

RESEARCH NOTE

First Report of *Simplicillium cylindrosporum* Isolated from Soil in Korea

Sangkyu Park¹, Seung-Yeol Lee¹, Jae-Jin Lee¹, Hyang Burm Lee², Leonid Ten¹, Hee-Young Jung^{1*}

¹School of Applied Biosciences, Kyungpook National University, Daegu 41566, Korea

²College of Agriculture and Life Sciences, Chonnam National University, Gwangju 61186, Korea

*Corresponding author: heeyoung@knu.ac.kr

Abstract

The fungal strain KNU16-006 was isolated from field soil in Daegu, Korea. The isolate was identified as *Simplicillium cylindrosporum* on the basis of its morphological characterization and phylogenetic analysis using internal transcribed spacer regions of rDNA. This species has not been previously reported in Korea.

Keywords: Cordycipitaceae, *Simplicillium cylindrosporum*, Soil fungi

The genus *Simplicillium* Gams W. & Zare R. was separated from the former *Verticillium* sect. *Prostrata* to accommodate entomogenous and fungicolous verticillium-like anamorphic fungi that mainly produce simple, solitary phialides [1]. Currently, *Simplicillium* belongs to the family Cordycipitaceae and includes approximately ten species [2, 3]. Members of this genus have been isolated from the soil, marine sediment, diseased plant tissues, fungi, nematodes, mushrooms, and human nails worldwide [1-6]. Some species of the genus *Simplicillium*, such as *S. lanosoniveum* and *S. lamellicola*, are parasites of rusts, such as soybean rust, coffee rust, and rust of the evergreen woody shrub *Elaeagnus latifolia*, and can be used as biological control agents [1, 7, 8]. Additionally, *S. lanosoniveum* and *S. lamellicola* cause plant diseases. *S. lanosoniveum* is a causal agent of brown spot on *Salvinia auriculata* and *Sa. molesta* [9] and *S. lamellicola* causes gill mildew and brown spots on *Agaricus bisporus* [1]. Generally, most species in the genus *Simplicillium*, including five recently described taxa, i.e., *S. aogashimaense*, *S. cylindrosporum*, *S. obclavatum*, *S. subtropicum*, and *S. sympodiophorum*, are not pathogenic to plants [2].

While screening for unrecorded fungal species in field soil in Daegu, Korea, a fungal strain, KNU16-006, was isolated. A soil sample (1 g) was suspended in 10 mL of sterile distilled water, and the prepared suspension was vortexed, serially diluted, and spread onto potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. The plates were incubated at 25°C for 3 days. For purification, single colonies on the plates were transferred to new

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plates, followed by incubation on PDA at 25°C. One isolate, KNU16-006, was selected for further morphological and molecular phylogenetic analyses.

The isolate KNU16-006 was subjected to molecular identification based on the internal transcribed spacer (ITS) region. For this purpose, genomic DNA was extracted from the mycelia using the HiGene Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea). The ITS region, including 5.8S, was amplified with the primers ITS1F and ITS4 [10]. PCR amplification was initiated with 2 min of denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 45 sec, and elongation at 72°C for 1 min. Amplification was completed with a final extension at 72°C for 7 min. The amplified PCR products were purified using ExoSAP-IT and sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) to obtain partial ITS region sequences of KNU16-006 consisting of 616 bp. BLAST searches against the GenBank database revealed that the ITS sequence of KNU16-006 has high similarity (i.e., 99.3~99.5%) with the ITS sequences of various strains of *Simplicillium cylindrosporum* (Fig. 1). This relationship was also evident in a phylogenetic tree including the ITS sequences of related *Simplicillium* strains obtained from GenBank. Evolutionary distance matrices were obtained using the neighbor-joining algorithm with Kimura's two-parameter model [11]. Tree topology was inferred using the neighbor-joining method in MEGA7 [12], and branch support was assessed by bootstrapping with 1,000 replicates. The isolate KNU16-006 clustered with seven *S. cylindrosporum* strains in phylogenetic trees constructed based on ITS region sequences, confirming their close relationship at the species level (Fig. 1). The nucleotide sequence of the ITS region of isolate KNU16-006 was deposited in GenBank (accession number LC228053).

