Journal of Microbiology (2013) Vol. 51, No. 5, pp. 704–708
DOI 10.1007/s12275-013-3361-9

NOTE

Two Novel *Talaromyces* Species Isolated from Medicinal Crops in Korea

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(Received July 8, 2013 / Accepted September 25, 2013)

Two novel biverticillate *Talaromyces* species, *T. angelicus* and *T. cnidii*, were collected from the medicinal crops *Angelica gigas* and *Cnidium officinale*, respectively, in Korea. Phylogenetic analyses with the nuclear ribosomal internal transcribed spacer (ITS) region and the β-tubulin gene as well as morphological analyses revealed that the two species differ from any known *Talaromyces* species. *Talaromyces angelicus* is related to *T. flavovirens* in the phylogeny of the ITS region, but the new species is grouped together with *Penicillium liani* and *T. pinophilus* in terms of its β-tubulin phylogeny, and its growth rate on Czapek yeast autolysate differs from that of *T. flavovirens*. *Talaromyces cnidii* is phylogenetically similar to *T. siamensis*, but exhibits differences in the morphologies of the colony margin, metulae, and conidia.

Keywords: *Talaromyces angelicus*, *Talaromyces cnidii*, morphological characteristics, molecular phylogenetics

Penicillium subgenus *Biverticillium* is characterized by its symmetrical, biverticillate conidiophores and phylogenetically distinct from other *Penicillium* subgenera: *Aspergilloides*, *Furcatum*, and *Penicillium* (Samson *et al*., 2011). Malloch (1985) suggested that morphological and ecological factors and anamorph-telemorph connections indicated that the *P.* subgenus *Biverticillium* needs to be separated from the genus *Penicillium*. Species of the *Talaromyces* genus were first introduced by Benjamin (1955) as one of sexual stages of *Penicillium* and morphologically characterized by producing soft yellow ascomata that is composed of interwoven hyphae. Previous phylogenetic studies revealed that *P.* subgenus *Biverticillium* and the genus *Talaromyces* form a monophyletic group that is distinct from the genus *Penicillium* based on the nuclear ribosomal internal transcribed spacer (ITS) regions, small subunit nuclear ribosomal DNA, and/or large subunit ribosomal DNA (Berbee *et al*., 1995; Ogawa *et al*., 1997; Peterson, 2000). The recent review of Samson *et al*. (2011) described *P.* subgenus *Biverticillium* and the genus *Talaromyces* as a taxonomically unified group, and transferred all accepted species of *P.* subgenus *Biverticillium* to the genus *Talaromyces* in accordance with the concept of unified nomenclatural system of fungi. In addition, Yilmaz *et al.* (2012) revised *T. purpurogenus* complex and introduced three new species based on polyphasic approaches including morphology, extrolite production, and multi-gene phylogeny, while Visagie *et al.* (2012) reported a new combination *T. flavovirens* connected via a newly recognized sexual stage and its known synnematous stage.

In this study, we collected two *Talaromyces* species that exhibit biverticillate conidiophores during an investigation of the occurrence of *Penicillium* species in medicinal crops in Korea. Investigations of cultural and morphological characteristics and phylogenetic analyses of the ITS region and β-tubulin gene sequences performed in this study revealed that these two species differ from any previously known *Talaromyces* species. Here, they are proposed as *Talaromyces angelicus* sp. nov. and *Talaromyces cnidii* sp. nov. with morphological and molecular examinations.

Talaromyces angelicus KACC (Korean Agricultural Culture Collection) 46611 and *T. cnidii* KACC 46617 were isolated on dried roots of *Angelica gigas* and *Cnidium officinale*, respectively, in storage houses in Pyeongchang and Jecheon in Korea, respectively. Briefly, the washed roots of *Angelica gigas* and *Cnidium officinale* were dried for 3–4 weeks in storage houses. The roots were ground using a sterilized coffee grinder and plated on antibiotic-amended potato dextrose agar (PDA). Two fungi were isolated from conidia and conidiophores of the *Talaromyces* species on PDA and transferred to malt extract agar (MEA). The isolates were incubated for 7 days at 25°C and maintained in the KACC. A morphological observation was conducted with following the methodology of Pitt (1979, 2000) and Frisvad and Samson (2004). Two isolates were inoculated at three points on Czapek yeast autolysate (CYA) agar, MEA, and yeast extract sucrose (YES) agar, and incubated at 25°C for 7 days in darkness (Figs. 1 and 2). The colony diameters were

