RESEARCH ARTICLE

First Report of *Plectosphaerella sinensis* Isolated from Soil in Korea

Ally Hassan Wajihi[1], Hyo-Won Choi[2], Seung-Yeol Lee[1], Hee-Young Jung[1]*

[1]School of Applied Biosciences, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea

[2]Crop Protection Division, National Institute of Agricultural Sciences, Wanju 55365, Korea

*Corresponding author: heeyoung@knu.ac.kr

ABSTRACT

A fungal isolate denoted NC14-264 was isolated from the soil in Jeongeup, Jeollabuk-do, Korea. Species in genus *Plectosphaerella* are pathogenic to several plant species, leading to fruit, root, and collar rot and collapse. In this study, a strain NC14-264 belonging to the *Plectosphaerella* was isolated from the soil and identified. Colonies were moderately grown, reaching 54 mm in diameter on potato dextrose agar, 49 mm on malt extract agar, and 55 mm on oatmeal agar at 25°C after 10 days of incubation. Most *Plectosphaerella* species are distinguishable morphologically by irregular chlamydospores and different proportions of phialides and conidia. Based on morphological features and phylogenetic analysis using the internal transcribed spacer region and partial 28S rRNA gene sequences, the isolated fungus was identified as *Plectosphaerella sinensis* belonging to the Plectosphaerellaceae. This is the first report of *P. sinensis* in Korea.

Keywords: Plectosphaerellaceae, *Plectosphaerella sinensis*, Soil-inhabiting fungi

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INTRODUCTION

The genus *Plectosphaerella* was established by Klebahn [1] to contain *Plectosphaerella cucumeris* Klebahn isolated from young cucumber plants in Germany. Recently, a new family named Plectosphaerellaceae W. Gams, Summerbell, and Zare has been introduced to include *Plectosphaerella*, *Acrostalagmus*, *Gibellulopsis*, *Musicillium*, and *Verticillium* [2]. *Plectosphaerella* has been recommended as the approved generic name in former publications rather than its anamorphic name *Plectosporium* Palm et al. [3]. Species in the genus *Plectosphaerella* are pathogenic towards several plant species, leading to fruit, root, and collar rot and collapse. Species such as *P. cucumerina* are mainly found in the soil or decayed plant material [4]. However, *P. cucumerina* has been infrequently described as a fungal pathogen on many crops, such as potato, tobacco, tomato, muskmelon, soybean, and pepper [4], or as endophytes inhabiting plant tissues without showing observable symptoms [5].

The asexual states of *Plectosphaerella* species are differentiated in terms of the proportion of

conidial shape and dimensions, ratio of septate conidia, and presence or absence of chlamydospores [6-10] as well as internal transcribed spacer (ITS) region sequence data. However, significant distinction has been observed in the cultural and morphological characteristics between species in the genus *Plectosphaerella* [3, 10]. To describe species in this genus, sequences of the ITS region have been widely used, but large variations were observed in the ITS region of *Plectosphaerella* species [10]. Thus, multi-locus phylogenetic analysis based on the partial 28S rRNA gene, calmodulin, elongation factor 1-α, and β-tubulin gene was conducted to clarify species delimitations in *Plectosphaerella* [11]. In this study, we isolated an undescribed fungal species belonging to the genus *Plectosphaerella*, performed phylogenetic analysis, and described the morphological and cultural characteristics of this undescribed isolate.

MATERIALS AND METHODS

Soil samples collection and fungal strains isolation

In 2017, a soil sample was collected from the field in Jeongeup, Jeollabuk-do, Korea (35°33'08.5"N, 126°52'05.5"E). The collected soil sample was air-dried and stored in a plastic bag at 4℃ prior to use. A conventional dilution planting technique was applied to isolate the fungus [12]. One gram of soil sample was mixed with 10 mL of sterile distilled water. The suspension was vortexed and diluted, and a serially diluted sample was prepared. Subsequently, 100 μL of suspension was spread onto potato dextrose agar (Difco, Detroit, MI, USA).

