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NOTE

Penicillium daejeonium sp. nov., a New Species Isolated from a Grape and Schisandra Fruit in Korea

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Two isolates of monoverticillate *Penicillium* species were collected from a grape and schisandra fruit in Korea. Multigene phylogenetic analyses with the nuclear ribosomal internal transcribed spacer (ITS) region and genes encoding β-tubulin (*benA*) and calmodulin (*cmd*), as well as morphological analyses revealed that the two isolates are members of the *P. sclerotiorum* complex in *Penicillium* subgenus *Aspergilloides*, but different from species of the *P. sclerotiorum* complex. The isolates are closely related to *P. cainii*, *P. jacksonii*, and *P. viticola* in terms of their multigene phylogeny, but their colony and conidiophore morphologies differ from those of closely related species. The name *P. daejeonium* is proposed for this unclassified new species belonging to the *P. sclerotiorum* complex in subgenus *Aspergilloides*.

***Keywords*:** *Penicillium daejeonium* sp. nov., morphological characteristics, molecular phylogenetics

Species of *Penicillium* occur in a wide range of habitats such as soils, foods, indoor and outdoor air, fruits, and water (Domsch *et al.*, 1980; Fischer and Dott, 2003). Some *Penicillium* species damage and cause decay in postharvest fruits such as grape, apple, and citrus, which results in economic loss. In particular, mycotoxins produced by *Penicillium* species contaminate juices and sauces from both healthy and partially rotten fruits (Agrios, 2005). Species of *Penicillium* subgenus *Aspergilloides* are mainly characterized by strictly or predominantly monoverticillate conidiophores with phialides borne directly on the stipe and only one branch point between the stipe and conidial chain (Pitt, 1979). The distinction defined by producing the monoverticillate or biverticillate penicilli is not consistently obvious in some species because metulae may be observed on some stipes. This characteristic prompted Pitt and Hocking (2009) to modify the taxonomic classification of species of *Penicillium* subgenus *Aspergilloides*.

Phylogenetic analyses of *Penicillium* subgenus *Aspergilloides* with molecular evidence have commonly used the nuclear ribosomal internal transcribed spacer (ITS) region and large subunit ribosomal DNA sequences (Peterson, 2000, 2004; Peterson *et al.*, 2003, 2004). *Penicillium sclerotiorum* complex in *Penicillium* subgenus *Aspergilloides* was recently phylogenetically and morphologically revised (Rivera and Seifert, 2011). Those authors Rivera and Seifert (2011) addressed seven phylogenetic distinct species including three new species (*P. cainii*, *P. jacksonii*, and *P. johnkrugii*) in the complex using multigene phylogenetic analyses with the ITS region, and genes encoding β-tubulin (*benA*), calmodulin (*cmd*), cytochrome C oxidase subunit 1 (*cox1*), and translation elongation factor 1-α (*tef1-α*). Rivera *et al.* (2012) subsequently reported two new *Penicillium* species in the *P. sclerotiorum* complex (i.e., *P. guanacastense* and *P. mallochii*) isolated from Costa Rican caterpillars. The recent study of Visagie *et al.* (2013) refined the concepts of the species in *Penicillium* section *Sclerotiora* that includes species of the *P. sclerotiorum* complex and introduced five new *Penicillium* species, including one species (*P. vanoranjei*) of the *P. sclerotiorum* complex.

Our studies of the etiology of postharvest diseases of fruits and medicinal crops in Korea have identified a new *Penicillium* species belonging to the *P. sclerotiorum* complex in *Penicillium* subgenus *Aspergilloides*. This new species is given the name *P. daejeonium* based on cultural and morphological characteristics and phylogenetic analyses of ITS, *benA*, and *cmd* gene sequences, as described below.

Strains KACC (Korean Agricultural Culture Collection) 46609 and KACC 46610 were isolated from *Penicillium* rot of grape and schisandra fruits in storage houses in Daejeon and Sangju, Korea, respectively. They were detected based

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on initial symptoms of soft, watery, and discolored spots on a fruit, with white mold eventually becoming visible on the surface of the fruit near the center of the spot with conidia. The fungus was isolated from the conidia and conidiophores of *Penicillium* species and transferred to malt extract agar (MEA). The isolates were cultured for 7 days at 25°C and stored in the KACC. The isolates were examined morphologically using the methodology of Pitt (1979, 2000) and Frisvad and Samson (2004). Czapek yeast autolysate (CYA) agar, MEA, and yeast extract sucrose (YES) agar were used for the identification, and cultures were incubated under the conditions described by Pitt (1979, 2000) and Frisvad and Samson (2004). The microscopic observations and measurements were made from slide preparations stained with 3% KOH. The morphological characteristics were identified with the aid of differential interference contrast microscopy.