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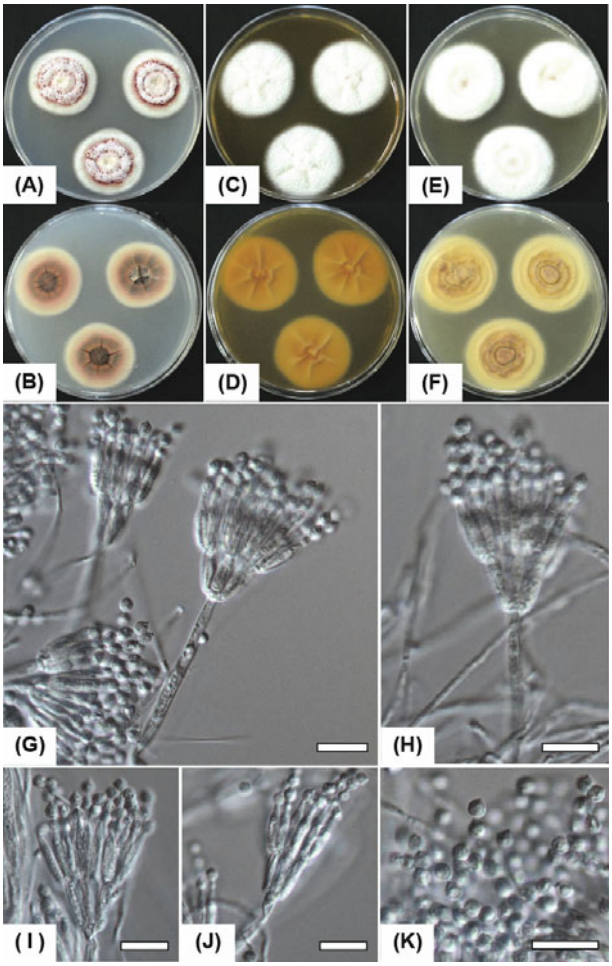

Fig. 1. ***Talaromyces angelicus*** **(KACC 46611).** Seven-day-old colonies on CYA (A, B), MEA (C, D), and YES (E, F), conidiophores (G–J), and conidia (K). White bars=10 μm.

measured and their appearances, pigmentations, and the reverse side of culture were assessed. In addition, the temperature-growth responses of the isolates were studied on CYA at 15°C, 30°C, and 37°C for 7 days in darkness. Cultures were also grown for up to 3 weeks in order to examine their ascomata production abilities in accordance with Samson *et al.* (2011). Our morphological observations were conducted with the aid of a light microscope (Eclipse E600, Nikon, Japan) equipped with differential interference contrast. Microscopic measurements were made using image-capturing software (Scion Image beta 4.0.2, Scion Corporation, USA). Mycelia were scraped from colonies grown on MEA after 7–9 days and freeze-dried for DNA extraction. Genomic DNA was extracted using the method described by Cubero *et al.* (1999). The ITS region and β-tubulin gene were amplified using primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White *et al.*, 1990), and Bt2a (5′-GGT AAC CAA ATC GGT GCT GCT TTC-3′) and Bt2b (5′-ACC CTC AGT GTA GTG ACC CTT GGC-3′) (Glass and Donaldson, 1995), respectively. PCR products were sequenced using the same primers with the BigDye terminator cycle sequencing kit (Applied Biosystems, USA) following the manufacturer's instructions and run on a gene analyzer (ABI Prism 310, Applied Biosystems). The ITS region and β-tubulin gene sequences of strains from *Talaromyces* species were obtained from GenBank for phylogenetic analyses based on the studies of Samson *et al.* (2011), Yilmaz *et al.* (2012), and Visagie *et al.* (2012). The sequences generated in the present study were proofread, edited, and merged into comparable sequences with sequences retrieved from GenBank using MEGA 5.1 (Tamura *et al.*, 2011). The software MAFFT v7.045 with the "L-INS-I" option was used for the initial sequence alignments (Katoh and Toh, 2008), which were subsequently refined using MEGA 5.1. Maximum likelihood (ML) analyses were performed using MEGA 5.1 and the node confidence was determined using bootstrapping with 1000 replicates for the ITS region and β-tubulin respectively (Figs. 3 and 4). The ITS region and β-tubulin gene sequences of *T. angelicus* KACC 46611 and *T. cnidii* KACC 46617 were deposited in GenBank with accession numbers KF183638– KF183641.

