First Report on Isolation of *Penicillium adametzioides* and *Purpureocillium lilacinum* from Decayed Fruit of Cheongsoo Grapes in Korea

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Two species, *Penicillium adametzioides* and *Purpureocillium lilacinum*, were isolated from decayed grapes (cv. Cheongsoo) in Korea. Each species was initially identified by phylogenetic analysis of a combined dataset of two genes. Internal transcribed spacer (ITS) and β-tubulin (BT2) genes were used for identification of *Penicillium adametzioides*, and ITS and partial translation elongation factor 1-α (TEF) genes were used for identification of *Purpureocillium lilacinum*. Morphologically, they were found to be identical to previous descriptions. The two species presented here have not been previously reported in Korea.

KEYWORDS: Grape, Morphology, *Penicillium adametzioides*, Phylogenetic analysis, *Purpureocillium lilacinum*

Introduction

The hyphomycete genus *Penicillium* is a large ubiquitous anamorphic genus with economic importance for industry, medicine, and ecology [1]. Some species of *Penicillium* are destructive pathogens that can cause spoilage and decay of fruit [2, 3]. An association of grape berries with several species of *Penicillium* has been reported [4-6].

Difficulty in identification of filamentous fungi is particularly pronounced in the genus *Penicillium* [7]. Due to lack of information on teleomorph states and similarities of morphological criteria between anamorphic states, utilization of traditional taxonomic methods has been difficult [8]. However, Pitt and Hocking [3] suggested that identification of *Penicillium* to species level could be accomplished using strains grown under standardized conditions. Based on cultural or morphological characteristics, *Penicillium* has been divided into several divisions (subgenera) and sections [9]. Multigene sequence analysis is used widely in identification of *Penicillium* species and study of intraspecific and interspecific relationships [10-13]. In particular, Houbraken and Samson [9], who determined the phylogeny between *Penicillium* and other members of the family *Trichocomaceae*, in which the best-known species belong to the genera *Aspergillus*, *Penicillium*, and *Paecilomyces*, proposed a new system for classification of *Penicillium*, including 25 sections and revealed division of the *Trichocomaceae* into three separate families: *Aspergillaceae*, *Thermoascaceae* (including *Paecilomyces*), and *Trichocomaceae* (including *Penicillium*).

*Paecilomyces* can be differentiated from *Penicillium* by its phialides, which taper into a long distinct neck and by its divergent or tangled conidial chains [14]. A new divergent genus, *Purpureocillium* which includes the medically important *Paecilomyces liacinus* was established in 2011 by Luangsara-Ard *et al.* [15]. They described both morphological and molecular characteristics of the new combination of *Purpureocillium lilacinum*. Based on phylogenetic analysis of the partial 18S rRNA gene, the type species *Purpureocillium lilacinum* belonged to *Ophiocordycipitaceae*, while the type species *Paecilomyces variotii* of *Paecilomyces* belonged to *Trichocomaceae*.

In 1993, a grape cultivar ‘Cheongsoo’, a seedless white table grape with good quality and good productivity, was released in Korea [16, 17]. During 2010, fruit rot disease of Cheongsoo grapes was observed in storage houses in Yuseong, Daejeon, Korea. The disease initially occurs at the point of rupture of fruit berry (the area connecting the pedicle and the fruit berry) or on wound. It causes fruit rot, with brown, discolored skin. Several species of fungi, including two species, *Penicillium adametzioides* and *Purpureocillium lilacinum*, were encountered and identified during investigation of the fungal community of healthy and decayed fruit berries from Cheongsoo grapes. In this study, description of the two species was based on molecular and morphological characteristics, which have
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not yet been reported in Korea.

Materials and Methods

Fungal isolation. Decayed fruits of Cheongsoo grapes were collected from a grape storage house in Yuseong, Daejeon, Korea. Healthy and weakly infected grapes were washed with tap water and cut in the middle. The berries were sterilized with sodium hypochlorite (2% available chlorine) for 5 min, rinsed three times in sterile distilled water, and dried in a laminar air flow chamber. Five sterile samples with the point of rupture downside were placed on potato dextrose agar (PDA; Difco, Detroit, MI, USA). To inhibit bacterial growth, the PDA media were supplemented with the antibiotic streptomycin sulfate. Five replications of the assay using either weakly infected or healthy fruit were performed. Emergent colonies were collected at 25°C after 7 days of incubation. Pure cultures were maintained in PDA slant tubes and deposited in the Culture Collection of Chungnam National University (CNU) Fungi Herbarium.

DNA sequence analysis. The method described by Deng et al. [18] was used for isolation of genomic DNA from mycelium. Primers ITS5 and ITS4 were used for amplification of the internal transcribed spacer (ITS) region [19]. In addition, primers Bt2a and Bt2b were used for amplification of the β-tubulin (BT2) gene of the Penicillium isolate CNU 111554 [20] and primers EF-728F and EF2 were used for amplification of the translation elongation factor 1-α (TEF) gene of the Purpureocillium isolate CNU 111556 [15].

