RESEARCH ARTICLE

Penicillifer diparietisporus: a New Record from Field Soil in Korea

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ABSTRACT

A fungus was isolated from field soil collected from Daegu, Korea. The colony of the isolated fungus showed short, branched, and light to dark yellow pigments with hyaline, yellowish red to orange brown aerial mycelia. In addition, the fungus produced solitary to aggregated perithecia, ovoid to pyriform, short neck, and asci as well as biseriately arranged ascospores. Phylogenetic analysis using the internal transcribed spacer region and translation elongation factor 1-α sequences and morphological characteristics identified the isolated fungus as *Penicillifer diparietisporus*, which belongs to the family Nectriaceae. To our knowledge, this is the first report of *Penicillifer diparietisporus* in Korea.

Keywords: Nectriaceae, *Penicillifer diparietisporus*, Soil fungi

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2018 September, 46(3): 227-233
https://doi.org/10.4489/KJM.20180029

Received: August 11, 2018
Revised: August 18, 2018
Accepted: August 18, 2018

INTRODUCTION

Approximately 2,700 fungal species from 240 genera are currently recognized in eight families in the order Hypocreales [1,2]. The species produce light- to bright-colored, ostiolate, perithecial ascomata containing unitunicate asci with hyaline ascospores. The asexual morphs found in nature are, most frequently, moniliaceous and phialidic [3-7]. The fungal species are globally found in various environments; they are of great importance in agriculture and medicine and extensively exploited for industrial and commercial applications [5]. Moreover, several species have been reported as important opportunistic human pathogens [8-10], whereas others produce mycotoxins that are of medical concern [5].

The purpose of this study was to screen for unreported fungal species in field soil in Korea. This study provides information for further studies on the use of such organisms in agriculture and medicine as well as for industrial applications. On the basis of morphological and molecular characteristics, the strain KNU16-010, identified as *Penicillifer diparietisporus*, was reported for the first time in Korea.

MATERIALS AND METHODS

Collection of soil samples

In 2016, samples were collected from field soil during the screening of fungal species in Daegu, Korea (35°54′11.3″N, 128°36′56.1″E). A sterile trowel and spatula were used to collect the soil samples from a depth of 15~30 cm. The collected soil samples were placed in polythene zipper bags and transferred to the laboratory. Then, the soil samples were prepared for serial dilutions, and 1 g of soil was diluted with 10 mL of sterile distilled water. The serial dilutions were performed until a concentration of 10^{-3} was achieved, and, then, 100 μL of each sample was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated for 2~3 days at 25℃. Single germinating fungal colonies were transferred to fresh PDA plates and incubated under the same conditions. The strain KNU16-10 was selected for further morphological and molecular phylogenetic analyses.

Morphological characterization

To study the cultural and morphological characteristics, PDA, oatmeal agar (OA; Difco), and malt extract agar (MEA; Difco) were used; the incubation period was 10 days at 25℃. Then, cultural characteristics such as colony color, fungal growth, and texture were observed and recorded, and the morphological characteristics were observed under a light microscope (BX-50; Olympus, Tokyo, Japan).

Genomic DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from 7-day-old colonies of the strain KNU16-010 grown on PDA by using the HiGene Genomic DNA Prep kit (BIOFACT, Daejeon, Korea), according to the manufacturer's instructions. Partial gene sequences were amplified by the internal transcribed spacer (ITS) region, ITS1/ITS4, and translation elongation factor (TEF) 1-α gene, EF1-728F/EF2, as described previously [11-13]. Then, the amplified PCR products were purified with EXOSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Solgent (Daejeon, Korea). The similarities of the sequences were analyzed using BLAST of NCBI. The sequences obtained from KNU16-010 were deposited in NCBI GenBank (accession numbers LC387549 and LC387601 for the ITS region and TEF gene, respectively).

Phylogenetic analysis

The consensus sequences were compared with other sequences in the NCBI database by using BLAST to determine the percentage of shared sequence identity with other sequences of fungal species. The alignments were performed using MEGA 7.0 [14] with 1,000 bootstrap replicates, and the evolutionary distance matrices were generated based on Kimura's neighbor-joining algorithm model [15].

