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RESEARCH NOTE

Sporothrix stylites: A New Record from Field Soil in Korea

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Abstract

The fungal strain, designated KNU16-008, was isolated from field soil in Chungcheongnam-do, Korea. The isolated fungi was characterized by morphological and phylogenetic analyses. Isolated fungus showed typical morphological characteristics of the genus *Sporothrix*. Based on its phylogenic analysis using internal transcribed spacer (ITS) of rDNA and β-tubulin gene sequences, the strain KNU16-008 was identified as *Sporothrix stylites*. This species has not been previously reported in Korea.

Keywords: Phylogenetic analysis, Soil fungi, *Sporothrix stylites*

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The genus *Sporothrix* was established by Hektoen and Perkins [1] who isolated the fungus from infected boy's finger, for which the binomial *Sporothrix schenckii* was introduced. Fungal species that related to the genus *Sporothrix* have a long history of phylogeny and recently summarized by de Beer et al. [2]. As a result, based on phylogenetic analyses of four gene regions they recognized 51 taxa under the genus *Sporothrix*, with six species complexes. Members of the *Sporothrix* originate from a wide variety of environments, including wood, plant debris, peat moss, human clinical materials, but mostly also from soil [2, 3]. Amongst *Sporothrix* taxa there are clinically relevant species such as *S. brasiliensis*, *S. globosa* and *S. mexicana* [4], insect pathogens such as *S. insectorum* [5] as well as saprophytic species such as *S. stylites* and *S. lignivora* [3]. A member of *S. pallida* species complex, *S. stylites*, was isolated from pine pole at soil level and was characterized as producing micro- to semimacronematous, solitary, straight conidiophores and nonseptate, hyaline, smooth, thin-walled conidia [3]. Dark secondary conidia are absent in *S. stylites* therefore its cultures do not darken with age.

During our studies of microbial communities in field soil in Buyeo-gun, Chung-

cheongnam-do, Korea, several fungal strains were isolated. With distinctive morphology, one isolate, KNU16-008, was selected for further morphological study and molecular phylogenetic analysis. Based on its morphological characteristics and phylogenetic analysis, this isolate was identified as *Sporothrix stylites* and named *S. stylites* KNU16-008. To the best of our knowledge this fungus has not been previously reported in Korea.

Collected soil sample (1 g) was suspended in 10 mL of sterile distilled water, and prepared suspension was vortexed, serially diluted, and then spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates. The plates were incubated at 25°C for 3 days. Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an incubation on PDA at 25°C.

During the past decade, species of *Sporothrix* were distinguished mainly by the molecular phylogenetic anlaysis, primarily using the internal transcribed spacer (ITS) sequences of rDNA [3, 6]. Other gene regions such as β-tubulin (BT) [3, 7], calmodulin (CAL) [4] or ribosomal large subunit (LSU) [8] were also used as markers for phylogenetic analysis in *Sporothrix*. In the present phylogenetic analysis, ITS regions and BT gene sequences of related *Sporothrix* taxa were obtained from the GenBank (Table 1). The recovered sequences were aligned with the ITS and BT sequences of isolate KNU16-008 using the program Clustal X. Gaps and 5′ and 3′ ends of the alignments were edited

Table 1. *Sporothrix* and *Ophiostoma* species used in phylogenetic analysis, with the GenBank accession numbers of their ITS regions of rDNA and β-tubulin gene sequences

Species	Strain	ITS	β-Tubulin
Sporothrix stylites	CMW 14544	EF127884	EF139097
Sporothrix stylites	CMW 14541	EF127881	EF139094
Sporothrix brasiliensis	CBS 130106	KC113212	AM116955
Sporothrix brasiliensis	IPEC 17943	FN549902	AM116935
Sporothrix brasiliensis	CBS 130107	KC113214	AM116952
Sporothrix inflata	CMW 12529	AY495428	AY495439
Sporothrix inflata	CMW 12527	AY495426	AY495437
Sporothrix globosa	CBS 130104	KC113225	AM116959
Sporothrix globosa	CBS 130116	KC113226	AM116962
Sporothrix lignivora	CMW 18600	EF127890	EF139104
Sporothrix lignivora	CMW 18597	EF127887	EF139101
Sporothrix schenckii	CBS 130098	KC113215	AM116917
Sporothrix schenckii	CBS 130103	KC113222	AM116915
Sporothrix variecibatus	CMW 23060	DQ821569	DQ821572
Sporothrix variecibatus	CMW 2543	DQ821567	DQ821573
Sporothrix stylites	KNU16-008	MF673228	MF673229
Ophiostoma nigrocarpum	CMW 651	AY280490	AY280480

ITS, internal transcribed spacer.

manually using the BioEdit program. Evolutionary distance matrices for the neighbor-joining algorithm were calculated using Kimura's two-parameter model [9]. Tree topologies were inferred by the neighbour-joining, maximum-likelihood, and maximum-parsimony methods in the program MEGA7 [10], with bootstrap values based on 1,000 replications.

A BLAST search in the NCBI database revealed that ITS regions and BT gene sequences of KNU16-008 matched with those of *Sporothrix stylites* CMW 14544 (EF127884, EF139097) with 99.6 and 100% similarities, respectively. Phylogenetic tree based on the combined ITS rDNA and BT gene sequences confirmed the affiliation of the isolate to *Sporothrix stylites*; KNU16-008 was clustered together with *S. stylites* CMW 14544 and CMW 14541 in a monophyletic clade with the maximum bootstrap value. This phylogenetic relationship was supported by three tree-inferring methods employed in this study (Fig. 1).