The isolate KNU16-006 was cultured at 25°C, and colony characteristics, such as color, shape, and size, were recorded. After 11 days of incubation on a PDA plate, the colony was 5.6~5.7 cm in diameter. The isolate produced a white floccose aerial mycelium and a creamy, slightly brownish pigment at the bottom of the medium (Fig. 2A, 2B). To observe the hyphae and conidia, the conventional slide culture method was applied, with some modifications [13]. A clean PDA block was cut from a fresh PDA plate and placed on a sterilized slide glass in an empty petri dish. A small piece of gauze soaked with sterile water was also placed in the petri dish to provide humidity. The sides of the PDA block were inoculated with the fungus, and a sterilized cover glass was placed on the top of the block. The petri dish with the slide culture was sealed and incubated at 25°C for 14 days. When the fungus grew, the cover glass was carefully detached from the PDA block, placed on a new slide glass, and fixed with 85% lactic acid. The remaining culture on the slide glass was also observed by removing the PDA block and mounting with 85% lactic acid. The morphology of the isolate was examined under an Olympus BX50 light microscope (Tokyo, Japan). Phialides were mainly solitary, arose from aerial hyphae, tapered towards the apex, without basal septum, and their sizes were $16\sim37 \times 1.1\sim1.9 \mu\text{m}$ (Fig. 2C, 2D). Conidia were

produced in small globose or subglobose heads at the apex of the long phialides. They were mostly cylindrical, smooth-walled, and one-celled. Conidia were 2.9–4.8 μm in length and 1.1–1.9 μm in width (Fig. 2E, 2F). As shown in Table 1, these morphological characteristics of isolate KNU16-006 were consistent with those previously reported for *S. cylindrosporum* [2], strongly supporting the results of the phylogenetic analysis of KNU16-001 based on

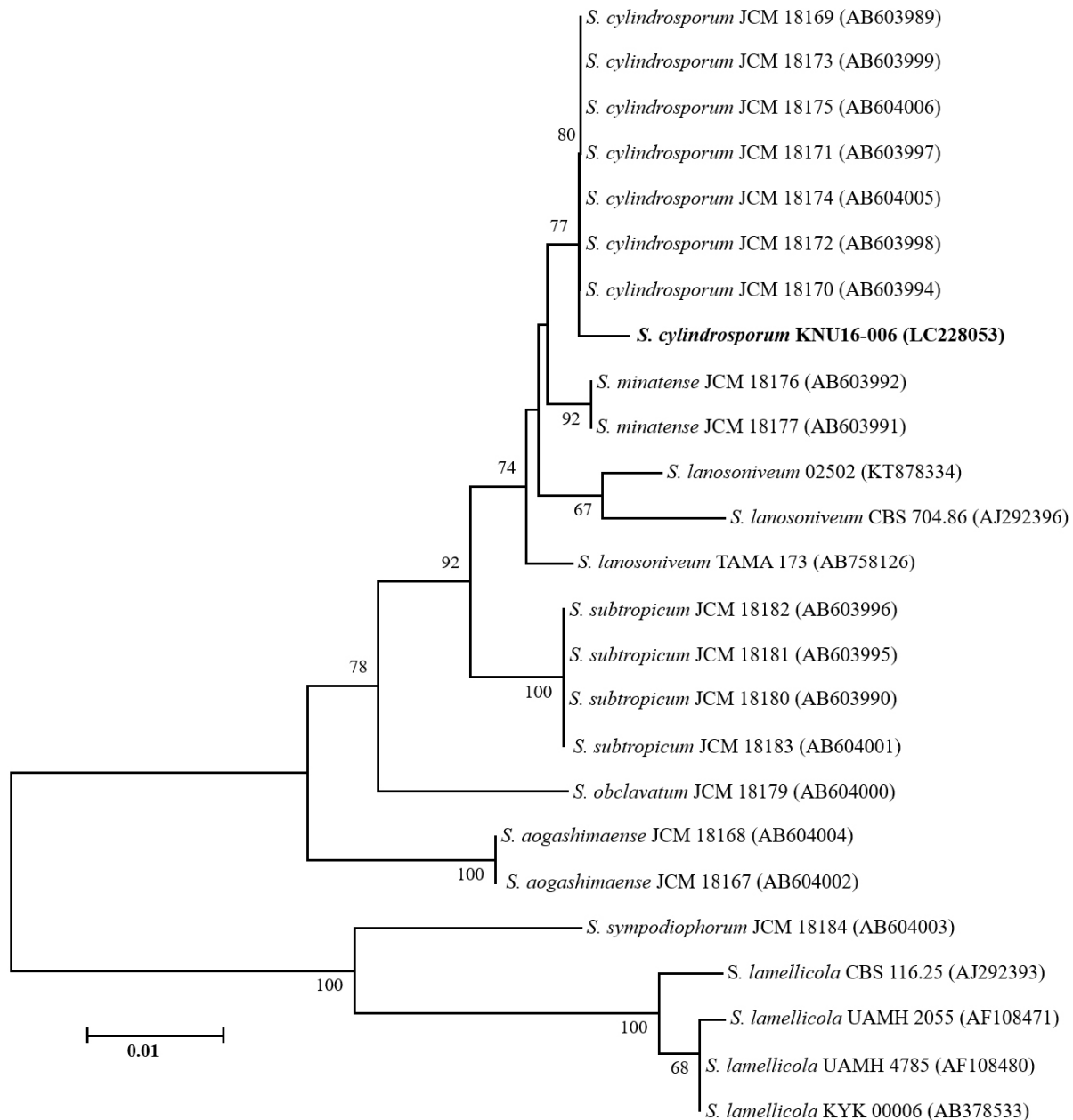


Fig. 1. Neighbor-joining phylogenetic tree based on the internal transcribed spacer region sequences showing the relationships between *Simplicillium cylindrosporum* KNU16-006 and members of the genus *Simplicillium*. The strain isolated in this study is indicated in bold. Accession numbers are shown in parentheses. Bootstrap values (based on 1,000 replications) greater than 60% are shown at the nodes. Bar, 0.01 substitutions per nucleotide position.

