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A New Record of *Pseudeurotium bakeri* from Crop Field Soil in Korea

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ABSTRACT : *Pseudeurotium bakeri* KNU14-21 isolated from crop field soil is a previously unreported fungal species in Korea. This fungal species was identified through morphological and molecular characteristics. Based on the shape and size of conidia and conidiophores as well as the internal transcribed spacer region of rDNA, the isolate was confirmed to be *Pseudeurotium bakeri*. This species has not been previously reported from Korea, and this is the first report of its incidence in Korea.

KEYWORDS : Characteristics, Morphology, *Pseudeurotium bakeri*, Species

Introduction

Pseudeurotium bakeri is an ascomycetous fungus belonging to the *Pseudeurotiaceae* family. The general features of this fungal species include having hyaline or brown ascospores in a thin walled ascus [1]. *Pseudeurotium bakeri* has been reported as an endophytic fungus having a cosmopolitan distribution, and can survive in high concentrations of diesel fuel in soil [2]. The conidial phase of *P. bakeri* has been noted as *sporothrix*, *beauvaria*, or *acremonium*-like structures [3].

Soil fungi play vital ecological roles as decomposers, pathogens, and symbionts [4]. Fungi have a cosmopolitan distribution and grow in a vast area of habitats. Despite their ecological importance, little is known about their distribution and biodiversity. Therefore, we collected fungal strains to discover the diversity of fungi in the crop field soil from Suncheon-si, Jeollanam-do, Korea. Among the fungal isolates, *Pseudeurotium bakeri*, which has not been reported officially from Korea, was encountered. The purpose of this study was to describe the isolated fungal species of *Pseudeurotium* by morphologically comparing it with previously described *Pseudeurotium* spp.

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Materials and Methods

Sampling of soil and fungal isolation

Soil samples were obtained from different areas of Suncheon-si, Jeollanam-do, Korea. Each soil sample was taken at a depth of 1~15 cm. All the collected soil samples were stored in plastic bags and stored at 4°C. The fungi were isolated by the conventional dilution method [5] and cultured on potato dextrose agar (PDA; Difco, Detroit, MI, USA) supplemented with 100 μg chloramphenicol (bacteriostatic agent) per liter of PDA for 5~7 days at 26°C. For further use, pure cultures of PDA slants were maintained at 4°C at the plant microbiology and biotechnology lab of Kangwon National University.

Morphological examination

Morphological features of the isolate were observed in PDA by performing one point inoculation in 9 cm petri plates. Inoculated plates were incubated in the dark at 26°C. The morphological characteristics were observed

Table 1. Morphological comparison between the studied isolates and the previously described isolate of *Pseudeurotium bakeri*

Characters		Present isolate *Pseudeurotium bakeri*	*P. bakeri*[a]
Colony	color	White in back and light yellow in front side	NA
	growth pattern	Colonies on PDA are moderate in growth, irregular	NA
Conidia	size	4.8~5.5 × 3.0~3.2 μm	5.0~5.7 × 3.2~3.5 μm
	shape	Subglobose or ellipsoid	Glabrous,subglobose, ellipsoid or obvoid
	conidial head	Loosely columnar	Loosely columnar
Conidiophores	structure	Aerial or substrate mycelium	Aerial or substrate mycelium
Terminal conidiogenous cells		9~21 μm, lageniform	10~22 μm, lageniform

NA, Not Available; PDA, potato dextrose agar.
[a] Source of description [10].

by using a differential contrast microscope. The color, size, and shape of the microstructures of the isolate were observed by using an HK3.1 CMOS digital camera (KOPTIC, Seoul, Korea) attached to an Olympus BX50F-3 microscope (Olympus, Tokyo, Japan). Scanning electronic microscopy images of the conidia and conidiophores were taken by a LEO Model 1450VP Variable Pressure (SEM; Carl Zeiss, Oberkochen, Germany).

DNA extraction, PCR amplification, sequencing and data analysis

Genomic DNA was extracted from fully grown mycelia on PDA media. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. For amplification of the internal transcribed spacer (ITS) gene region, primers ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [6] were used. The amplified PCR products were sequenced using an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were compared with reference ITS sequences deposited in GenBank at the National Center for Biotechnology Information (NCBI) using the basic local alignment search tool (BLAST) [7]. The nucleotide sequence obtained from the isolate KNU14-21 was deposited in GenBank and was assigned an accession number KP055601. The isolates used for the construction of the phylogenetic tree are shown in Table 2. The neighbor-joining relationship was analyzed using MEGA 6.0 [8]. A neighbor-joining tree was constructed using the Kimura 2-parameter substitution model [9]. The branch robustness was tested by 1,000 replicates of bootstrap analysis.