The dataset of the ITS region consisted of 43 species and 480 characters, and that of the β-tubulin gene consisted of 23 species and 459 characters. The ML tree of the ITS region

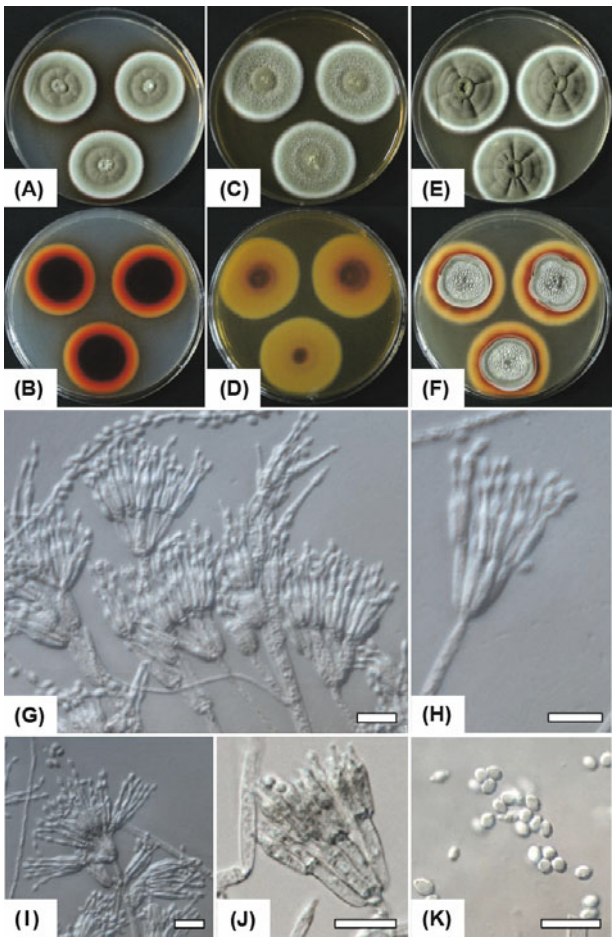

Fig. 2. ***Talaromyces cnidii*** **(KACC 46617).** Seven-day-old colonies on CYA (A, B), MEA (C, D), and YES (E, F), conidiophores (G–J), and conidia (K). White bars=10 μm.