Morphological observations

To examine the growth rate of the fungal colonies, the petri dishes were incubated at 25℃ for 5 days. After 2~3 days, fungal colonies were isolated, and NC14-264 was selected among the isolated strains for subsequent analysis. To evaluate cultural and morphological characteristics, the fungal strain was incubated on potato dextrose agar (PDA), 2% malt extract agar (MEA), and oatmeal agar (OA) at 25℃ for 10 days. Morphological structures were observed on OA media, measured, and photographed after 10 days under a light microscope (BX-50; Olympus, Tokyo, Japan). The NC14-264 isolate was deposited at the National Institute of Biological Resources as ZEVCFG0000000095.

DNA extraction, PCR amplification and Sequencing

On the PDA plate, total genomic DNA was extracted from the mycelia of a fungal strain NC14-264 using the HiGene Genomic DNA Prep kit (BIOFACT, Daejeon, Korea) according to the manufacturer's instructions. The primers LROR and LR5 [13] were used to amplify the partial 28S rRNA gene, and the ITS1F/ITS4 [14, 15] primer pair was used to amplify ITS region sequences. Amplifications were performed in an Applied Biosystems PCR machine using the following parameters: 94℃ for 3 min, followed by 35 cycles at 94℃ for 40 sec, an annealing

temperature dependent on the gene amplified (52℃ for large subunit, 54℃ for ITS) for 60 sec, and 72℃ for 120 sec, with a final extension at 72℃ for 10 min [11]. The amplified PCR fragments were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA). Finally, 503- and 566-bp ITS region and partial 28S rRNA gene sequences were obtained from NC14-264 and deposited in NCBI GenBank under accession numbers LC405939 and LC405940, respectively. Reference sequences were obtained from GenBank under the accession numbers shown in Table 1.

Phylogenetic analyses

For molecular analysis of this strain, the phylogenetic tree was constructed based on ITS region and partial 28S rRNA gene sequences using the maximum parsimony method by MEGA 6 software with a bootstrap of 1,000 replicates [16]. The identity of the isolate NC14-264 was confirmed by BLAST analysis, revealing 100% similarity to the partial 28S rRNA sequence of *P. sinensis* ACCC 39144 and 100% similarity to the ITS region sequence of *P. sinensis* ACCC 39145.

RESULTS AND DISCUSSION

Phylogenetic results based on the combined ITS gene and partial 28S rRNA gene sequences revealed a clear separation between *P. sinensis* and other *Plectosphaerella* spp. [17]. In the phylogenetic tree, the representative isolate NC14-264 was clustered within a clade containing reference isolates of *Plectosphaerella sinensis* (Fig. 2).

Taxonomic descriptions and microphotographs of morphological structures of the isolate NC14-264 are shown in Table 2 and Fig. 2. Colonies grew moderately, reaching 54 mm in diameter on PDA, 55 mm on OA, and 49 mm on MEA at 25℃ for 10 days of incubation.

Table 1. A list of *Plectosphaerella* strain and reference strains of related species in this study

Species	GenBank accession numbers		
	Collection/Strain no.	ITS	LSU
P. alismatis	CBS 113362	JF780523	KY662261
P. cucumerina	3F24	KY401603	KY399832
P. cucumerina	3F43	KY401605	KY399830
P. delsorboi	CBS 116708	DQ825986	EF543843
P. pauciseptata	Plect 186	HQ238971	HQ239012
P. plurivora	Plect 63	HQ238970	HQ239011
P. plurivora	Plect 32	HQ238969	HQ239010
P. populi	CBS 139624	KR476751	KR476784
P. ramiseptata	Plect 158	HQ238963	HQ239049
P. sinensis	ACCC 39144	KX527889	KX527892
P. sinensis	ZEVCFG0000000095	LC405939	LC405940
Colletotrichum gloeosporioides	CBS 953.97	GQ485605	JN940399

ITS, internal transcribed spacer; LSU, large subunit.