Results and Discussion

Morphological features of the isolate KNU14-21 assessed by optical and scanning electron microscopes are shown in Fig. 1. The colony grew moderately, attaining a diameter of 25~32 mm. The isolate had a smooth colony surface. The colony was white in color at the backside and light yellow on the front side. The center of the isolate was slightly powdery (Fig. 1A, 1B). The conidiophores were produced from the aerial or substrate mycelium (Fig. 1F). Short side-branches of the conidiophores were observed (23~57 μm in length) (Fig. 1G). Conidia were subglobose or ellipsoid (Fig. 1E). The size of the conidia was 4.8~5.5 × 3.0~3.2 μm (Table 1). Conidia were present in a chain-like structure (Fig. 1H). The conidial head was loosely columnar. The terminal conidiogenous cells were 9~21 μm in size and lageniform. The morphological description of our study isolate reasonably fits with the description of *P. bakeri* [10]. Formation of conidia and conidiophores by the isolate is similar to that of the *Teberdinia* state of *P. bakeri* reported by Sogonov et al. [10].

To confirm the identification, molecular analysis was carried out. ITS gene region was used for determination of the phylogenetic relationship with the previously described *Pseudeurotium* species. Our results showed that the isolate is most closely related to *P. bakeri* (CBS878.71), with a bootstrap value of 99% (Fig. 2). Additional sequence similarity analysis of the ITS region using BLAST search also supported that KNU14-21 is *P. bakeri* (Table 3). Although this is a common fungal species with a cosmopolitan distribution, this is the first report of its incidence in Korea.

Since *Pseudeurotium bakeri* has been reported as a

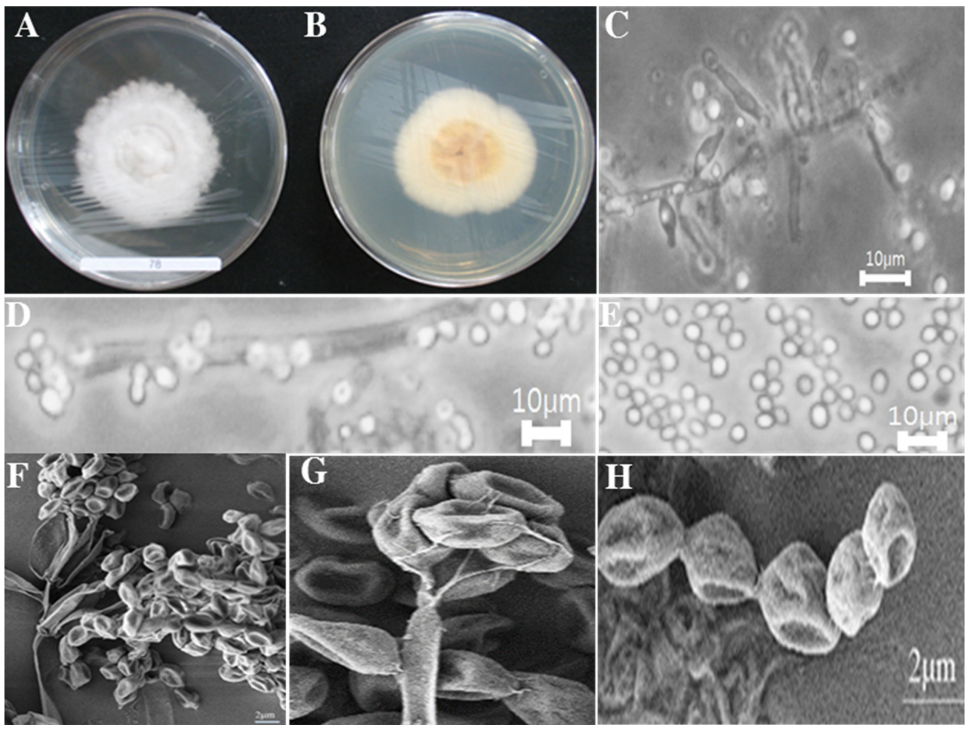

Fig. 1. Morphological characteristics of *Pseudeurotium bakeri* KNU14-21 grown for 7 days on potato dextrose agar. A, reverse view; B, obverse view; C, D, Simple microscopy picture of conidiophores; E, Simple microscopy picture of conidia; F, G, Scanning electronic microscopic pictures of conidiophores; H, Scanning electronic microscopic pictures of conidia.

Table 2. Sequences of *Pseudeurotium* and allied species used in this study with their GenBank accession numbers

Serial No.	Species	Isolate No.	GenBank accession No.
1	*Pseudeurotium hygrophilum*	CBS 102670	NR111128
2	*Teberdinia hygrophila*	CBS 102671	AY129292
3	*Pseudeurotium ovale var. ovale*	CBS 389.54	AY129289
4	*Pseudeurotium bakeri*	CBS 878.71	AY129287
5	*Pseudeurotium zonatum*	CBS 329.36	NR111127
6	*Thelebolus globosus*	CBS 113940	NR138367
7	*Pseudeurotium desertorum*	CBS 986.72	AY129288
8	*Collophora rubra*	CBS120873	NR119747
9	*Collophora capensis*	CBS120879	NR137726
10	*Collophora hispanica*	CBS 128568	NR111680
11	*Pseudofabraea citricarpa*	CBS 130297	KR859279
12	*Phialocephala virens*	CBS 452.92	NR103564
13	*Phacidium pseudophacidioides*	CBS 590.69	KJ663853
14	*Phacidium grevilleae*	CBS139892	NR137977
15	*Allantophomopsis lunata*	CBS137781	NR132922
16	*Geomyces auratus*	CBS 108.14	NR11187
17	*Pseudogymnoascus destructans*	ATCC MYA-4855	NR111838
18	*Pseudogymnoascus appendiculatus*	UAMH 10509	NR137875
19	*Gymnostellatospora frigida*	UAMH 9304	NR111200
20	*Gymnostellatospora alpina*	UAMH 9430	NR111201