The Wizard PCR prep kit (Promega, Madison, WI, USA) was used for purification of successfully amplified PCR products. Both DNA strands of the amplified DNA fragments were sequenced with the same PCR primers. Sequences of the two genes for each isolate were concatenated in a single nucleotide alignment. The CLUSTAL X program [21] was used for alignment of the resulting sequences and relevant sequences available in the GenBank database with manual adjustment when necessary. Maximum likelihood analysis was performed using RAxML 7.2.8 HPC BlackBox [22] with the GTRGAMMA model for nucleotide substitution at the CIPRES web portal [23]. Editing of the trees obtained was performed in Mega v 5.05.

Morphology. For the two species, three-point inoculation was performed in 9 cm plastic Petri dishes using a dense conidial suspension on Czapek yeast extract agar (CYA; K,HPO4 1 g, Czapek concentrate 10 mg, yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 L), malt extract agar (MEA; OXOID; Basingstoke, Hampshire, England), and yeast extract agar (YES; Bacto; Dickinson Co., Sparks, MD, USA). For determination of cultural characteristics, including growth rate and texture of colony, pigmentation, and exudates, plates were incubated in the dark at 25°C for 7 days. Conidial morphology on MEA media was measured and compared with previous descriptions. In addition, the growth ability of each isolate was examined on CYA at 5°C and 37°C for 7 days.

Results and Discussion

Phylogenetic analysis. Combined alignment of Penicillium adametzioides and related species resulted in a data matrix of 1,014 sites containing 517 sites of the ITS region and 497 sites of the β-tubulin gene. Alignment of Purpureocillium lilacinum and relevant species generated a data matrix of 1,186 sites containing 593 sites of the ITS region and 593 sites of the TEF gene. Based on phylogenetic analysis, two maximum likelihood trees were obtained (Figs. 1 and 2). Presence of the isolates CNU 111554 and CNU 111556 was observed in monophyletic clades of Penicillium adametzioides (Fig. 1) and Purpureocillium lilacinum (Fig. 2), respectively, and was supported with high bootstrap values (99%).

The ITS gene sequence of the isolate CNU 111554 (accession No. JQ627629) was found to be identical to those of the Penicillium adametzioides isolates, including the type strain CBS 313.59; however, its β-tubulin gene sequence (accession No. JQ627630) differed at several sequence sites. The species was found to be closely related to Penicillium angulare and was distinct from...
Penicillium sclerotiorum, which is grouped in a distinct clade. *Penicillium adametzioides* was proposed as a synonym of *Penicillium sclerotiorum* by Stolk and Samson [24]. It was noted as a phylogenetically distinct species by Peterson [25] and accepted by Houbraken and Samson [9]. Rivera and Seifert [13] agreed with Houbrakening and Samson [9] and asserted that *Penicillium adametzioides* was a separate species belonging to the *Sclerotiora* section in the subgenus *Aspergilloides* of *Penicillium*.

Sequences of the ITS gene of the isolate CNU 111556 (accession No. JQ627631) and the β-tubulin gene (accession No. JQ627632) were 100% identical to the ex-type strain CBS 284.36 of *Purpureocillium lilacinum*. According to the report by Luangsa-Ard et al. [15], the isolate CNU 111556 was found to be present in one of the two clades in *Purpureocillium lilacinum* with 97% bootstrap value support. Our results were similar, showing a close phylogenetic association between *Purpureocillium lilacinum* and *Isaria takamizusanensis*.

**Morphology.** Morphological characteristics of the two isolates indicated that they were two different species; *Penicillium adametzioides* and *Purpureocillium lilacinum*. Taxonomic descriptions and morphological structures of each species are shown in Figs. 3 and 4.

*Penicillium adametzioides* Abe ex Smith, 1963 (Fig. 3).  
**Colonies on CYA after 7 days at 25°C** (Fig. 3A): 34–36 mm in diameter, sulcate in the center with ambiguous radial sulcation, dense and velutinous with white mycelium at the margins, glaucous grey to greenish grey, exudates absent, strong soluble pigment production, pale brown in color, and reverse pale luteous to orange.

**Colonies on MEA after 7 days at 25°C** (Fig. 3B): 26–28 mm in diameter, radial sulcation, velutinous with white mycelium at the margins, glaucous grey, exudates absent, entire margin, and reverse pale luteous to orange.  
**Colonies on YES after 7 days at 25°C** (Fig. 3C): 22–24 mm in diameter, radial sulcation, floccose, white to glaucous grey, exudates produced in light, yellow droplets, entire margin, and reverse pale luteous.

Conidiophores were typically monoverticillate on MEA, borne on basal felted hyphae, or from an agar surface. Stipes were simple, smooth-walled, septate, and normally measured 50–100 (~150) × 1.7–3 (~4) μm with vesicles measuring 3–5.5 μm in width. Phialides, which were ampulliform, measured 8–11 × 1.7–2.5 (~2.8) μm with collula measuring 0.5–1.5 μm. Conidia, which were produced in catenation, were globose to subglobose, smooth-walled, and measured 2–3 (~3.4) μm in diameter.

