RESEARCH ARTICLE

Isolation and Identification of Three Newly Reported Ascomycete Fungal Species Isolated from Soil in Korea

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ABSTRACT

In this study, three fungal isolates belonging to the phylum Ascomycota under classes Leotiomycetes, Eurotiomycetes, and Sordariomycetes were isolated from soil in Korea. These species were designated as KNUF-22-003, KNUF-22-005, and KNUF-20-NI016, respectively, and identified based on their phylogenetic relationships and morphological characteristics. The isolates were confirmed through molecular phylogenetic analyses of their internal transcribed spacer (ITS) region, 28S rDNA large subunit (LSU), and actin (*ACT1*) gene sequences. Cultural and morphological characteristics of strains KNUF-22-003, KNUF-22-005, and KNUF-20-NI016 were matched with *Chaetomella oblonga* CBS110.78^{T}, *Oidiodendron chlamydosporicum* CBS403.69^{T}, and *Sarocladium subulatum* CBS217.35^{T}, respectively. To the best of our knowledge, this is the first report on *C. oblonga*, *O. chlamydosporicum*, and *S. subulatum* in Korea.

Keywords: *Chaetomella oblonga*, morphology, *Oidiodendron chlamydosporicum*, phylogenetic analysis, *Sarocladium subulatum*

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2024 June, 52(2): 97-108
https://doi.org/10.4489/kjm.520202

Received: March 29, 2024
Revised: June 03, 2024
Accepted: June 07, 2024

INTRODUCTION

Soil fungi significantly contribute to ecosystems by acting as decomposers, generating diverse enzymes that facilitate the breakdown of organic matter and maintain nutrient balance. Furthermore, these key regulators in the ecosystem have a substantial influence on both plant diversity and productivity [1]. Ascomycota is the largest phylum of fungi that contains 93,000 species under 20 classes, including Eurotiomycetes, Leotiomycetes, and Sordariomycetes [1]. The phylum Ascomycota is well known for its diverse ecological characteristics and includes saprophytes, symbionts, and pathogens that are associated with soils, insects, plants, fungi, and other invertebrates [2]. The genus *Chaetomella* belonging to the phylum Ascomycota, class Leotiomycetes, order Chaetomellales, and family Chaetomellaceae was introduced by Fuckel in 1870 based on the production of the fruiting body pycnidium [3]. This genus was initially documented in Europe but was also observed in other regions, such as the United Kingdom and

the United States. Its presence in different geographical locations indicates its adaptability and survival capabilities [4]. Morphologically, fungi belonging to this genus produce black pycnidia that generally open by a raphe; have acropleurogenous conidiogenous cells; and have nonseptate, hyaline, usually fusiform to falcate, rarely ellipsoid conidia [5]. The genus *Oidiodendron* belonging to the phylum Ascomycota, class Eurotiomycetes, and family Myxotrichaceae was introduced by Robak in 1932 [6]. This genus is well known as a globally distributed genus that is commonly found in various habitats, such as soil and various cellulosic substrates, and is occasionally present in lichens or the air [7]. The primary morphological attributes of this genus include upright and distinct dark conidiophores, which exhibit a high degree of branching at their upper regions to generate fertile hyphae. Moreover, these conidiophores fragment in a basipetal manner at their lower regions, resulting in the formation of arthroconidia [7]. The genus *Sarocladium* belonging to the phylum Ascomycota, class Sordariomycetes, order Hypocreales, and family Sarocladiaceae constitutes the soil of agricultural ecosystems [8]. This genus was first reported by Gams and Hawksworth in 1975 [9]. Several species belonging to the genus *Sarocladium*, including *S. brachiariae* and *S. spinificis*, are known to be saprophytic or endophytic fungi that live on Poaceae plants, such as bamboo, rice, and maize, or in the soil [10]. In addition, while some members of this genus have traditionally been regarded as significant phytopathogens, the genus also consists of opportunistic human pathogens [11]. A morphological characteristic of this genus is phialides that are one-celled, cylindrical, hyaline conidia; have a narrow cylindrical shape; hardly taper toward the apices; and lack collarettes [12]. The objectives of this study aimed to analyze the unidentified fungi isolated from soil in Korea and to determine their phylogeny and morphology. To this end, we isolated three morphologically distinct fungal strains during an investigation of unrecorded fungal species in Korea.

