RESEARCH ARTICLE

First Record of a Rare Species Belonging to Genus *Penicillago* (*Penicillaginaceae*, *Eurotiales*) from Korea

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ABSTRACT

During surveys of *Eurotiales* fungi from soil habitats, a fungal strain, CNUFC U8-51, was isolated. Based on the morphological characteristics and phylogenetic analyses of a combined dataset of four loci (β-tubulin, calmodulin, internal transcribed spacer, and RNA polymerase II second largest subunit), the isolate was identified as *Penicillago mirabilis*. To our knowledge, this is the first record of the genus *Penicillago* in Korea, as well as the first record of *P. mirabilis* in the country. Herein, descriptions, illustrations, and results of the phylogenetic analyses of this species are provided.

Keywords: *Eurotiales*, *Penicillago*, Phylogeny, Taxonomy

INTRODUCTION

Eurotiales is a relatively large order of ascomycete fungi comprising species that have been widely used in food fermentation and biotechnology for the production of enzymes, organic acids, and pharmaceuticals. However, some species in this order are also associated with food spoilage, mycotoxin production, indoor contamination, and opportunistic infections [1–4].

At present, *Eurotiales* comprises four families (*Aspergillaceae*, *Penicillaginaceae*, *Thermoascaceae* and *Trichocomaceae*), 26 genera (*Acidotalaromyces*, *Ascospirella*, *Aspergillago*, *Aspergillus*, *Dendrosphaera*, *Evansstolkia*, *Hamigera*, *Leiothecium*, *Monascus*, *Paecilomyces*, *Penicillago*, *Penicilliopsis*, *Penicillium*, *Phialomyces*, *Pseudohamigera*, *Pseudopenicillium*, *Rasamsonia*, *Sagenomella*, *Sclerocleista*, *Talaromyces*, *Thermoascus*, *Thermomyces*, *Trichocoma*, *Warcupiella*, *Xerochrysium* and *Xeromyces*), and 1,393 species [5].

The genus *Penicillago* (*Penicillaginaceae*, *Eurotiales*) was introduced to accommodate the taxonomically confusing species *Penicillium nodositatum* [6]. Subsequently, three additional species were added: *P. kabunica*, *P. mirabilis*, and *P. moldavica* [7]. Four species, *P. kabunica*, *P. mirabilis*, *P. moldavica*, and *P. nodositata*, are currently listed in Index Fungorum (http://www.indexfungorum.org/names/names.asp) [8]. However, to date, no species of this genus has been reported in Korea.

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Morphologically, species of *Penicillago* are characterized by the production of *Penicillium*-like conidiophores, which contain ampulliform phialides with relatively long, narrow necks and green conidia. They are unable to grow on Czapek yeast autolysate agar (CYA) when incubated at 37°C and exhibit moderate growth on creatine sucrose agar (CREA) without acid production [6,7].

During our investigation of fungal diversity in soil in Korea, we identified a newly recorded species, *Penicillago mirabilis*, belonging to the order *Eurotiales*, based on morphological characteristics and multi-gene phylogenetic analyses. Descriptions, illustrations, and results of phylogenetic analyses of this species are provided.

MATERIALS AND METHODS

Sampling and isolation

Soil samples were collected from Ulleung Island, Korea (37°30′23.0″N 130°51′25.9″E). One gram of soil was mixed with 9 mL sterile distilled water. Serial dilutions of the mixtures were then prepared (from 10^{-1} to 10^{-5}). For each dilution, a 100 µL aliquot was dispensed onto potato dextrose agar (PDA; Becton, Dickinson and Co., Sparks, MD, USA) supplemented with neomycin (50 mg/L). The plates were incubated at 25°C in the dark for 7 days. Individual fungal colonies were transferred to new PDA plates and incubated at 25°C for 3–7 days. Pure isolates were obtained and stored on PDA slants at 4°C and in 20% glycerol at −80°C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea. The isolated strain was also deposited in the Collection of National Institute of Biological Resources (NIBR), Incheon, Korea.

Culture and morphological descriptions

Three-point inoculations were performed on CYA, malt extract agar (MEA), yeast extract sucrose agar (YES), oatmeal agar (OA), and CREA [9,10]. All media were incubated at the standard temperature of 25°C for 7 days. Additional plates were incubated at 30 and 37°C. Macroscopic and microscopic features were studied after 7 days. Slides were prepared using 60% lactic acid, and excess conidia were removed using 96% ethanol. An Olympus BX53 microscope with differential interference contrast optics (Olympus, Tokyo, Japan) was used to obtain digital images.