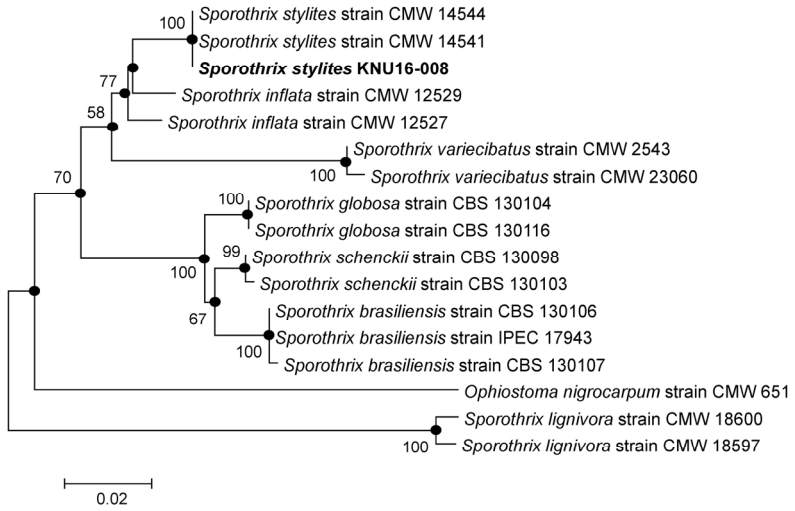

Fig. 1. Phylogenic relationship between *Sporothrix stylites* (KNU16-008) and allied species of *Sporothrix* and *Ophiostoma* taxa, constructed using the neighbor-joining method for the combined internal transcribed spacer (ITS) rDNA and β-tubulin gene sequences. Bootstrap values (based on 1,000 replications) greater than 50% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and the maximum-parsimony algorithms. Bar means 0.02 substitutions per nucleotide position.

Morphology of the isolate was examined under an Olympus CX31 light microscope (Olympus, Tokyo, Japan). The isolate KNU16-008 was cultured at 25°C, and colony characteristics such as color, shape and size were recorded. After 14 days of incubation on PDA agar, colony was 3.4~3.5 cm in diameter, pale yellowish in color, smooth, compact and flat (Fig. 2A, 2B). The colony did not darken with age. Conidia were 2.4~3.2 × 1.3~1.7 μm, nonseptate, hyaline, smooth, oval to fusiform with a pointed base (Fig. 2C, 2D). These morphological characteristics of isolate KNU16-008 are in good agreement with those of

Sporothrix stylites reported by de Meyer et al. [3] (Table 2), which is in line with the phylogenetic results of KNU16-008.

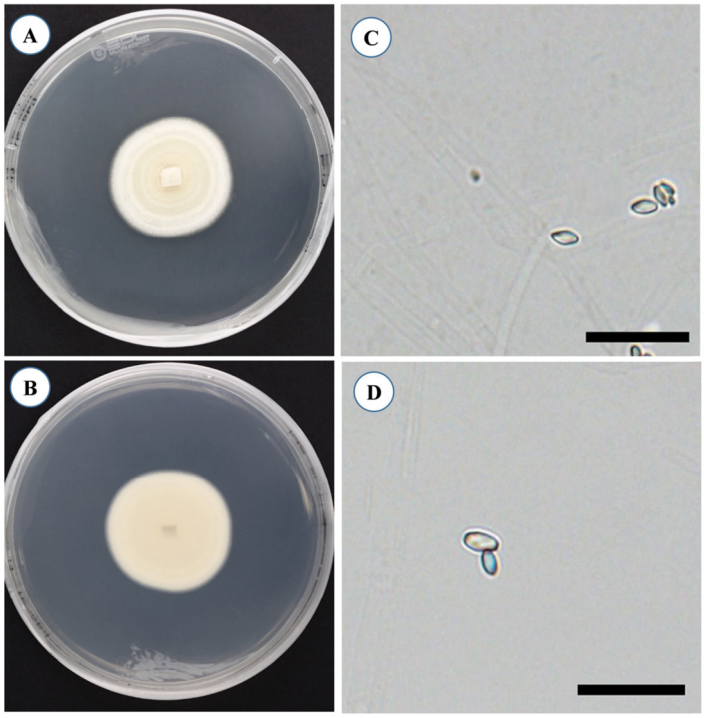

Fig. 2. Cultural and morphological characterization of *Sporothrix stylites* KNU16-008. A, colony in front; B, colony in reverse; C, D, microscopic pictures of conidia (scale bars: C, D = 10 μm).

Table 2. Morphological characteristics of *Sporothrix stylites* isolated in this study

Characteristics		KNU16-008	*Sporothrix stylites*[a]
Colony	Color	pale yellowish	more straw than buff
	Size	34 mm in 14 days on PDA	37 mm in 14 days on 2% MEA
	Texture	smooth, compact, and flat	usually smooth, compact, flat or furrowed brain-like
Conidia	Shape	nonseptate, hyaline, smooth, oval to fusiform with a pointed base	nonseptate, hyaline, smooth and thin-walled, guttiliform to fusiform with a pointed base
	Size	2.4~3.2 × 1.3~1.7 μm	(2~)2.5~4(~5.5) × (1~)1.5~2(~2.5) μm

PDA, potato dextrose agar; MEA, malt extract agar.
[a]Source of description [3].

This is the first report on *Sporothrix stylites* in Korea. The type species of genus *Sporothrix*, *S. schenckii* is known as pathogen of human sporotrichosis and other species such as *S. brasilensis*, *S. globosa* and *S. mexicana* also show human infectivity [4, 11]. There were no reported cases of human infection by *S. stylites*, but that could not eliminate

the possibility of infections that may have been erroneously ascribed to other *Sporothrix* species. Further studies are needed to confirm a low infectious potential of *S. stylites*.

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