RESEARCH ARTICLE

A New Record of *Epicoccum draconis* Isolated from the Soil in Korea

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ABSTRACT

A fungal isolate US-18-11 was isolated from the soil in Uiseong, Korea. The mycelium growth measured after 7 days of incubation at 22°C on malt extract agar (MEA) and oatmeal agar (OA) media was 42-43 mm and 41-44 mm in diameter, respectively. The fungal colony formed white to dull green aerial mycelia that were floccose with regular margins and olivaceous black with leaden gray patches on the reverse side. The conidia were hyaline to brown in color, ellipsoidal to ovoid, guttulate, abundant, globose, solitary, or confluent measuring 3.2-7.2×1.1-2.3 µm. A BLAST search of the large subunit (LSU), internal transcribed spacer (ITS) region, second largest subunit of DNA-directed RNA polymerase II (RPB2) and β-tubulin (TUB2) gene sequences revealed that the isolate US-18-11 has similarities of 99, 100, 97, and 99% with those of *Epicoccum draconis* CBS 186.83, respectively. A neighbor-joining phylogenetic tree constructed based on the concatenated dataset of above-mentioned sequences showed that isolate US-18-11 clustered with *Epicoccum draconis* CBS 186.83 in the same clade. Based on the results of morphological, cultural, and phylogenetic analysis, the isolate US-18-11 was identical to the previously described *E. draconis* CBS 186.83. To our knowledge, this is the first report of *E. draconis* in Korea.

Keywords: Dothideomycetes, *Epicoccum draconis,* Morphological characteristic, Phylogeny

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2020 March, 48(1): 39-45
https://doi.org/10.4489/KJM.20200004

Received: January 15, 2020
Revised: March 18, 2020
Accepted: March 23, 2020

INTRODUCTION

The genus *Phoma,* since it was first identified over 170 years ago, has been considered one of the largest fungal genera with over 3,000 infrageneric taxa [1,2]. Moreover, it is very well-known because it includes several fungal pathogens associated with plants. Most species in this genus cause mainly leaf and stem spots on plants and usually live in the environment as saprobic soil organisms, but become pathogenic when the conditions are suitable [3,4]. In addition, other species of the genus *Phoma* are pathogenic to humans, cattle, and fish [5,6]. They are also responsible for production of toxic secondary metabolites that may indirectly affect human and animal health [7]. *Epicoccum draconis* (syn. *Phyllosticta draconis*, *Phoma draconis*) was first acknowledged by Chen et al. [8]. It is distributed worldwide as pathogenic fungi causing diseases in

plants and can thrive in the soil, air, animals, and humans [9,10]. *E. draconis* was first reported in Rwanda, Africa, from the wild *Dracaena* spp. and later found in the cultivated species of the *Dracaena* in Europe, India, and North America. This species is known as causing agent of leaf spot and dieback in *Cordyline* spp. [11]. The main objective of the study was to study the morphological and molecular characteristics of the isolate US-18-11 and compare it with the previously reported *E. draconis*.

MATERIALS AND METHODS

Soil sample collection and fungal isolation

Soil samples were collected from a tobacco (*Nicotiana tabacum* L.) field in Uiseong (N36°25'14.7", E128° 45'34.1"), Korea. The soil sample was collected whenever all the tobacco were harvested. A sterile spatula was used to collect the soil sample at a depth of 15-30 cm. The soil sample was placed in a polythene zipper bag, transferred to the laboratory, and stored at 4°C until analysis. For analysis, one gram of the soil was suspended in 10 mL of sterile distilled water. The suspension was vortexed, serially diluted, and spread on potato dextrose agar (PDA; Difco, MI, USA). Individual colonies that developed on the agar were purified by subculture on fresh PDA and incubated at 25°C until mycelium development. The pure cultures were preserved on PDA slants at 4°C. The isolate US-18-11 was selected for subsequent morphological and molecular analysis.

Morphological and cultural characterization

The colony characteristics of isolate US-18-11 such as color, shape and size were recorded after 7 days of incubation at 22°C [12] on malt extract agar (MEA; Difco, MI, USA) and oatmeal agar (OA; Difco, MI, USA) while morphological characteristics were studied after 14 days. A light microscope (BX-50 light microscope-Olympus, Tokyo, Japan) was used to observe the morphological characteristics of the isolate.