DNA extraction, PCR, and DNA sequencing

Fresh mycelium was scraped from the margins of colonies on PDA overlaid with cellophane after 7 days incubated at 25°C. Genomic DNA was extracted using a Solg™ Genomic DNA Prep Kit (Solgent Co. Ltd., Daejeon, Korea) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region of rRNA, β-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) were amplified using primers V9G/ITS4 [11,12], Bt2a/Bt2b [13], CF1L/CF4 [14], and RPB2-5F/RPB2-

7cR [15], respectively. The PCR products were purified using an AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea). DNA sequencing was performed at Macrogen (Daejeon, South Korea) using the same primers used for PCR.

Phylogenetic analyses

The generated sequences, together with reference sequences for closely related species based on the BLAST results, were aligned using MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) [16] and then manually edited in MEGA 7 [17]. Phylogenetic analyses were based on maximum likelihood performed in IQ-TREE 2 and bootstrap analyses using 1,000 replicates [18]. The tree figures were created using FigTree v1.3.1. Bootstrap support values were higher than 70% in the branches. All sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov) (Table 1).

Table 1. Strains and GenBank accession numbers used for phylogenetic analysis in this study

Species	Strain no.	GenBank accession number			
		ITS	*BenA*	*CaM*	*RPB2*
Penicillago kabunica	CBS 575.90^{T}	MN431415	MN969438	MN969357	MN969217
Penicillago mirabilis	CBS 624.72^{T}	MN431416	MN969439	MN969358	MN969218
Penicillago mirabilis	**CNUFC U8-51**	**PZ327461**	**PZ338222**	**PZ338223**	**PZ338224**
Penicillago moldavica	CBS 574.90^{T}	MN431417	MN969440	MN969359	MN969219
Penicillago nodositata	CBS 333.90^{T}	KC790403	KC790399	MN969361	MN969220
Penicillago sp.	FMR 16442	LT899788	LT898313	LT899772	LT899806
Penicillago sp.	FMR 15296	LT899787	LT898312	LT899771	LT899805
Penicillago sp.	DTO 119-A2	N/A	N/A	MT066181	MT066178
Penicillago sp.	DTO 216-G1	N/A	MF974887	MT066182	MT066179

Bold letters indicate strains and accession numbers determined in this study. ITS: internal transcribed spacer; *BenA*: β-tubulin; *CaM*: calmodulin; *RPB2*: RNA polymerase II second largest subunit; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, The Netherlands; CNUFC: Chonnam National University Fungal Collection, Gwangju, Korea; DTO: Internal Culture Collection of the CBS-Fungal Biodiversity Center; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; N/A: not available. ^{T}ex-type strain.

RESULTS

Phylogenetic analyses

Phylogenetic analyses of *Penicillago* species were performed using a concatenated dataset of four loci (*BenA*, *CaM*, ITS, and *RPB2*) (Fig. 1). The multi-gene analysis included nine taxa. The concatenated alignment consisted of 2,880 characters (including alignment gaps): 481, 527, 895, and 977 characters were used in *BenA*, *CaM*, ITS, and *RPB2*, respectively. Multi-gene phylogenetic analysis revealed that our strain (CNUFC U8-51) clustered with an ex-type strain of *Penicillago mirabilis* (Fig. 1).