For phylogenetic analyses, cultures were grown on MEA medium for DNA extraction. Mycelia were scraped from colonies after 7–9 days and freeze-dried for DNA extraction. Genomic DNA was extracted using a previously described method (Cubero *et al.*, 1999). ITS, *benA*, and *cmd* 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), 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), and CF1 (5′-AGG CGG AYT CTY TGA CYG A-3′) and CF4 (5′-TTT YTG CAT CAT RAG YTG GAC-3′) (Peterson, 2004), respectively. The PCR mixture contained 0.5 pmol of each primer, dNTPs at 0.2 mM, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of Taq polymerase, and 15 ng of template DNA. The PCR products were purified using the Wizard PCR Preps kit (Promega, USA) and directly sequenced with the BigDye terminator cycle sequencing kit (Applied Biosystems, USA) following the manufacturers' instructions. The same primer sets used in PCR amplification were used to sequence both DNA strands with an ABI Prism 310 Genetic Analyzer (Applied Biosystems). For the phylogenetic analyses, sequences of strains from the *P. sclerotiorum* complex and related species used in the studies of Rivera and Seifert (2011), Nonaka *et al.* (2011), and Visagie *et al.* (2013) were obtained from GenBank. The sequences obtained in this study were proofread, edited, and merged into comparable sequences using Mega v5.05 software (Tamura *et al.*, 2011). Sequences generated from materials in this study and retrieved from GenBank were initially aligned using MAFFT v7.045 (Katoh *et al.*, 2009) with the L-INS-I option, and the alignment was refined manually using Mega v5.05. Phylogenetic trees were constructed with Mega v5.05 using maximum likelihood (ML) analysis, and a bootstrap analysis was performed with 1,000 replicates. The resulting ITS region and *benA* and *cmd* sequences of KACC 46609 and KACC 46610 were submitted to GenBank with accession numbers of JX436489–JX436494.

The culture and morphological characteristics of the two isolates examined in the present study differed from those of any known *Penicillium* species, which is consistent with the multigene phylogeny described in detail below. Therefore, *P. daejeonium* is here described as a new species of *Penicillium*.