Genomic DNA extraction, PCR amplification and phylogenetic analysis

For molecular identification based on multiple gene sequences, we extracted the genomic DNA of the isolate US-18-11 from fungal mycelium growing on MEA medium following the manufacturer's instructions for the HiGene Genomic DNA prep kit (BIOFACT, Daejeon, Korea). PCR amplification was performed to amplify four molecular markers. The primers ITS1F and ITS4 for the internal transcribed spacer (ITS) region, LROR and LR7 for the 28S rDNA (LSU) gene, Btub2Fd and Btub4Rd for the partial beta-tubulin (TUB2) gene and *rpb2*-5F2 and *rpb2*AM-7cR for the second largest subunit of DNA-directed RNA polymerase II (RPB2) gene region were used for the amplification [13-19]. Then, the amplified PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced (Macrogen, Daejeon, Korea). The similarities of obtained sequences were analyzed using basic local alignment search tool (BLAST) in the National Center for Biotechnology Information (NCBI) and Genetyx-Win ver. 10.0 (Genetyx Corporation, Tokyo, Japan). The partial sequences of ITS region,

LSU, RPB2 and TUB2 genes of allied species of *E. draconis* were retrieved from NCBI to perform the phylogenetic analysis (Table 1). The obtained sequences of US-18-11 were deposited in NCBI GenBank under the accession number of LC496555 for ITS region, LC500288 for LSU, LC500353 for RPB2 and LC500354 for TUB2 genes. The sequences of our isolate were compared with reference sequences from the NCBI GenBank database by using BLAST. The fungal strains used for a construction of the phylogenetic tree are summarized in Table 1. Based on the Kimura's neighbor-joining algorithm model, the evolutionary distance matrices were generated using the program MEGA 7 with bootstrap values based on 1,000 replications [20].

Table 1. GenBank accession numbers of fungal strains used for phylogenetic analyses.

Species name	Strain number	GenBank assession numbers			
		LSU	ITS	RPB2	TUB2
Epicoccum brasiliense	CBS 120105	GU238049	GU237760	KT389627	GU237588
E. draconis	CBS 186.83	GU238070	GU237795	KT389628	GU237607
E. draconis	**NIBRFGC000502241**	**LC500288**	**LC496555**	**LC500353**	**LC500354**
E. henningsii	CBS 104.80	GU238081	GU237731	KT389629	GU237612
E. huancayense	CBS 105.80	GU238084	GU237732	KT389630	GU237615
E. nigrum	CBS 125.82	GU237974	FJ426995	KT389631	FJ427106
E. nigrum	CBS 173.73	GU237975	FJ426996	KT389632	FJ427107
E. pimprinum	PD 771028	GU237977	FJ427050	KT389633	FJ427160
E. plurivorum	CBS 558.81	GU238132	GU237888	KT389634	GU237647
E. sorghinum	CBS 179.80	GU237978	FJ427067	KT389635	FJ427173
E. sorghinum	CBS 627.68	GU237979	FJ427072	KT389636	FJ427178
Paraboeremia adianticola	CBS 187.83	GU238035	GU237796	KP330401	GU237576

LSU, 28S rDNA; ITS, internal transcribed spacer region; TUB2, beta-tubulin gene; RPB2, RNA polymerase II gene.
The newly generated sequences are indicated in bold.

RESULTS AND DISCUSSION

Morphology of the isolate US-18-11

The isolate US-18-11 was observed on MEA, and OA media after 14 days at 22°C. The diameters of the colonies on MEA and OA were 42-43 mm and 41-44 mm, respectively. These characteristics were compared with those of previously described *E. draconis* CBS 186.83 (Table 2). On MEA, colonies formed white-to-dull green aerial mycelium, floccose texture, and regular margins and appeared olivaceous black with leaden grey patches on the reverse side. On OA, colonies showed regular margins with white-to-grey olivaceous aerial mycelium, floccose texture, and were tufted (Fig. 1A-D). Morphological characters were described after 14 days of incubation (Fig. 1E-H). Hyphae were hyaline-to-light brown, thin-walled, smooth, and septate. Pycnidia were immersed, globose to subglobose or obpyriform, and pale brown-to-dark brown with a size of 167-173 μm. Conidia were hyaline-to-brown in color, ellipsoidal to ovoid, and guttulate conidia were abundant, globose, solitary, or confluent with a size of 3.2-7.2×1.1-2.3 μm (n=50). Comparing to *E. draconis* CBS 186.83, the colony on MEA was dull green-to-grey olivaceous, greenish, with olivaceous margins, and the reverse side was olivaceous black with leaden grey patches with olivaceous to greenish olivaceous margins.