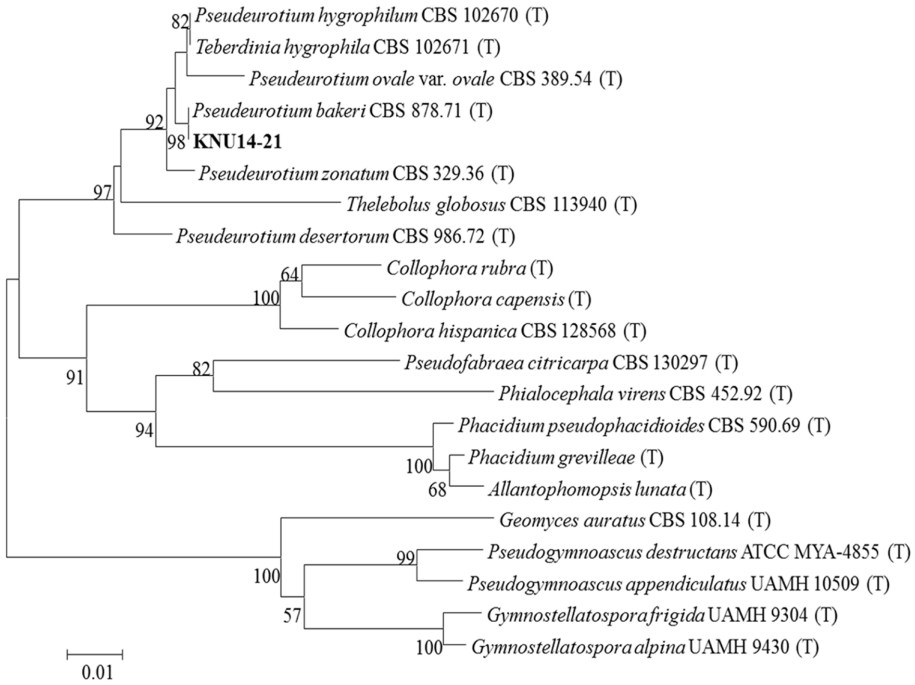

Fig. 2. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-28S rDNA sequences of *Pseudeurotium bakeri* KNU14-21 obtained from crop field soil in Korea. The phylogenetic tree was constructed using the MEGA 6 program. The sequence obtained in the study is shown in boldface. The mark (T) indicates type strain. Numerical values (> 50) on branches are the bootstrap values as percentage of bootstrap replication from a 1,000 replicate analysis. The scale bar represents the number of substitutions per site.

Table 3. Identification of fungal isolate to species level with reference species based on the analyses of internal transcribed spacer gene sequence

Gene	NIBR No.	GenBank accession No.	GenBank library strain	Sequence similarity (%)
ITS rDNA	NIBRFG0000142402	KP055601	*Pseudeurotium bakeri* (the present isolate)	98
	CBS878.71	AY129288	*Pseudeurotium bakeri* (the closest isolate)	

ITS, internal transcribed spacer.

fungal species resistant to biodiesel contaminated soil [2], this finding has important biotechnological implications. However, the main purpose of our present study was limited to identify and describe the isolate morphologically and molecularly. Further studies on its use and implications in the field of biotechnology are thus needed.

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REFERENCES

1. Cannon PF, Kirk PM. Fungal families of the world. Wallingford: CAB International. 2007.
2. Flewelling AJ, Johnson JA, Gray CA. Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Bot Mar 2013:56:287-97.
3. Ferrari BC, Zhang C, van Dorst J. Recovering greater fungal diversity from pristine and diesel fuel contaminated sub-Antarctic soil through cultivation using both a high and a low nutrient media approach. Front Microbiol 2011;2:217.
4. Swift MJ, Heal OW, Anderson JM. Decomposition in terrestrial ecosystems. Berkeley: University of California Press; 1979.
5. Davet P, Rouxel F. Detection and isolation of soil fungi. Enfield: Science Publishers; 2000.
6. White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR

protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
7. National Center for Biotechnology Information. Basic Local Alignment Search Tool [Internet]. Bethesda (MD): National Library of Medicine; 2015 [cited 2016 June 15]. Available from: https://blast.ncbi.nlm.nih.gov/Blast.cgi.
8. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725-9.
9. Kimura MA. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980;16:111-20.
10. Sogonov MV, Schroers HJ, Gams W, Dijksterhuis J, Summerbell RC. The hyphomycete *Teberdinia hygrophila* gen. nov., sp. nov. and related anamorphs of *Pseudeurotium* species. Mycologia 2005;97:695-709.