The aims of this study were to collect indigenous soil-derived *Trichoderma* and report previously unrecorded species of the same in Korea. A phylogenetic tree of *Trichoderma* spp. based on the translation elongation factor 1 alpha gene (EF1α) sequences was constructed to classify the collected species. Morphological observations of the macro- and microscopic characteristics were performed to describe the unrecorded *Trichoderma* spp.

**MATERIALS AND METHODS**

**Collection of soil-derived *Trichoderma* cultures.** Seven soil samples were collected from three sites: a fir forest in Odaesan National Park, Republic of Korea (37°44’08.3” N, 128°35’23.3” E; in Jan, Apr, Jul, and Oct 2013); Hinoki cypress forest in Jangseong-gun, Jeolnam-do, Republic of
Korea (35°22’40.5” N, 126°44’35.5” E; in Jun 2013); and a wetland beside the Han River in Seoul, Republic of Korea (37°35’13.8” N, 126°49’03.4” E; on 19 Feb 2014).

To isolate fungal cultures, soil solutions (1/1,000) were made from each soil sample and sterilized distilled water (DW) with serial dilution. For each soil sample, 1 mL of diluted soil solution was inoculated onto a 90 mm-plate containing malt extract agar (malt extract 20 g, agar 15 g, DW 1 L; Difco, Detroit, MI, USA) with 0.01% streptomycin sulfate for prevention of bacterial contamination. Recognizable fungal colonies were transferred onto new malt extract agar (malt extract 20 g, agar 15 g, DW 1 L; Difco, Detroit, MI, USA) by Macrogen (Seoul, Korea).

Phylogenetic analysis. Genomic DNA of collected Trichoderma spp. was extracted with an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea) according to the manufacturer’s protocol. PCR amplification of the EF1α region was performed with primers EF1-728F (5’-CAT CGA GAA GTT CGA GAA GG-3’) [12] and TEF1 rev (5’-GCC ATC CTG GGA GAT ACC AGC-3’) [13] according to previous described method [10]. DNA sequencing was performed using the Sanger method with a 3730xl DNA Analyzer (Life Technology, Carlsbad, CA, USA) by Macrogen (Seoul, Korea).

The obtained EF1α sequences were proofread and aligned with the selected reference sequences from GenBank using MAFFT 7.130 [14] and modified manually with MacClade 4.08 [15]. Datasets were tested by MrModeltest 2.3 using the Akaike Information Criterion (AIC) with default options [16]. The GTR + I + G model was chosen under the AIC criteria as a result of the test. Bayesian analysis was performed with MrBayes 3.2.1 [17]. The phylogenetic tree was created according to previously described methods [18].

Analysis of phenotype. Cultures were grown on 9-cm plastic Petri dishes contained 20 mL of corn meal dextrose agar (CMD; corn meal 20 g, glucose 20 g, agar 18 g, DW 1 L), potato dextrose agar (PDA; potato dextrose agar 39 g, DW 1 L; Difco), or synthetic nitrogen-poor or nutrient-poor agar (SNA; sucrose 0.2 g, glucose 0.2 g, KNO3 1 g, KH2PO4 1 g, MgSO4•7H2O 0.5 g, NaCl 0.5 g, agar 12 g, DW 1 L). To analyze colony characteristics, a 6-mm inoculum plug was placed at about 1.5 cm from the edge of the Petri dish. The cultures were grown at room temperature (20–25°C) [10]. After 1 wk, pictures of colonies were taken using a NEX-5R digital camera (Sony, Tokyo, Japan). The observations were carried out for 15–20 days of growth. The Munsell color system was used for color standards [19]. Morphological analyses of microscopic characteristics were performed with an Olympus BX51 light microscope (Olympus, Tokyo, Japan). Photographs of conidiophores and conidia were taken using the same microscope mounted with an Olympus DP20 microscopic camera, and measurements were made using 3% KOH mounts. At least 30 units of each parameter were measured for each collection. To ensure reliable data, 5% of the measurements from each end of the range were removed. The isolates were deposited at the National Institute of Biological Resources (NIBR; Incheon, Korea).