Colonies on OA became buff, plane, and portioned with regular margins (Fig. 1C, 1D). Hyphae were colorless, septate, branched, and commonly 1.7~3.9 μm (mean = 2.4 μm) wide. Conidiophores, formed by hyphal coils or from prostrate hyphae developing on the surface of the media, were occasionally branched and solitary. Conidiogenous cells were phialidic, detached, determinate, and smooth with an indistinct collarette. They were also flask-shaped, normally widened at the bottom, solitary, hyaline-to-subhyaline, and 4.8~15.9 × 2.0~4.1 μm in size (mean = 12.2 × 2.4 μm). Conidia formed in smarmy heads on the upper part of the phialides and were ellipsoid with a slight apiculate base, 0~1-septate, smooth-to-finely coarsened, and 6.1~9.8 × 1.8~4.4 μm in size. Chlamydospores were terminal or intercalary, uneven, thick-walled, and 4.3~10.2 μm in diameter. The morphological and cultural characteristics of NC14-264 matched those of *P. sinensis* ACCC 39144 (Table 2).

Many species in genus *Plectosphaerella* are well-known as not only important pathogens of several plant species, causing fruit, root, and collar rot and collapse [18], but also as endophytes in symptomless plant tissue and soil [5]. *Plectosphaerella* species are distinguished based on the proportion of their conidial shape and dimensions, ratio of septate conidia, and presence or absence of chlamydospores [6-10]. Large differences have been observed in the cultural and morphological characteristics between species in the genus *Plectosphaerella*. [3, 10].

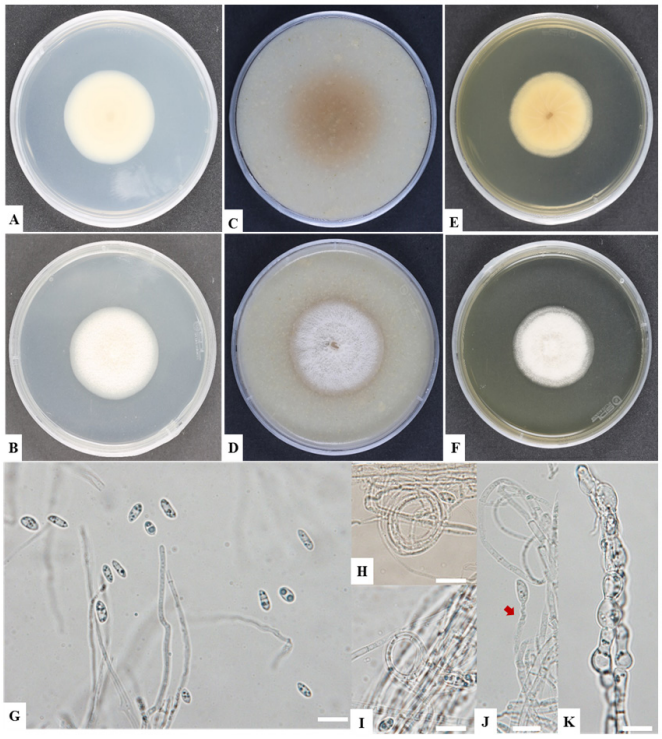

Fig. 1. Cultural and morphological characteristics of *Plectosphaerella sinensis* NC14-264. A, B, Colonies on potato dextrose agar; C, D, Colonies on oatmeal agar; E, F, Colonies on malt extract agar; G, conidia; H, I; Conidiophores from hyphal coils; J, Conidiophores (arrow denotes conidiophore); K, Chlamydospores (scale bars = 10 μm).