RESULTS AND DISCUSSION

Morphology of the strain KNU16-010

To observe the morphological characteristics, the strain KNU16-010 was cultured on PDA, OA, and MEA at 25℃ for 10 days, and the diameter of the colonies was 28.7~29.7 mm, 32.0~32.7 mm, and 31.9~32.5 mm, respectively (Fig. 1). The morphology of the strain KNU16-010 was compared with previous descriptions of *Penicillifer diparietisporus* [16] and *Pseudonectria diparietospora* [17] (Table 1). *Pseudonectria diparietospora* produced reddish brown colonies on PDA [17]. Moreover, *Neocosmospora arxii* (basionym: P*seudonectria diparietispora*) colonies are composed of hyaline or sometimes yellow-pigmented, branched, septate, smooth-walled, hyphae that are 2.5~5 μm wide, and they produce semi-immersed to immersed perithecia, scattered or aggregated in small groups, yellowish orange to yellowish brown, broadly ovoid to somewhat pyriform, 220~340 × 160~270 μm in diameter, almost glabrous, conical shaped with short neck [18]. However, our strain formed orange brown to yellowish red colonies in all the media, and PDA was used to observe the mycological characteristics (Fig. 1). The fungal colonies showed wavy mycelia at the edges and rough wart-like structures. They also formed septate, irregular, and short hyphae, and the average width of the hyphae was 2.42 μm. They produced submerged perithecia, solitary to aggregated, yellowish orange to yellowish brown, ovoid to pyriform, smooth, conical shaped with short neck (Fig. 2A, 2C). Asci clavate, 70.9~81.1 × 16.3~19.7 μm, thin wall, and 8-spored (Fig. 2D, 2E). Ascospores were oval, septate, guttulate, 2-celled, distichous, double wall, biseriate, tubular, hyaline and pale green, smooth and blunt at both ends with a diameter (n = 30) of 17.9~30.7 × 6.7~10.9 μm and no conidia (Fig. 2F, 2G). The ascospores were distichous, elliptical, continuous, hyaline, with thick, double wall, smooth 18~24 × 12~14 μm and no

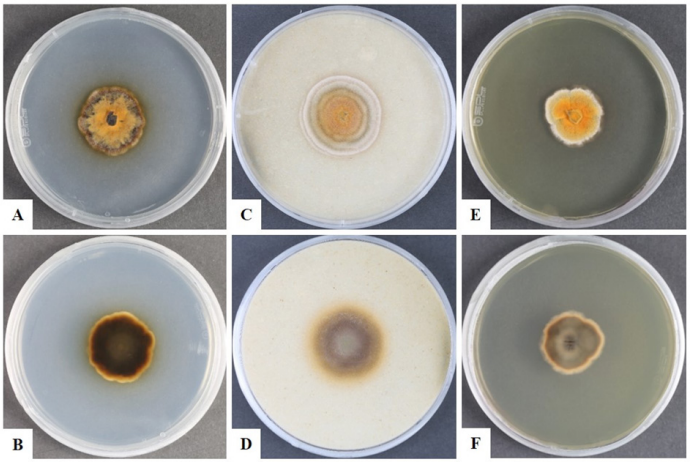

Fig. 1. Cultural characteristics of KNU16-010. A, B, Front and reverse sides of the colony on potato dextrose agar; C, D, Front and reverse sides of the oatmeal agar; E, F, Front and reverse sides of the malt extract agar.

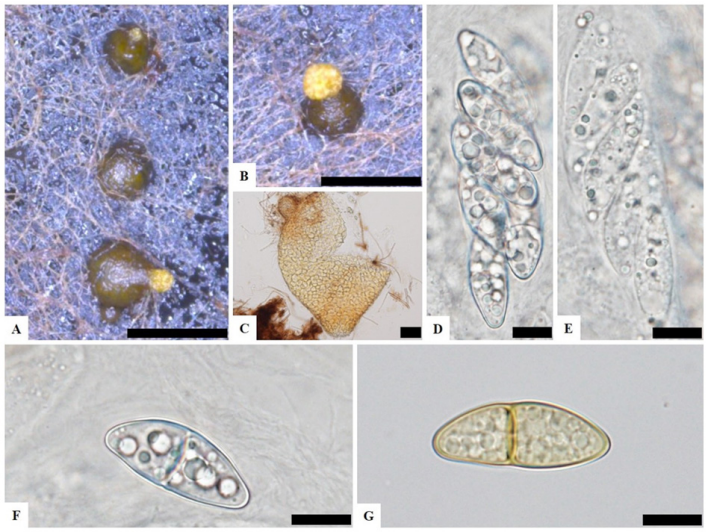

Fig. 2. Morphological characteristics of KNU16-010. A, B, Mature perithecia on potato dextrose agar; C, Perithecium; D, E, Asci; F, G, Ascospores (scale bars: A, B = 500 μm, C = 50 μm, D~G = 10 μm).