RESULTS AND DISCUSSION

Isolation and identification of soil-derived Trichoderma spp. A total of 26 Trichoderma cultures were isolated (Table 1). The Trichoderma spp. were identified by phylogenetic analysis based on the EF1α region (Fig. 1), and the tree obtained was similar to that of other studies [20-23]. The sequences in the phylogenetic tree were classified into five subgroups: Harzianum, Strictipilosa, Longibrachiatum, Pachybrasioides, Brevicompactum, and Viride-Kongingii group; however, three species: T. helicum Bissett, C. P. Kubicek & Szakács; T. virens (J. H. Mill., Giddens & A. A. Foster) Arx; and T. alboluteascens had a single clade [20, 21]. Our Trichoderma isolates were identified as 12 species with their own species clade in the phylogenetic tree. Three isolates, KUC21203, KUC20205, and KUC21207, were in a

| Identity      | Location                | KUC No.                  |
|---------------|-------------------------|--------------------------|
| T. alboluteascens | Odaesan, Gwangwon-do | KUC21115\*; KUC21168\* |
| T. asperelloides    | Gangseo-gu, Seoul       | KUC21191\*, KUC21194\*, KUC21197\*, KUC21208\* |
| T. asperellum       | Gangseo-gu, Seoul       | KUC21206, KUC21212     |
| T. atroviride       | Gangseo-gu, Seoul       | KUC21202                |
| T. hamatum         | Gangseo-gu, Seoul       | KUC21204                |
| T. harzianum       | Gangseo-gu, Seoul       | KUC21203, KUC21205, KUC21207 |
| T. longibrachiatum  | Gangseo-gu, Seoul       | KUC21196                |
| T. orientale       | Gangseo-gu, Seoul       | KUC21210                |
| T. polyporum       | Odaesan, Gwangwon-do   | KUC21103, KUC21131 |
| T. tomentosum       | Odaesan, Gwangwon-do   | KUC21111\*              |
| T. spirale          | Gochang-gun, Jeollabuk-do | KUC21268\*         |
| T. virens           | Gangseo-gu, Seoul       | KUC21192, KUC21193, KUC21200, KUC21209, KUC21211, KUC21215 |

*New record species in Korea.
Fig. 1. The 50% majority-rule consensus tree which contains 75 taxa and 785 characters obtained from the bayesian analysis based on the translation elongation factor 1 alpha gene. Numbers above branches indicate posterior probabilities. Fungal cultures examined in this study are in bold. Sequences of type specimens are indicated by asterisk symbols (*).
clade with an ex-type strain of *T. harzianum* Rifai with 100% posterior probabilities (PP). Six isolates were identified as *T. virens* (= *H. virens*) with high PP (100%). KUC21196 and KUC21210 were identified as an ex-type strain of *T. longibrachiatum* Rifai (= *H. sagamiensis*) with 100% of PP. KUC21103 and KUC21131 were placed into a monophyletic clade with *T. polysporum* (Link) Rifai (= *H. pachybasioides*) with 54% PP. KUC21204 was in a clade with *T. hamatum* (Bonord.) Bainier and 100% PP. The remaining five species, *Trichoderma albolutescens*, *T. asperelloides*, *T. orientale*, *T. spirale*, and *T. tomentosum*, have not previously been reported in Korea. For this first report, they are described with macro- and micro-observations.

To the best of our knowledge, including these five new records, 27 species of *Trichoderma* have been reported in Korea to date [6-11]. However, we presume that the diversity of Korean *Trichoderma* is greater than 100 species, because this study of only three sites revealed five unrecorded species. Further studies throughout the Republic of Korea are required to understand the potential diversity of *Trichoderma*.

**Taxonomy.**

*Trichoderma albolutescens* Jaklitsch, Fungal Divers. 48: 202 (2011) (Fig. 2).