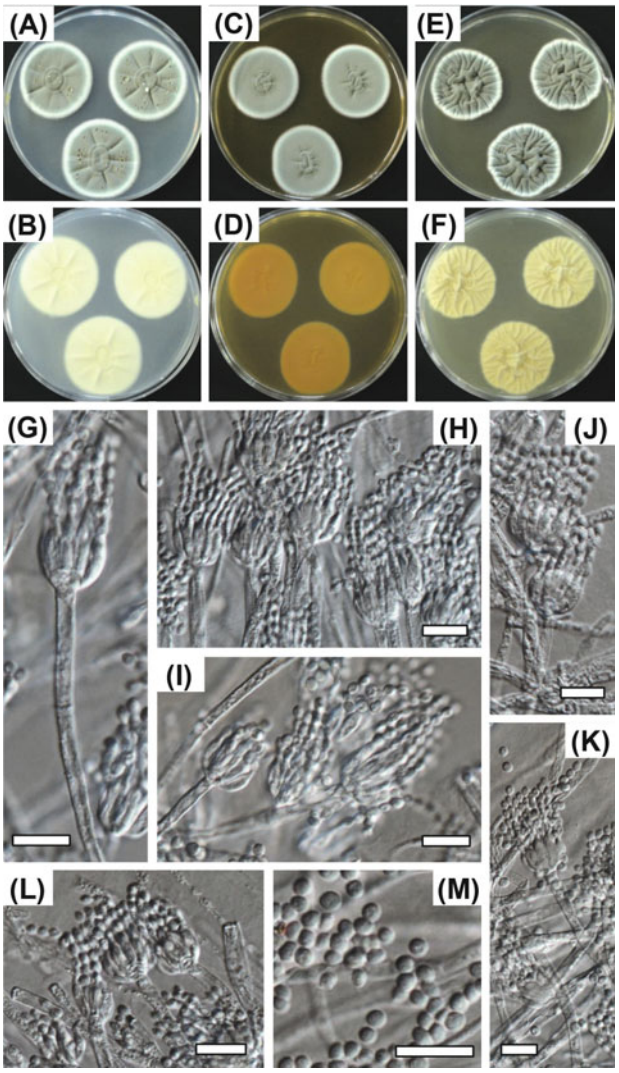

Fig. 1. *P. daejeonium* KACC 46609. 7-day old colonies on (A) CYA, (B) MEA, (C) YES, (D-I) conidiophores, (J) Conidia. White bar=10 μm.

Taxonomy

Penicillium daejeonium S.H. Yu & H.-K. Sang, sp. nov (Fig. 1). MycoBank MB 561572

Etymology: The use of '*daejeonium*' refers to Daejeon, the city in the Republic of Korea from where the species originates.

Description: Colonies grown 7 days on CYA at 25°C 36–41 mm diam., plane, velutinous, grey green to deep grey green, colony margin on the surface, a 2–3 mm peripheral white band of hyphae, sporulation moderate, moderate amount of clear to pale yellow exudate present, colony reverse cream. On CYA, colonies 8–10 mm diam after 7 days at 15°C. No growth or germination of conidia on CYA at either 30°C or 37°C. Colonies grown 7 days on MEA at 25°C 30–35 mm diam, plane, velutinous, colored grey green to deep grey green, marginal ring white 1–2 mm, moderate to heavy sporulation, exudate absent, colony reverse cream. Colonies grown 7 days on YES at 25°C 33–36 mm diam, plane, velutinous, deep grey green, marginal ring white 2-3 mm, mod-