**Culture examined:** CNU 111554 was isolated from Cheongsoo grapes, Deajeon, Korea.

**Note:** The present fungus did not grow on CYA at 5°C and 37°C. Cultural and conidial morphology of the present

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**Fig. 2.** Phylogenetic tree for *Purpureocillium lilacinum* and its related species generated from maximum likelihood analysis of combined data on internal transcribed spacer and translation elongation factor 1-α (TEF) gene sequences. Numbers above the nodes indicate bootstrap values (> 50%) from 1,000 replicates. The bar indicates the number of substitutions per position. The letter T indicates ex-type strain and the present *Penicillium* isolate is marked in bold. The out group is *Paecilomyces marquandii* CBS 182.27.

**Fig. 3.** Morphology of the isolate CNU 111554 (*Penicillium adametzioides*). Colonies grown on Czapek yeast extract agar (A), malt extract agar (B), and yeast extract agar (C) after 7 days at 25°C; penicilli (D–F), conidia (G) (scale bars: D, E = 20 μm, F, G = 10 μm).
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Isolate was in agreement with the previous description of *Penicillium adametzioides* [26]. It showed morphological similarity to *Penicillium sclerotiorum* and has been designated in the *Penicillium sclerotiorum* complex [24]. Based on phylogenetic analysis, the species has lately been proposed as a separate species [9, 13] that is closely related to *Penicillium angulare* but is distinguished by restricted growth rates, production of pigment in CYA, and morphologically by the length of conidiophores [10]. Presence of the fungus in soil from Japan has been reported [10]. In addition, airborne fungus in Turkey [27] and peach tree twigs in Spain [28] have also been reported; however, it has not been reported in Korea.

**Purpureocillium lilacinum** (Thom) Luangs-ard, Hou-braken, Hywel-Jones and Samson 2011 (Fig. 4).

Colonies on CYA after 7 days at 25°C (Fig. 3A): 32–34 mm in diameter, polygonous circular or subround sulcation in the center, with radial sulcation, strong floccosity, rosy vinaceous, and reverse white to buff.

Colonies on MEA after 7 days at 25°C (Fig. 3B): 31–33 mm in diameter, circular sulcation in the center, sometimes with ambiguous radial sulcation, rosy vinaceous, and reverse orange to fulvous.

Colonies on YES after 7 days at 25°C (Fig. 3C): 24–26 mm in diameter, pentagonal sulcation in the center, with radial sulcation, white to pale vinaceous, and reverse buff to pale luteous.

Conidiophores were erect, arising mainly from sub-merged hyphae, with occasional formation of synnemata. Stalks were smooth or roughened, consisting of verticillate branches with whors of two to four phialides, and measured 2–3 (~4) µm in width. Conidiophores of branches measured 6–17 × 1.9–2.6 µm. Phialides were cylindrical or ellipsoidal in the lower part, narrowing abruptly into a short neck approximately 1 µm wide, and measured 6–11 (~13) × 1.5–2.5 µm. Conidia, which were observed in short divergent chains, were ellipsoidal to fusiform, more or less apiculate, hyaline, and smooth-walled, and measured 2–3 (~3.5) × 1.9–2.7 µm.

Formation of typical conidial structures was observed near the agar, with either solitary phialides or 2–4 in verticils, which varied in length. The phialides had the shape of typical *Purpureocillium lilacinum* phialides, or were very long (up to 30 µm) and *Acremonium*-like. Conidia, which were primarily cylindrical, and occasionally slightly curved or ellipsoidal, measuring 3–17 (~20) × 1.2–2.5 µm, formed in ‘slimy heads’ on these *Acremonium*-like structures with variable size. Chlamydospores were absent.

**Culture examined:** CNU 111556 was isolated from Cheongsoo grapes, Deajeon, Korea.

**Note:** No growth was observed for the present fungus at 5°C and 37°C. Morphology of the present isolate was in agreement with the description of *Purpureocillium lilacinum* [Penicillium lilacinum (Thom) 1910, Paecilomyces lilacinus (Thom) Samon 1974, and Paecilomyces nostocoides Dunn 1983] which is a dimorphic species with the ability to form an *Acremonium*-state on agar media [15, 29]. Based on overall morphology and spore color, the species is similar to *Paecilomyces marquandii* [14]. However, phylogenies show them to be separate in two families of the Hypocreales [30]. The species was found to be closely related to *Isaria takamizusanensis*, which is associated with insects having purple-colored conidia. Luangs-Ard et al. [15] reported that it could not be separated between harmful and beneficial isolates of *Purpureocillium lilacinum*.

The species is commonly a saprobic species isolated from soil, decaying vegetation, insect and insect larvae, nematodes, humans, animals, and (indoor) air in many countries. Colonization of this species can occur on materials such as catheters and plastic implants. In addition, it can contaminate antiseptic creams and lotions, and cause infection in immunocompetent and immunocompromised...
patients. It can also serve as a biological control agent for control of nematodes [15]. This is the first study to report on isolation of *Purpureocillium lilacinum* from decayed fruit of Cheongsoo grapes in Korea.

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