Colonies on CMD colony sterile, circular, hyaline at first, turned white to pale orange yellow (10YR9/2) color after 1 wk aerial mycelium abundant, no distinct odor. On PDA colony sterile, circular, thin, zonate, hairy, aerial mycelium abundant, white to light gray (10YR7/2), odor slightly like mushrooms. On SNA hyaline, forming white cottony spots; margin hairy; no pigment, no distinct odor. Conidiation capricious, after 1 wk; compact, white pustules 1–3 mm diameter. Conidiophores with complex, mostly symmetric, often distinctly rectangular branching. Phialides (3.5–)4.0–7.5(–8) × (2–)2.5–3.5(–4.5) μm, (1.5–)2–3(–4) μm wide at the base, ampulliform, short, mostly inequilateral or curved upwards. Conidia (3.2–)3.5–5(–5.7) × (1.6–)1.8–2.5 μm, hyaline, oblong or cylindrical.

**Specimens examined:** Republic of Korea, Gangwon-do, Pyoengchang-gun, Odaesan National Park, 37°44’08.3” N, 128°35’23.3” E, topsoil of fir forest, Apr 2013, Seokyoon Jang (KUC21115, GenBank KX912191); Oct 2013, Seokyoon Jang (KUC21168, GenBank KX912194).

**Known distribution:** Austria, Germany [21], Spain [24], Republic of Korea.

**Note:** This species has sterile and greyish to greyish-brown colonies with white cottony mycelia. It is easily distinguishable from other Korean *Trichoderma* spp. with its green spores. It cannot grow at temperatures over 30°C.

KUC21115, KUC21168, and *T. albolutescens* CBS 119286 are monophyletic with high PPs (100% of PP) in our phylogenetic tree (Fig. 1).

*Trichoderma asperelloides* Samuels, Mycologia 102: 961 (2010) (Fig. 3).

Colonies on CMD conidiophores produced in pustules, conidia pale yellow (5Y8/4) to yellow (7Y7/8). On PDA conidiophores produced in pustules, aerial mycelium abundant, white to light gray (10YR7/2), odor slightly like mushrooms. On SNA hyaline, forming white cottony spots; margin hairy; no pigment, no distinct odor. Conidiation capricious, after 1 wk; compact, white pustules 1–3 mm diameter. Conidiophores with complex, mostly symmetric, often distinctly rectangular branching. Phialides (3.5–)4.0–7.5(–8) × (2–)2.5–3.5(–4.5) μm, (1.5–)2–3(–4) μm wide at the base, ampulliform, short, mostly inequilateral or curved upwards. Conidia (3.2–)3.5–5(–5.7) × (1.6–)1.8–2.5 μm, hyaline, oblong or cylindrical.

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KUC21115, KUC21168, and *T. albolutescens* CBS 119286 are monophyletic with high PPs (100% of PP) in our phylogenetic tree (Fig. 1).

**Taxonomy.**

*Trichoderma albolutescens* Jaklitsch, Fungal Divers. 48: 202 (2011) (Fig. 2).

Colonies on CMD colony sterile, circular, hyaline at first, turned white to pale orange yellow (10YR9/2) color after 1 wk aerial mycelium abundant, no distinct odor. On PDA colony sterile, circular, thin, zonate, hairy, aerial mycelium abundant, white to light gray (10YR7/2), odor slightly like mushrooms. On SNA hyaline, forming white cottony spots; margin hairy; no pigment, no distinct odor. Conidiation capricious, after 1 wk; compact, white pustules 1–3 mm diameter. Conidiophores with complex, mostly symmetric, often distinctly rectangular branching. Phialides (3.5–)4.0–7.5(–8) × (2–)2.5–3.5(–4.5) μm, (1.5–)2–3(–4) μm wide at the base, ampulliform, short, mostly inequilateral or curved upwards. Conidia (3.2–)3.5–5(–5.7) × (1.6–)1.8–2.5 μm, hyaline, oblong or cylindrical.

**Specimens examined:** Republic of Korea, Gangwon-do, Pyoengchang-gun, Odaesan National Park, 37°44’08.3” N, 128°35’23.3” E, topsoil of fir forest, Apr 2013, Seokyoon Jang (KUC21115, GenBank KX912191); Oct 2013, Seokyoon Jang (KUC21168, GenBank KX912194).

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KUC21115, KUC21168, and *T. albolutescens* CBS 119286 are monophyletic with high PPs (100% of PP) in our phylogenetic tree (Fig. 1).