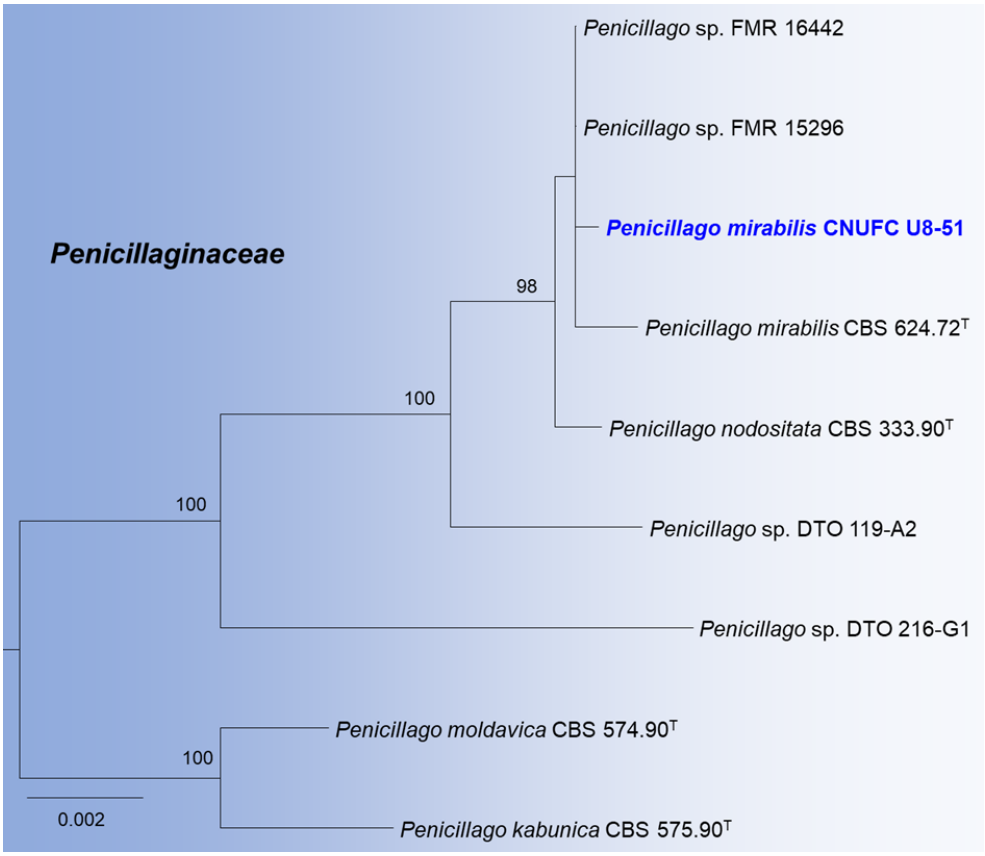

Fig. 1. Phylogram generated using maximum likelihood (ML) analysis based on combined β-tubulin (*BenA*), calmodulin (*CaM*), internal transcribed spacer (ITS), and RNA polymerase II second largest subunit (*RPB2*) sequence data for *Penicillago* species. Branches with >70% ML bootstrap support are indicated. *Penicillago kabunica* CBS 575.90 and *P. moldavica* CBS 574.90 were used as the outgroups. The newly generated sequences are indicated in bold blue. Ex-type strains are marked with a superscript T.

Taxonomy

Penicillago mirabilis (Beliakova & Milko) Houbraken, Frisvad & Samson, Stud. Mycol. 95: 90 (2020) (Fig. 2).

Culture characteristics (7 days at 25°C): On CYA, colonies radially sulcate and protuberant at the center, mycelia white, texture velutinous, sporulation sparse, soluble pigments or exudates absent, reverse moderate yellowish pink. On MEA, colonies plane, texture floccose, mycelia white, sporulation moderate to dense, soluble pigments or exudates absent, reverse pale yellow. On YES, concentric rings toward the center, texture velutinous, mycelia white, sporulation very sparse or absent, soluble pigments or exudates absent, reverse yellowish brown and white toward the edges. On OA, colonies slightly raised at the center and cottony, mycelia white, sporulation sparse, soluble pigments or exudates absent, reverse white. On CREA, acid production absent.

Micromorphology: Conidiophores biverticillate. Stipes rough walled, 198–432 × 2.5–3.5 µm. Metulae three to five, divergent, 7.5–10.5 × 2.5–3.5(–4) µm. Phialides ampulliform, 6.5–10 × 2.5–3.5 µm. Conidia echinulate walled, subglobose, oval to broad fusiform, 2.6–3.0 × 2.5–2.8 µm. Ascomata not observed.

Colony diameters (mm), 7 days: CYA 25°C, 40–43; CYA 30°C, 24–26; CYA 37°C, no growth; MEA 25°C, 49–51; YES 25°C, 35–37; OA 25°C, 26–30; CREA 25°C, 26–31.

Material examined: Republic of Korea, Ulleung Island (37°30′23.0″N 130°51′25.9″E); from rhizosphere soil; May 1, 2019, culture CNUFC U8-51= NIBRFG0000509589.

Notes: Phylogenetic analysis of the combined *BenA*, *CaM*, ITS, and *RPB2* dataset indicates that our strain CNUFC U8-51 is most closely related to *P. mirabilis*. Pairwise comparisons between CNUFC U8-51 and the reference strain of *P. mirabilis* showed high sequence similarity among all loci examined, with identities of 565/566 bp (99.82%) for ITS, 436/437 bp (99.77%) for *BenA*, 490/491 bp (99.79%) for *CaM*, and 767/768 bp (99.87%) for *RPB2*.