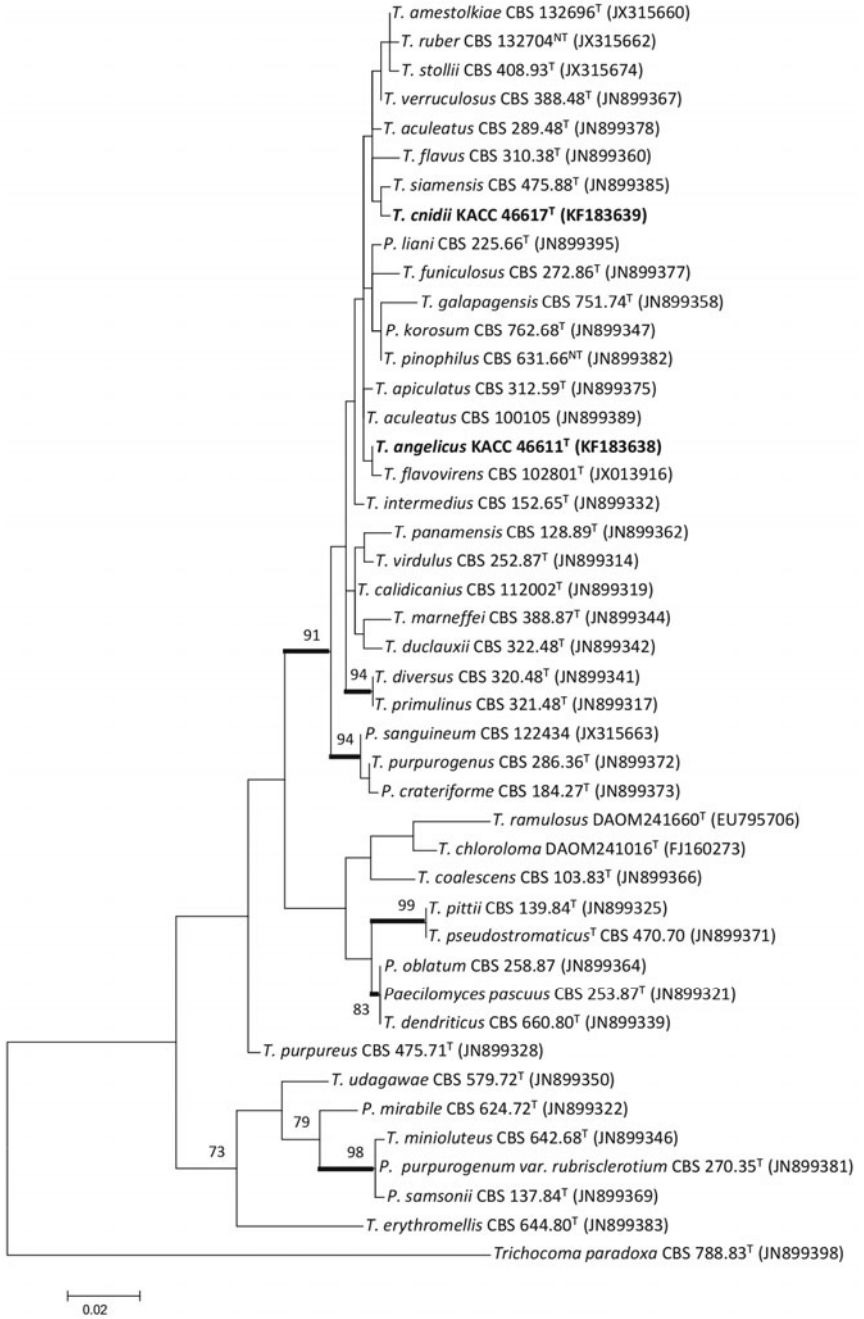

Fig. 3. Maximum likelihood constructed using MEGA 5.1 based on ITS sequences, showing the placements of *T. angelicus* and *T. cnidii* (in bold letter) and other closely related *Talaromyces* species. The bootstrap support percentages from the ML analysis are listed at the branching nodes. Bootstrap support values lower than 70% are not shown, and bold branches indicate bootstrap values higher than 80%. *Trichocoma paradoxa* was used as the outgroup. ^{T}= ex-type strain; ^{NT}= neo-type strain.

showed that *T. angelicus* KACC 46611 and *T. cnidii* KACC 46617 were members of the "clade1" subclade described by Samson *et al.* (2011). *Talaromyces angelicus* KACC 46611 formed a monophyletic group with *T. flavovirens* CBS 102801 without the support of bootstrap value (Fig. 3). Similarly, *Talaromyces cnidii* KACC 46617 and *T. siamensis* CBS 475.88 were grouped together without the support of bootstrap value (Fig. 3). The ML tree of the β-tubulin gene indicated that *T. angelicus* KACC 46611 is closely related with *P. liani* CBS 225.66 and *T. pinophilus* CBS 631.66, and *T. cnidii* KACC 46617 formed a well-supported monophyletic group with *T. siamensis* CBS 475.88 (Fig. 4). *Talaromyces angelicus* is close to *T. flavovirens* in the phylogenetic tree generated with the ITS region. In morphological comparison, *T. flavovirens* can grow to 11–13 mm after 7 days on CYA at 25°C, but this species is not able to grow on CYA at 37°C (Visagie *et al.*, 2012), whereas *T. angelicus* can grow to 29–36 and 26–29 mm at 25°C and 37°C, respectively (Fig. 1). *Talaromyces cnidii* is phylogenetically related to *T. siamensis*, but the ITS region and β-tubulin sequences of *T. cnidii* differ from those of *T. siamensis* at eight and six nucleotide positions, respectively. Manoch and Ramirez (1988) described *Talaromyces siamensis* as a novel species isolated from Thailand soil, and it is morphologically similar to *T. cnidii* in exhibiting velutinous colonies on CYA and MEA and smooth-walled conidiophores. However, *T. siamensis* differs from *T. cnidii*