**Taxonomy.**

*Trichoderma asperelloides* Samuels, Mycologia 102: 961 (2010) (Fig. 3).

Colonies on CMD conidiophores produced in pustules, conidia pale yellow (5Y8/4) to yellow (7Y7/8). On PDA conidiophores produced in pustules, aerial mycelium abundant, white to light gray (10YR7/2), odor slightly like mushrooms. On SNA hyaline, forming white cottony spots; margin hairy; no pigment, no distinct odor. Conidiation capricious, after 1 wk; compact, white pustules 1–3 mm diameter. Conidiophores with complex, mostly symmetric, often distinctly rectangular branching. Phialides (3.5–)4.0–7.5(–8) × (2–)2.5–3.5(–4.5) μm, (1.5–)2–3(–4) μm wide at the base, ampulliform, short, mostly inequilateral or curved upwards. Conidia (3.2–)3.5–5(–5.7) × (1.6–)1.8–2.5 μm, hyaline, oblong or cylindrical.

**Specimens examined:** Republic of Korea, Gangwon-do, Pyoengchang-gun, Odaesan National Park, 37°44’08.3” N, 128°35’23.3” E, topsoil of fir forest, Apr 2013, Seokyoon Jang (KUC21115, GenBank KX912191); Oct 2013, Seokyoon Jang (KUC21168, GenBank KX912194).

**Known distribution:** Austria, Germany [21], Spain [24], Republic of Korea.

**Note:** This species has sterile and greyish to greyish-brown colonies with white cottony mycelia. It is easily distinguishable from other Korean *Trichoderma* spp. with its green spores. It cannot grow at temperatures over 30°C.

KUC21115, KUC21168, and *T. albolutescens* CBS 119286 are monophyletic with high PPs (100% of PP) in our phylogenetic tree (Fig. 1).
abundant, conidia pale yellow (5Y8/4) to yellowish green (5GY6/4), no distinctive odor or diffusing pigment. Colonies on SNA abundant conidia formation, scattered, 1–1.5 mm diameter pustules, no distinctive odor or pigment observed, pustules yellow (7Y7/8) to yellowish green (5GY6/4). Conidiophores smooth central axis from which secondary branches arise, secondary branches tending to be paired but also commonly asymmetric, all branches terminating in a single phialide or a whorl of 2–4 divergent phialides. Phialides 5–10.5 μm long, (2–)2.5–3.5 μm at the widest, 1–2.5(–3) μm wide at the base, flask-shaped. Conidia dark green, subglobose, 3.5–4.5(–5) × 3–3.9 μm.

Specimens examined: Republic of Korea, Seoul, 37°35′13.8″ N, 126°49′03.4″ E, soil of wetland beside Han River, 19 Feb 2014, Seokyoon Jang (KUC21191, GenBank KX912195; KUC21194, GenBank KX912198; KUC21197, GenBank KX912201; KUC21208, GenBank KX912212).

Known distribution: China [24], Korea.

Note: Morphologically, this species is very similar to and indistinguishable from Trichoderma asperellum [24]. Moreover, our isolates of Trichoderma asperellum and T. asperelloides were isolated from the same source. On the other hand, they are clearly separated into two clades with high support (100% PP) in the phylogenetic tree (Fig. 1). Molecular biological approaches are necessary to identify these two species.

Trichoderma orientale (Samuels & Petrini) Jaklitsch & Samuels, Mycotaxon 126: 151 (2014) (Fig. 4).

Colonies on CMD with few aerial mycelium, conidial production forming in broad concentric rings, light greenish gray (10GY7/1) to dark grayish green (10GY4/2); diffusing pale yellow (5Y8/3) to olive yellow (5GY6/6) pigment. On PDA aerial mycelium uniformly cottony, conidial production forming in broad concentric rings, light greenish gray (10GY7/1) to dark greenish gray (5G4/2); no distinctive odor, diffusing pale yellow (5Y8/3) to olive yellow (5GY6/6) pigment. Colonies on SNA conidia forming in abundant, scattered, 1–1.5 mm diameter pustules, light olive green (10Y6/2) to dark olive green (5GY3/4); no distinctive odor or pigment. Conidiophores primary branches tending to produce phialides singly, either directly from the branch itself or terminating a short secondary branch. Phialides typically not held in whorls. Phialides typically cylindrical, straight, (5.5–)7–12 μm long, (1.5–)2–4(–4.5) μm at the widest, 1.5–3 μm at the base. Conidia oblong to ellipsoidal, 3.5–4.5 × 2.4–3 μm, smooth. Chlamydospores not observed.