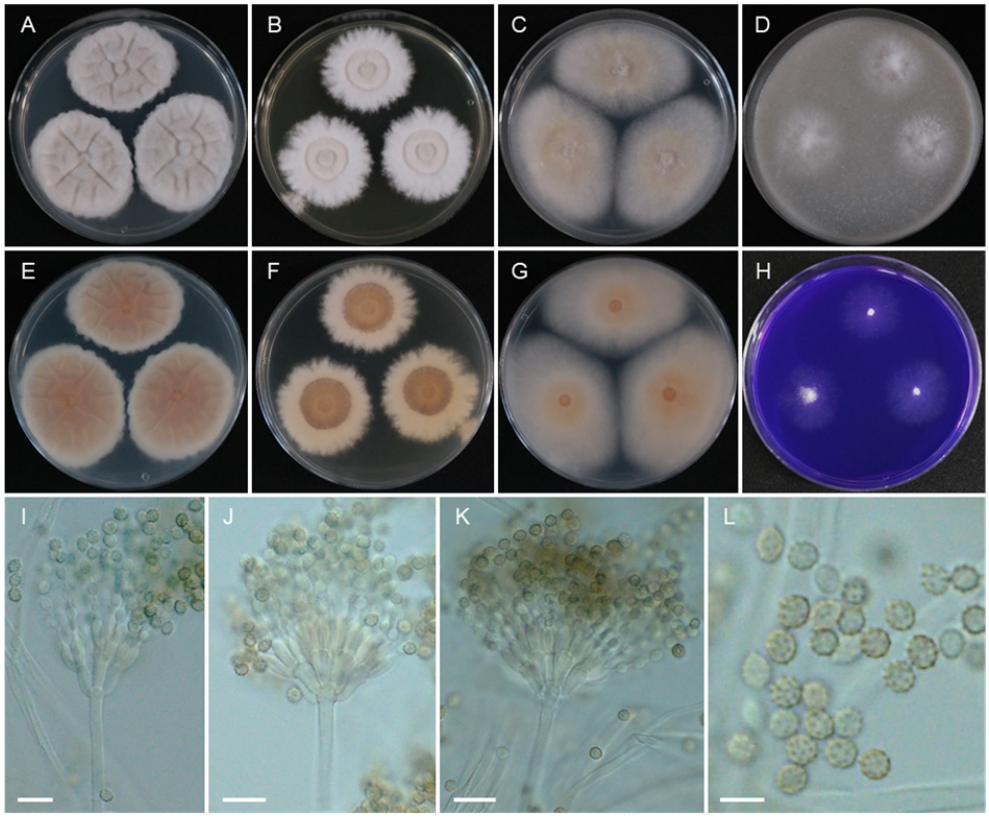

Fig. 2. *Penicillago mirabilis* (CNUFC U8-51). A, E: Colonies on Czapek yeast autolysate agar; B, F: Colonies on yeast extract sucrose agar; C, G: Colonies on malt extract agar; D: Colonies on oatmeal agar; H: Colonies on creatine sucrose agar. A–D, H: Obverse view; E–G: reverse view. I–K: Conidiophores; L: Conidia. Scale bars: I–K = 10 μm, L = 5 μm.

DISCUSSION

In the present study, we identified a rare species, *Penicillago mirabilis*, as a newly recorded species in Korea. This finding contributes to a broader understanding of the fungal diversity of *Eurotiales* in Korea.

Based on our multi-locus phylogenetic analysis, our strain CNUFC U8-51 is most closely related to *P. mirabilis*. The results of our molecular data analysis are consistent with the phylogeny presented by Houbraken et al. [7]. Morphologically, CNUFC U8-51 is consistent with the original description of *P.*

mirabilis. However, our strain shows slower colony diameter growth on YES than that of *P. mirabilis* (35–37 mm vs. 51–59 mm) [7]. Based on the molecular and morphological evidence together, we identify strain CNUFC U8-51 as *P. mirabilis*. This isolate was obtained from a soil sample, thereby expanding the known ecological range of the species and contributing additional molecular data for the genus *Penicillago*.

The ITS region has been proposed as a universal DNA barcode marker for fungi [19]. However, ITS rDNA alone is not sufficient to distinguish all species in *Eurotiales*. Therefore, *BenA* and *CaM* have been proposed as alternative markers [7,9,20]. Even with additional gene regions, this study shows that more may be required, as illustrated by the taxonomy of *P. mirabilis* and *P. nodositata*.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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