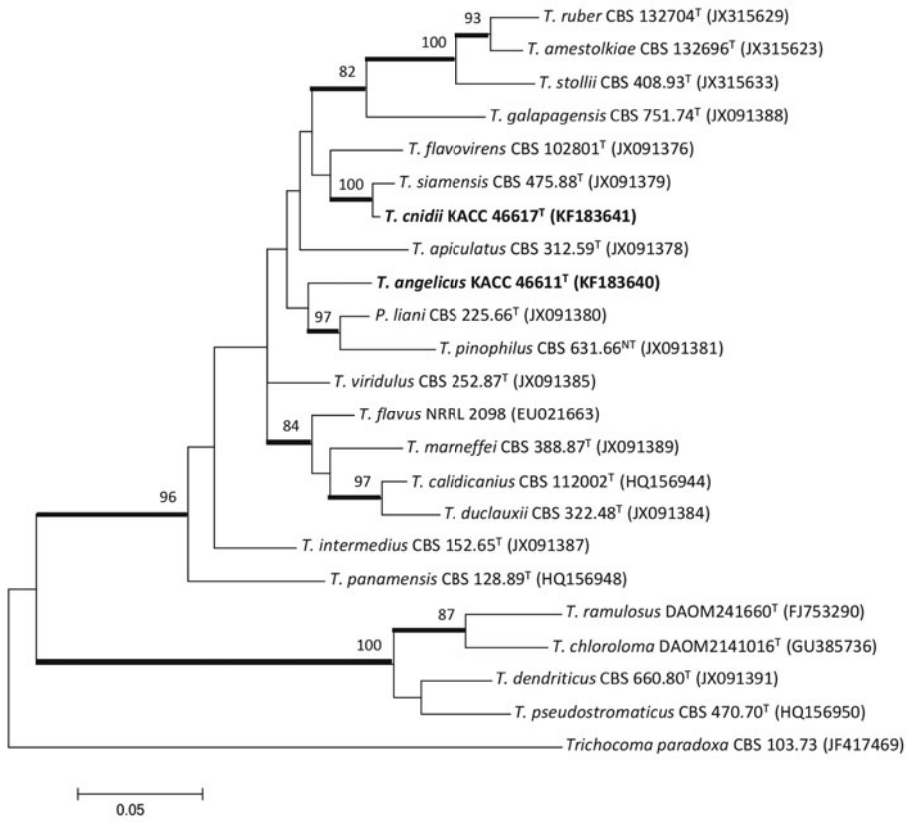

Fig. 4. Maximum likelihood constructed using MEGA 5.1 based on β-tubulin sequences, showing the placements of *T. angelicus* and *T. cnidii* (in bold letter) and other closely related *Talaromyces* species. The bootstrap support percentages from the ML analysis are listed at the branching nodes. Bootstrap support values lower than 70% are not shown, and bold branches indicate bootstrap values higher than 80%. *Trichocoma paradoxa* was used as the outgroup. T= ex-type strain; NT= neo-type strain.

due to its wider metulae (4–5 µm) and rough-walled conidia. Furthermore, the *T. siamensis* colony in the present study showed an undulating margin, whereas the *T. cnidii* colony showed a straighter margin (Fig. 2). In the observation to examine the sexual stages of these species, no ascomata were observed in cultures of *T. angelicus* and *T. cnidii* lasting up to 3 weeks. In *T. flavovirens*, ascomata were observed on dry *Quercus suber* leaf litter, but it could not be induced in culture (Visagie *et al*., 2012). The ascomata production abilities of the two novel species in nature remain to be determined. *Talaromyces angelicus* and *T. cnidii* appear to be asexual species, but the presence of mating-type genes needs to be examined using PCR amplification of MAT1 or MAT2 genes (Visagie *et al*., 2012) to provide the information on whether or not these species are heterothallic.