Table 1. Morphological characteristics of the strain KNU16-010 with reference to *Penicillifer diparietisporus*

Characteristics		KNU16-010[a]	*Penicillifer diparietisporus*[b]	*Pseudonectria diparietospora*[c]
Colony	Color	Yellowish to orange and dark brown in reverse on PDA. Light yellow in center, white edge and brown color in reverse on MEA.	Reddish white edge, changing to greenish yellow on OA and yellowish brown to brown, light brownish orange to dark brown on PDA. Dark brown color in reverse both media.	Reddish-brown color on PDA and colonies composed of tangled masses of conidial chains.
	Size (Diam.)	PDA: 28.7~29.7 mm; OA: 32.0~32.7 mm and MEA: 31.9~32.5 mm in 10 days at 25°C.	PDA: 22mm; OA:30 mm in 10 days at 25°C; MEA: N/A	N/A
	Shape	Edge margin irregular, wavy and inadequate aerial mycelium.	Edges submerged into irregular margin and scant of aerial mycelium.	Lightly floccose or plane and appressed and thin.
Asci	Size (Diam.) and Shape	Clavate, 70.9~81.1 × 16.3~19.7 μm, thin wall and 8-spored.	Clavate, 60~85 × 12~25 μm, unitunicate and 8-spored.	Broadly clavate to spindle shaped with very thin wall, short stalked, 60~80 × 19~22 μm and 8-spored.
Ascosspores	Size (Diam.)	17.9~30.7 × 6.7~10.9 μm.	21~25 × 12~15 μm.	18~24 × 12~14 μm.
	Shape and Color	Oval, septate, distichous, double wall, tubular, hyaline to pale green, smooth and blunt in both ends.	Biseriate, thick walled, broadly ellipsoidal, surrounded by a thin walled sheath collapsing at maturity and sometimes giving the ascospores roughened appearance.	Distichous, elliptical, continuous, hyaline, with thick, double wall, smooth.
Perithecia		Submerged, solitary to aggregated, yellowish orange to yellowish brown, ovoid to pyriform,smooth, conical shaped and short neck.	Solitary to densely aggregated, superficial, globose, ovoid to pyriform, 270~300 × 240~270 μm diameter, brown, red-orange to orange.	Superficial, transparent wall, globose-conoid with very short neck, smooth, yellowish-red, 400~500 × 300~400 μm.

PDA, potato dextrose agar; MEA, malt extract agar; OA, oatmeal agar; Diam., diameter; N/A, not available in previous references.
[a]Fungal strain studied in this paper.
[b]Sources of the descriptions [16].
[c]Sources of the descriptions [17].

conidia [17]. Thus, these morphological characteristics of the strain KNU16-010 were very similar to those of *Penicillifer diparietisporus*.

Notes: Currently, the genus *Viridispora* is composed of four species, *Viridispora diparietispora* (= *Penicillifer furcatus*), *Viridispora alata* (= *Penicillifer bipapillatus*), *Viridispora fragariae* (= *Penicillifer fragariae*), and *Viridispora penicilliferi* (= *Penicillifer macrosporus*), each with its own *Penicillifer* asexual morphs [7, 16, 19, 20]. Lombard et al. [21] reported that the epithet *Pseudonectria diparietispora* (1957) pre-dates that of *Penicillifer furcatus* (1991), and the basionyms are provided below.

Penicillifer diparietisporus (J.H. Miller, Giddens & A.A. Foster) Rossman, L. Lombard & Crous, comb. nov.

Basionym: *Pseudonectria diparietispora* J.H. Miller, Giddens & A.A. Foster, Mycologia 49:793. 1957.

≡ *Neocosmospora diparietispora* (J.H. Miller, Giddens & A.A. Foster) Rossman, Samuels & Lowen, Mycologia 85:699. 1993.

≡ *Viridispora diparietispora* (J.H. Miller, Giddens & A.A. Foster) Samuels & Rossman, Stud. Mycol. 42:167. 1999.

= *Neocosmospora arxii Udagawa*, Horie & P. Cannon, Sydowia 41:353. 1989.

= *Neocosmospora endophytica* Polishook, Bills & Rossman, Mycologia 83:798. 1991.