Table 2. Comparison of morphological characteristics of the isolate US-18-11 with the reference strain of *Epicoccum draconis*.

Characteristics		*Epicoccum draconis* US-18-11[a]	*Epicoccum draconis* CBS 186.83[b]
Colony	Color	MEA: Colony showed white to dull green aerial mycelium, floccose and regular margins and olivaceous black with leaden grey patches in reverse. OA: Colony showed regular margins with white to grey olivaceous aerial mycelium	MEA: Colony dull green to grey olivaceous, greenish olivacous margins, reverse olivaceous black with leaden grey patches with ochraceous to greenish olivaceous margins. OA: Colony colorless to rosy buff honey, greenish margins.
	Size	MEA: 42-43 mm, OA: 41-44 mm after 7 days	MEA: 45-53 mm, OA: 50-59 mm after 7 days
	Shape	MEA: Colonies showed regular margins	OA: Colony colorless to rosy buff honey, greenish margins.
Conidia	Size	3.2-7.2×1.1-2.3 µm	4.0-8.5×2.0-4.0 µm
	Shape	Ovoid, ellipsoidal, globose and guttulate	Ovoid, ellipsoidal to oblong, sub-cylindrical, epicoccoid conidia
Pycnidia	Shape and color	Pycnidia globose to subglobose, brown to dark brown	Pycnidia immersed, globose to subglobose or obpyriform, pale brown to dark brown
	Size	167-173 µm in diameter	90-220 µm in diameter

[a] Fungal isolate studied in this paper, [b]Source of description [11].

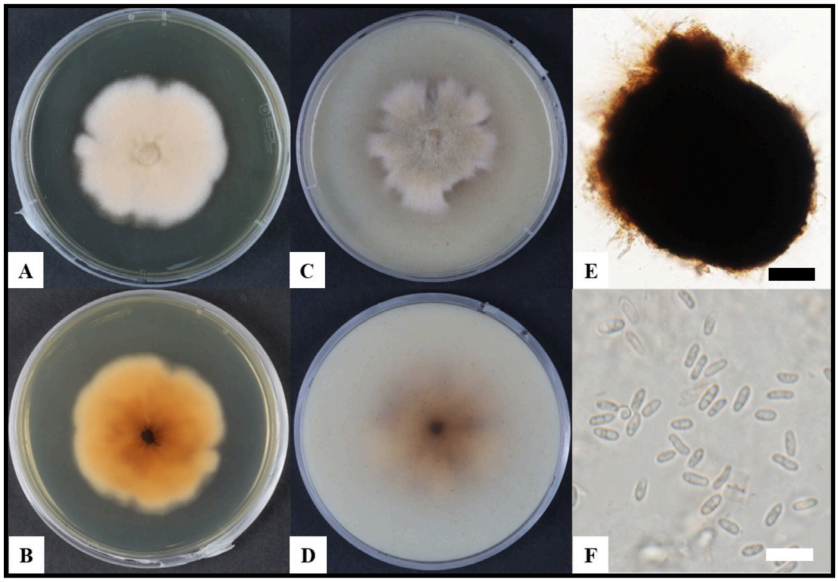

Fig. 1. Cultural and morphological characteristics of US-18-11. A, B, colony on malt extract agar, front and reverse sides, respectively; C, D, colony on oatmeal agar, front and reverse sides, respectively; E, pycnidium; F, conidia. Scale bar G=50 µm; H=10 µm.