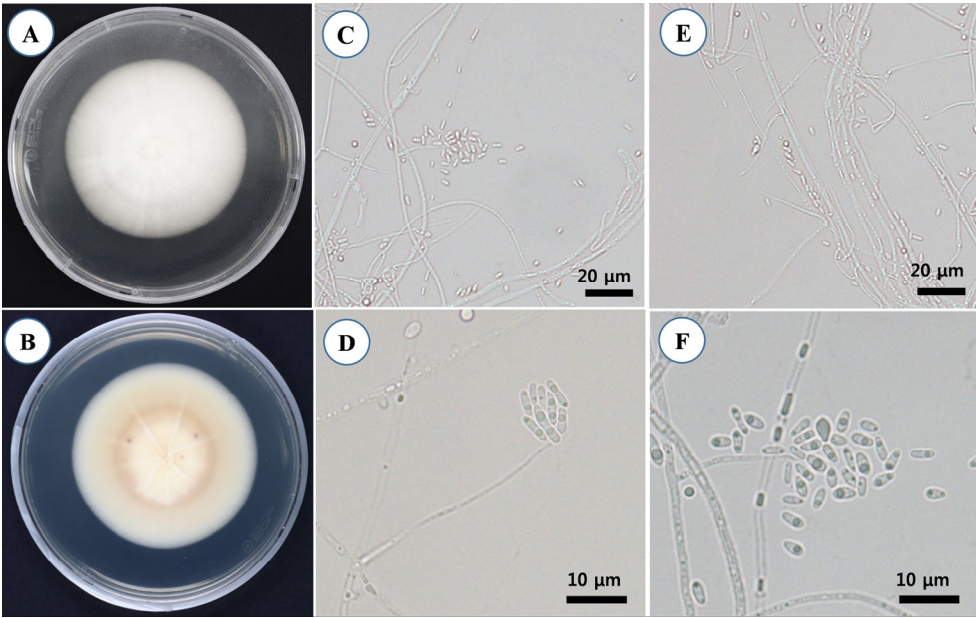


Fig. 2. Morphological characterization of *Simplicillium cylindrosporium* KNU16-006 via light microscopy. A, colony in front; B, colony in reverse; C, D, microscopic images of phialides; E, F, microscopic images of conidia.

ITS sequences. The fungal strain isolated in this study was deposited in the National Institute of Biological Resources (NIBR, <http://www.nibr.go.kr>) for further study (sample no. NIBRFG0000499835).

This is the first report on *S. cylindrosporium* in Korea. Members of the genus *Simplicillium* produce various bioactive compounds, such as cytotoxic linear peptides produced by *S. obclavatum* EIODSF 020 [6], antibacterial mannosyl lipids produced by *S. lamellicola* BCP [14], and antifungal pyrrolidine alkaloids produced by *S. lanosoniveum* TAMA 173 [15].

Table 1. Morphological characteristics of *Simplicillium cylindrosporium* isolated in this study and comparison with those reported previously

Characteristics		<i>Simplicillium cylindrosporium</i> isolated in this study	<i>Simplicillium cylindrosporium</i> ^a
Colony	Color	White, reverse slightly yellowish to brownish yellow	White, reverse slightly yellowish to brownish orange
	Size	5.6~5.7 cm after 11 days on PDA	2.1~2.2 cm after 7 days on PDA
	Shape	Convex, with floccose aerial mycelium, margin entire	Convex, with floccose aerial mycelium, margin entire
Conidia	Size (μm)	2.9~4.5(~4.8) × 1.1~1.9	3.0~4.5(~5.0) × 1.0~2.0
	Shape	Cylindrical, smooth-walled, 1-celled	Cylindrical, smooth-walled, 1-celled
Phialides	Size (μm)	16~37 × 1.1~1.9	17~32 × 1.2~2.0(~2.5)
	Shape	Mainly solitary, slender, arose from aerial hyphae, tapering towards the apex	Mainly solitary, produced on prostrate aerial hyphae, slender, tapering towards the tip

^aSource of description [2].
PDA, potato dextrose agar.

Thus, further studies of *S. cylindrosporum* KNU16-006 as a potential biocontrol agent and producer of bioactive compounds are needed.

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