= *Penicillifer furcatus* Polishook, Bills & Rossman, Mycologia 83:798. 1991.

Molecular phylogeny of the strain KNU16-010

Genetic sequences of the ITS regions and TEF1-α were analyzed to determine the evolutionary relationships (Fig. 3) with the strains obtained from GenBank (Table 2). After analyzing the nucleotide sequences, 523 bp and 438 bp sequences were obtained from the ITS regions and TEF1-α, respectively. The BLAST results showed 100% similarity with *Penicillifer diparietisporus* (CBS 376.59) and 99% with *Penicillifer diparietisporus* (KM231861) in the ITS regions and TEF1-α, respectively. The combined BLAST results suggested that the strain KNU16-010 showed 100% similarity with the strain *Viridispora diparietispora* (= *Penicillifer diparietisporus*) (CBS 102797) and *Penicillifer diparietisporus* (CBS 376.59, NR154310). The phylogenetic tree was constructed on the basis of the ITS regions and TEF1-α to the strain *Penicillifer diparietisporus* [21]. The strain KNU16-010 was closely clustered with *Penicillifer diparietisporus* (CBS 376.59), with a 100% bootstrap value (Fig. 3). The phylogenetic results support the fact that the strain KNU16-010 is *Penicillifer diparietisporus*.

Members of the family Nectriaceae are pleomorphic fungi that display both asexual and sexual morphs during their life cycles [22]. In this study, we used molecular analysis, which provides a considerable challenge to conventional fungal systematics. Thus, more genomic studies of members of the family Nectriaceae are urgently required. To our knowledge, this is the first report of *Penicillifer diparietisporus* isolated from field soil in Korea.

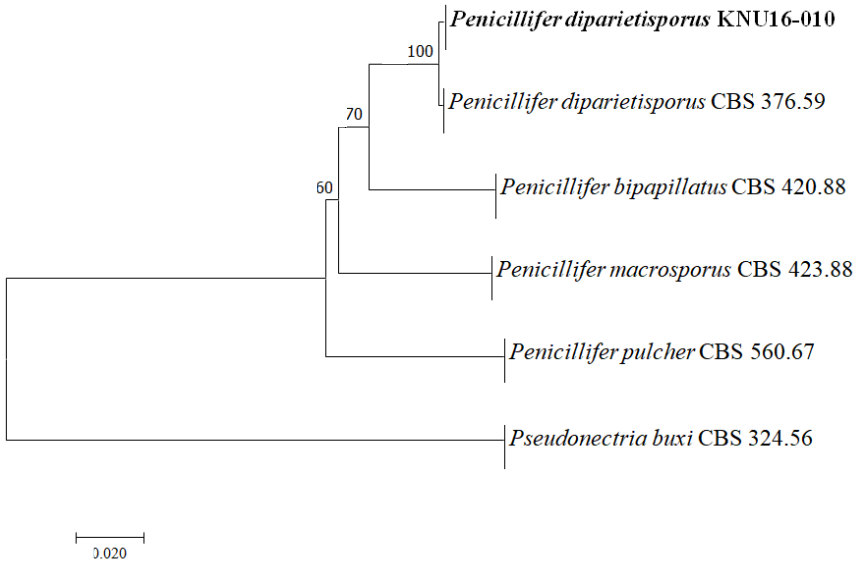

Fig. 3. Neighbor-joining phylogenetic tree based on the combined internal transcribed spacer sequences and translation elongation factor 1-α. *Pseudonectria buxi* CBS 324.56 was used as an outgroup. The strain isolated in this study is indicated in bold, and the bootstrap values are based on 1,000 replications. Bar, 0.02 substitutions per nucleotide position.

Table 2. GenBank accession numbers used in this study for the phylogenetic analyses

Species	Strains	GenBankaccession numbers	
		ITS	TEF
Penicillifer bipapillatus	CBS 420.88	KM231740	KM231860
Penicillifer diparietisporus	CBS 376.59	NR 154310	KM231861
Penicillifer macrosporus	CBS 423.88	KM231739	KM231859
Penicillifer pulcher	CBS 560.67	KM231742	KM231862
Penicillifer diparietisporus	KNU16-010	LC387549	LC387601
Pseudonectria buxi	CBS 324.56	KM231778	KM231909

ITS, internal transcribed spacer; TEF, translation elongation factor.

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea for the project on survey and discovery of indigenous fungal species.

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