Taxonomy

Talaromyces angelicus S.H. Yu, T.-J. An & H. Sang, sp. nov. Fig. 1.

MycoBank MB804807

Etymology: '*angelicus*' refers to *Angelica gigas*, the host of the species.

Description: Colonies grown 7 days on CYA at 25°C 29–36 mm diam., plane or radially sulcate, floccose, white to reddish white, sporulation light, moderate amount of clear exudate present, no soluble pigments produced, colony reverse cream to red. On CYA, colonies 4–5, 34–40, and 26–29 mm diam after 7 days at 15°C, 30°C, and 37°C, respectively. Colonies grown 7 days on MEA at 25°C, 35–45 mm diam., plane or radially sulcate, floccose, colored white, reddish white center, light sporulation, exudate and soluble pigment absent, colony reverse cream to reddish. Colonies grown 7 days on YES at 25°C 31–39 mm diam, plane or raially sulcate, floccose, colored white, reddish wither center, light sporulation, exudate often present clear to light yellow droplets on the surface, colony reverse yellowish cream, occasionally brown center. Conidiophores arised from subsurface hyphae, biverticillate. Stipes 80–300 × 2.5–4.0 µm, smooth walled, terminating in a whorl of 2–5 metulae. Metulae cylindrical, 9–12 × 2.0–3.0 µm. Phialides acerose, 9–13 × 2.0–3.0 µm. Conidia subglobose to ellipsodial, 3.0–4.2 × 2.5–3.7 µm, smooth to finely rough-walled.

Type strain: KACC 46611, Republic of Korea, Pyeongchang, Gangwon-do, on a dried angelica root, May 2009, S.H. Yu, T.-J. An & H.-K. Sang, deposited in Korean Agricultural Culture Collection (KACC).

Distribution: Area of Gangwon-do, Republic of Korea.

Habitat: Dried roots of *Angelica gigas*

Talaromyces cnidii S.H. Yu, T.-J. An & H.-K. Sang, sp. nov. Fig. 2.

MycoBank MB804809

Etymology: '*cnidii*' refers to *Cnidium officinale*, the host of the species

Description: Colonies grown 7 days on CYA at 25°C, 30–35 mm diam., plane, velutinous, olive green to green, colony margin on the surface, a 2–3 mm peripheral white band of hyphae, sporulation moderate, exudate absent or very few red droplets on the surface, strong red pigments produced,

colony reverse red to red black. On CYA, colonies 6–9, 39–42, and 17–20 mm diam after 7 days at 15°C, 30°C, and 37°C, respectively. Colonies grown 7 days on MEA at 25°C 38–43 mm diam, plane, velutinous, colored olive green, marginal ring white 1–2 mm, moderate sporulation, moderate amount of pale red exudate present, no soluble pigments produced, colony reverse cream to brown cream. Colonies grown 7 days on YES at 25°C, 40–45 mm diam., radially sulcate, velutinous, olive green, marginal ring white 2–3 mm, moderate to heavy sporulation, exudate absent or few red exudate present, soluble pigment absent, colony reverse red, mycelium and conidia grown on the reverse colony. Conidiophores arised from subsurface hyphae, biverticillate, appressed. Stipes 100–300 × 2.5–4.0 µm, smooth walled, terminating in a whorl of 3–5 metulae. Metulae cylindrical, 9–12 × 2.0–3.0 µm. Phialides acerose, 10.5–13 × 1.5–2.5 µm. Conidia ellipsodial, (2.2-) 3.0–4.0 × 2.0–2.5 (-3.2) µm, smooth to finely rough-walled.

Type strain: KACC 46617, Republic of Korea, Jecheon, Chungbuk, on a dried roots of cnidium, August 2010, S.H. Yu, T.-J. An and H.-K. Sang, deposited in Korean Agricultural Culture Collection (KACC).

Distribution: Area of Chungbuk, Republic of Korea.

Habitat: Dried roots of *Cnidium officinale*

This work was supported by Korean Agricultural Culture Collection (KACC) and the Next-Generation BioGreen 21 Program (PJ008154 and PJ009508) and Cooperative Research Program for Agricultural and Technology Development (PJ009241) from Rural Development Administration, Republic of Korea.

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