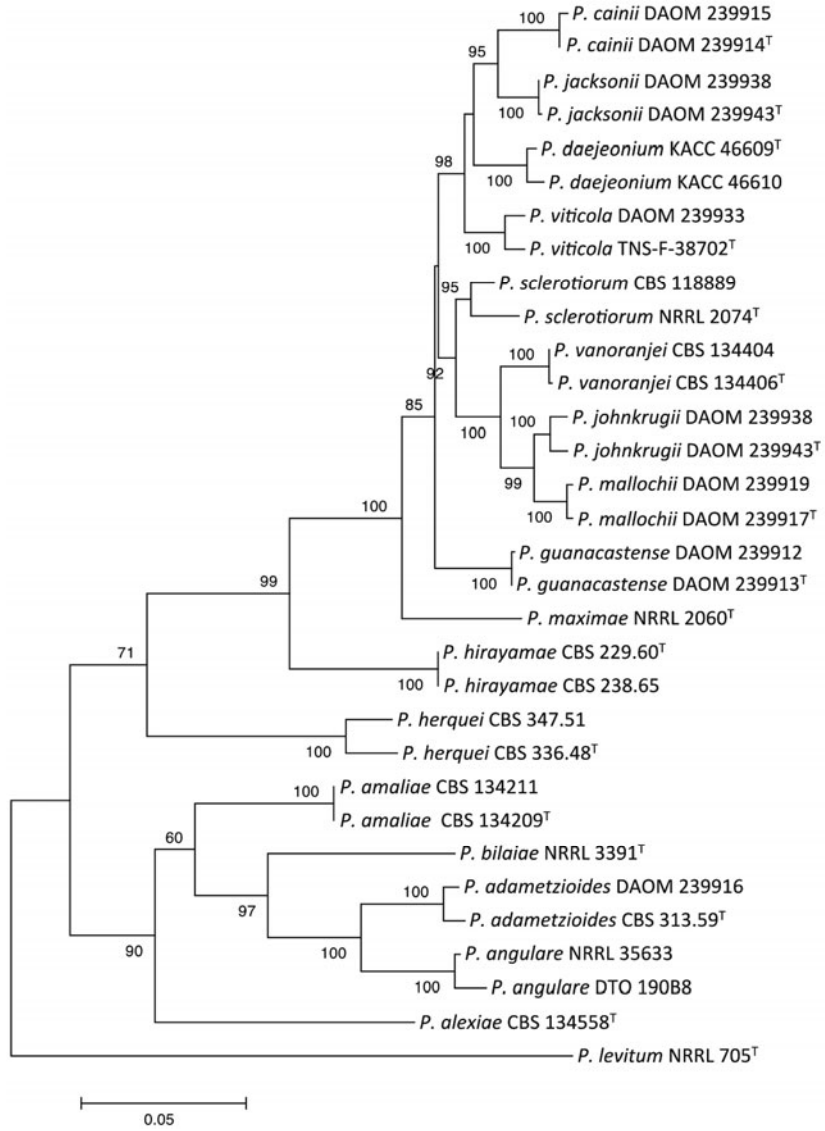

Fig. 2. Phylogenetic tree for *P. daejeonium* and its related species generated from maximum likelihood analysis of combine data on ITS, *benA* and *cmd* gene sequences. Numbers at nodes indicate percent bootstrap values (>70%) from 1,000 replicates. The bar indicates the number of substitutions per position and the letter T indicates ex-type strains. *P. levitum* NRRL 705 was used as an outgroup.

erate to heavy sporulation, exudate absent, colony reverse cream to yellowish cream. Conidiophores strictly simple, rarely with one lower branch-like metula 25–50 µm long. Stipes (50-) 100–400 × 2.0–3.5 (-4.0) µm, with vesicular apices up to 4–7 µm and walls smooth or finely roughed. Penicillus monoverticillate bearing 8–14 ampulliform, 8–12 × 2.0–2.8 µm phialides. Conidia globose to subglobose, 2.4–3.1 × 2.0–2.7 µm, smooth-walled.

Type strain: Republic of Korea, Daejeon, Yuseong-gu on *Penicillium* rot from grape fruits in August 2010 by S.H. Yu and H. Sang, deposited in KACC (holotype, KACC 46609).

Additional isolates examined: Republic of Korea, Gyeongbuk, Sangju, on *Penicillium* rot from schisandra fruits in September 2010 by T.-J. An (KACC 46610).

Distribution: Area of Daejeon and Gyeongbuk, Republic of Korea.

Habitat: Fruits of *Vitis* spp. (Cheongsoo grape cultivar) and fruits of *Schisandra chinensis* (schisandra).

The combined dataset of the ITS region, *benA*, and *cmd* consisted of 32 taxa of 18 species and 1,416 characters (512 for the ITS region, 426 for *benA*, and 478 for *cmd*). The ML tree showed that two isolates, KACC 46609 and 46610, formed a strongly supported monophyletic group comprising *P. cainii*, *P. jacksonii*, and *P. viticola*, with a bootstrap value of 98% (Fig. 2). The ITS region or *benA* sequences of these two isolates differed from each other only at one nucleotide position, while the *cmd* sequence differed at eight nucleotide positions. The results of morphological and molecular analyses of *Penicillium* species indicate that the new species is morphologically similar to *P. cainii* and *P. jacksonii* in velutinous colonies on CYA, MEA, and YES, and phylogenetically close to these two species. However, *P. cainii* differs from *P. daejeonium* with its orange to reddish orange reverse color on YES, and *P. jacksonii* can be distinguished by its high proportion of conidiophores with a single branch. Rivera and Seifert (2011) showed that *P. cainii* and *P. jacksonii* were closely grouped together in ML trees generated from the combined analyses of ITS, *benA*, *tef1-α*, and *cmd*.

The ML tree for the combined dataset of the ITS region, *benA*, and *cmd* obtained in this study strongly support the clade of *P. cainii* and *P. jacksonii*, and indicates that the two isolates of *P. daejeonium* are distinct from this clade (Fig. 2).

Penicillium viticola was first described as a novel species isolated from a grape in Japan (Nonaka *et al.*, 2011). *Penicillium daejeonium* and *P. viticola* were isolated from the same host and share phenotypic characteristics. However, the wall of conidiophores produced by *P. viticola* is rougher than that of *P. daejeonium*. Nonaka *et al.* (2011) demonstrated that *P. viticola* is closely related to *P. angulare* based on the phylogenetic tree generated from the calmodulin gene, while Rivera and Seifert (2011) considered it to be a sister group with *P. cainii* and *P. jacksonii*. *Penicillium daejeonium* is grouped with a clade including *P. cainii* and *P. jacksonii* and *P. viticola* (Fig. 2), which indicates that *P. daejeonium* is a new addition to the species comprising the *P. sclerotiorum* complex.

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