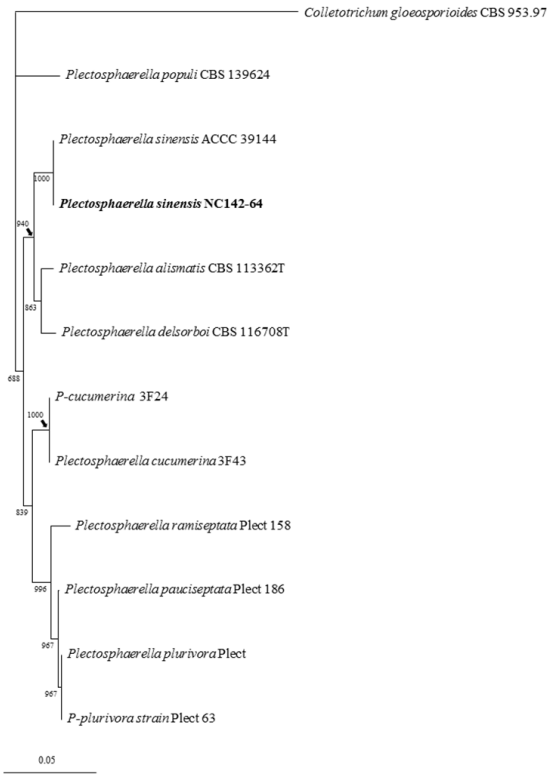

Fig. 2. One of the ten most parsimonious trees generated from maximum parsimony analysis of obtained sequences from the internal transcribed spacer region and the partial 28S rRNA genes. *Colletotrichum gloeosporioides* strain was used as an outgroup, and bootstrap values smaller than 80% were not shown. The fungal isolate used in this study is in bold. The scale bar indicates the number of nucleotide substitutions.

Table 2. Morphological characteristics of isolate NC14-264 compared with reference *Plectosphaerella sinensis*

Characteristics		Study isolate NC14-264[a]	*Plectosphaerella* sinensis [11]
Colony	Size (Diam.)	Colonies slightly growing, attaining 54 mm diam. on PDA, 49 mm on MEA, and 55 mm on OA at 25°C within 10 days of incubation.	Colonies moderately growing, reaching 39 mm diam. on PDA, 29 mm on MEA, and 32 mm on OA at 25°C for 10 days
	Shape and position	Colonies on OA flat, smooth, buff, portioned with regular margin	Colonies on OA buff, plane, lobed with regular margin
Hyphae	Size	1.7~3.9 µm (mean = 2.4µm) wide	2~4 µm (mean = 2.6µm) wide
	Color and septa	Colorless, septate	Hyaline, septate
	Shape and position	Branched	Branched
Conidiophores	Shape and position	Usually branched, emanated from hyphal coils developed on the surface of the media, or from prostate hyphae, solitary	Sometimes branched, produced from prostrate hyphae, or from hyphal coils growing on the agar surface, solitary
Conidia	Size	6.1~9.8×1.8~4.4 (mean = 7.5 × 3.1µm)	6~10×1.5~4 µm (mean = 8 × 2.8µm)
	Shape and position	Formed in smarmy heads on the upper parts of the phialides, ellipsoid having a slight apiculate base, 0~1-septate, smooth to finely coarsened	Formed in slimy heads at the apex of the phialides, ellipsoid with a slightly apiculate base, 0~1-septate, smooth to finely roughened
Chlamydospores	Size (Diam.)	4.3~10.2 µm	5~12 µm
	Shape and position	Terminal or intercalary, thick-walled, uneven	Intercalary or terminal, irregular, thick-walled

Source of description [11].
Diam., diameter; PDA, potato dextrose agar; MEA, malt extract agar; OA, oatmeal agar.
[a]Fungal isolates studied in this paper.

Plectosphaerella species are plant pathogens occupying mainly the plant parts, such as stems, roots, and leaves. However, our study revealed the occurrence and diversification of *P. sinensis* in soil. In a recent study [11], multi-gene phylogenetic analysis of the internal transcribed spacer (ITS) regions, calmodulin (CaM) genes, elongation factor 1-α (EF1) and part of beta-tubulin gene region (TUB) and morphological characteristics have been used to describe well-separated species of *Plectosphaerella*. In this study, however, the ITS region and partial 28S rRNA genes were useful to clearly distinguish among different species in this genus. However, further studies of *Plectosphaerella* species in Korea are needed to fully understand their occurrence, diversification, and characterization.

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