Molecular phylogeny of the isolate US-18-11

A phylogenetic tree of the isolate US-18-11 was constructed to determine the phylogenetic relation with its allied species. *Amplification of the* LSU, ITS, RPB2 and TUB2 loci yielded fragments of 1,268, 501, 884, and 830 bp, respectively. The BLAST search results revealed that the LSU, ITS region, RPB2 and TUB2 showed 99.7, 100, 97.1 and 99.3% similarities to that of *E. draconis* CBS 186.83 (GU238070, GU237795, KT389628 and GU237607). To check the phylogenetic relationship, the LSU, ITS, RPB2 and TUB2 sequences of allied *Epicoccum* spp. were retrieved from GenBank (Table 1). The phylogenetic tree based on a concatenated dataset of LSU, ITS, RPB2 and TUB2 sequences revealed that the isolate

US-18-11 clustered in the same clade with *E. draconis* CBS 186.83 (Fig. 2). The isolate US-18-11 was deposited at the National Institute of Biological Resources (NIBRFGC000502241). Currently, several species belonging to the genus *Epicoccum* have been reported as quarantine pathogens and concern a lot of serious problems to organizations that are involved in the plant health quarantine activities. According to Animal and Plant Quarantine Agency in Korea, the pathogen *Phoma draconis* (syn. *E. draconis*, *Phyllosticta draconis*) has been regulated as a quarantine pathogen causing leaf spot diseases of plants [21]. *P. andigena* and *P. tracheiphila* are considered as quarantine organisms in the European and Mediterranean regions [22], respectively, as well as *P. exigua* var. *foveata* in Southern America [23] and *P. macdonaldii* in Australia [24]. On the other side, some *Phoma* species are regarded as very helpful by acting as biocontrol agents of weeds. The *Phoma* species such *P. macrostoma* and *P. herbarum* are very effective in a control of broadleaf weeds [25]. In this study, *E. draconis* KNU-1811 was also isolated from a tobacco cultivated soil in Korea. Further researches on *Epicoccum* species have proved to affect vital aspects of the agricultural field and therefore a further research needs to be done to deepen our understanding of this fungal species.

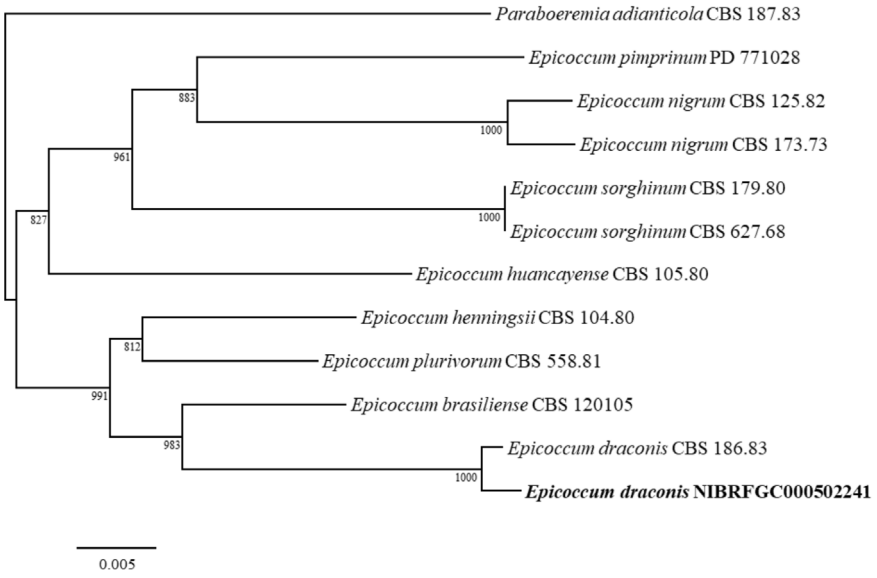

Fig. 2. Neighbor-joining phylogenetic tree based on the concatenated LSU, ITS, TUB2 and RPB2 gene sequences. The strain isolated in this study is shown in bold. Bootstrap values (based on 1,000 replications) higher than 70% are shown at the branch points. *Paraboeremia adianticola* CBS 187.83 was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. LSU, 28S rDNA; ITS, internal transcribed spacer regions; TUB2, beta-tubulin gene; RPB2, RNA polymerase II gene.

ACKNOWLEDGMENTS

This research was supported by the project on the survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR 201801105) under the Ministry of Environment, Republic of Korea.

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