Specimens examined: Republic of Korea, Seoul, 37°35′13.8″ N, 126°49′03.4″ E, soil of wetland beside Han River, 19 Feb 2014, Seokyoon Jang (KUC21216, GenBank KX912220).

Known distribution: China, New Zealand, South Africa [25], Ecuador, Spain [22], Korea.

Note: Trichoderma orientale KUC21216 matched the protologue description well, except for the protologue conidia being longer (4.0–)5.0–7.2(–10.5) μm [25]. This may be an intraspecies variation because we analyzed a single Korean T. orientale strain. To investigate this feature, other Korean strains need to be studies. KUC21216 and the type strain of T. orientale (GJS 88-81) are monophyletic with high support (97% PP) (Fig. 1).
Trichoderma spirale Bissett, Can. J. Bot. 69: 2408 (1991) (Fig. 5).
Colonies on CMD forming a few aerial mycelium, conidial production forming in broad concentric rings, very dark grayish green (5GY3/2); olive-coloured pigment (5Y4/3). On PDA aerial mycelium abundant, conidial production forming in broad concentric rings, grayish green (5GY5/2) to dark grayish green (5GY4/2); olive yellow pigment (2.5Y6/8). Colonies on SNA conidia forming in abundant, scattered, 1–2 mm diam pustules; dark grayish green (5GY4/
First Report of Five *Trichoderma* Species in Korea

2); no distinctive pigment. No distinctive odor. Conidiophores broad fertile branches arising from the base. The secondary branches arising from the fertile branches, containing 1–2 cells. Phialides arising in dense clusters, nearly doliiform, short, (3.7–)4–6.4(–7.4) μm long, 2.6–3.5(–4.3) μm widest, 1.9–2.8(–3.2) μm wide at the base. Conidia smooth, oblong to ellipsoidal, 4.1–5.1 × 2.5–2.8(–3) μm. Chlamydospores not observed.

**Specimens examined:** Republic of Korea, Jeollanam-do, Jangseong-gun, 35°22′40.5″ N, 126°44′35.5″ E, topsoil of Hinoki cypress forest, June 2013, Seokyoon Jang (KUC21268, GenBank KX912221).

**Known distribution:** Cosmopolitan [26].

**Note:** *Trichoderma spirale* KUC21268 matched well with the descriptions in the other study [26]. KUC21268 and a type strain of *T. spirale* (DAOM 183974) are monophyletic with high support (100% PP) (Fig. 1).

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*Trichoderma tomentosum* Bissett, Can. J. Bot. 69: 2412 (1991) (Fig. 6).

Colonies on CMD conidiophores forming pustules in circular around the edge of the colony, aerial mycelium abundant, pustules 0.5–1 mm diam., light grayish green (5GY6/2) to grayish green (5GY5/2); no distinctive odor or pigment. On PDA aerial mycelium uniformly cottony, conidial production forming in broad concentric rings, pale olive (10Y6/4); no distinctive odor or pigment. Conidia smooth, broadly ellipsoidal, (2.8–)3–3.3(–3.6) × 2.3–2.7 μm. Chlamydospores not observed.

**Specimens examined:** Republic of Korea, Gangwon-do, Pyeongchang-gun, Odaesan National Park, 37°44′08.3″ N, 128°35′23.3″ E, topsoil of fir forest, Apr 2013, Seokyoon Jang (KUC21111, GenBank KX912190).

**Known distribution:** Cosmopolitan [20, 26-28].

**Note:** *Trichoderma tomentosum* KUC21111 matched well with the other previous description [26]. It cannot grow at temperatures over 35°C. KUC21111 and a type strain of *T. tomentosum* (DAOM 178713a) are grouped with a monophyletic clade (99% of PP) (Fig. 1).

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