MATERIALS AND METHODS

Sample collection and fungal isolation

The fungal isolates used in this study were present in soil samples collected from Baekunsa, Gyeongsangbuk-do (36°27′14.14″N, 127°56′33.40″E); Suseo-ri, Gyeongsangbuk-do (36°11′31.4″N, 128°33′29.5″E); and Gapjangsa, Gyeongsangbuk-do (36°20′54.30″N, 128°10′38.23″E) in Korea. Soil samples were collected from the field at a depth of 15-30 cm using a pre-autoclaved sterile spatula, air-dried, and stored at 4°C in a plastic bag. For isolation, the soil samples (1 g) were suspended in 10 mL of sterile distilled water, vortexed gently, and serially diluted and a volume of 100 µL was spread on potato dextrose agar (PDA; Difco, Detroit, MI, USA) plates and incubated for 2-3 days at 25°C [13]. Germinating single colonies were then transferred to new PDA plates at 25°C. A pure culture of the strain was selected for further molecular analyses based on specific characteristics. The isolates KNUF-22-003, KNUF-20-NI016, and KNUF-22-005 were chosen for additional molecular analyses and cultural and morphological characterization. These isolates have been deposited at the National Institute of Biological Resources (NIBR) under the accession numbers NIBRFGC000509830, NIBRFGC000507847, and NIBRFGC000509831, respectively.

Cultural and morphological characterization

Cultural and morphological characteristics of the three fungal strains were assessed using cultural media based on the genus by following previous studies [4,7,12]. All three strains were cultured on PDA for 14 days at 25°C. KNUF-20-NI016 was also grown on oatmeal agar (OA; Difco, Detroit, MI, USA) for 14 days at 25°C, while KNUF-22-005 was also grown on cornmeal agar (CMA; Difco, Detroit, MI, USA) for 28 days at 25°C. The growth of the fungi was quantified, and details of the colonies, including their color, shape, and size, were noted. A light microscope (BX-50; Olympus, Tokyo, Japan) was used to study the morphological properties.

Genomic DNA extraction, PCR amplification, and sequencing

For the molecular identification of strains KNUF-22-003, KNUF-22-005, and KNUF-20-NI016, total genomic DNA was extracted using the HiGene™ Genomic DNA Prep Kit for fungi (Biofact, Daejeon, Korea). The internal transcribed spacer (ITS) region of the total genomic DNA extracted from the samples was amplified using ITS1F/ITS4 primer pairs [14,15], and the large subunit (LSU) region of 28S rDNA was amplified using LROR/LR5 primer pairs [16]. The actin (*ACT1*) gene was amplified using ACT1/ACT4 primer pairs [17]. Amplification was confirmed by electrophoresis on 0.7% agarose gels stained with ethidium bromide. The amplification products were purified using ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced by Bioneer Co. (Daejeon, Korea).

Phylogenetic analyses

Sequences obtained through sequencing were compared for similarity using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) database (Table 1). Phylogenetic trees were constructed based on combined sequences of ITS regions, LSU genes, and *ACT1* genes using the neighbor-joining (NJ) method [18] in MEGA version X [19]. The evolutionary distance matrices for the NJ analysis were generated according to Kimura's two-parameter model with bootstrap values based on 1,000 replications [20].

Table 1. GenBank accession numbers used for phylogenetic analyses in this study

Species name	Strain number	GenBank accession number		
		ITS	LSU	*ACT1*
Oidiodendron chlamydosporicum	CBS 132.72	MH860415	MH872153	-
Oidiodendron chlamydosporicum	CBS 403.69^{T}	MH859334	NG_064071	-
Oidiodendron chlamydosporicum	**KNUF-22-005**	**OR879279**	**OR794321**	**-**
Oidiodendron citrinum	CBS 127638	MH864649	MH876085	-
Oidiodendron citrinum	CBS 129229	MH865252	MH876692	-
Oidiodendron clavatum	21-64-D	LC683775	LC683776	-
Oidiodendron eucalypti	CPC 32659	NR_160624	NG_064544	-
Oidiodendron flavum	CBS 128269	MH864869	MH876316	-
Oidiodendron griseum	CBS 128807	MH865155	MH876594	-
Oidiodendron griseum	CBS 249.33	MH855427	LC639310	-
Oidiodendron maius	CBS 126948	MH864347	MH875791	-
Oidiodendron maius	CBS 928.73	MH860825	MH872559	-
Oidiodendron mellicola	CBS 143839	LT906544	LT978464	-
Oidiodendron mellicola	FMR 15680	LT906540	LT978465	-
Oidiodendron nigrum	CBS 321.31^{T}	MH855228	MH866680	-
Pseudogymnoascus roseus	CBS 395.65	NR_165894	NG_070577	-
Glomerella cingulata	AR3788	DQ286200	DQ286201	-
Chaetomella oblonga	CBS 110.78	MH861115	MH872875	-
Chaetomella oblonga	BPI 843552	AY487079	AY487083	-
Chaetomella oblonga	BPI 843553	AY487082	AY487080	-
Chaetomella oblonga	**KNUF-22-003**	**OR794272**	**OR794320**	**-**
Chaetomella pseudocircinoseta	CBS 145549	NR_165554	NG_067876	-
Chaetomella raphigera	BPI 843541	AY487076	AY487077	-
Chaetomella raphigera	BPI 843551	AY487087	AY487086	-
Chaetomella zambiensis	CPC 22465	KJ869130	KJ869187	-
Sarocladium bacillisporum	CBS 425.67^{T}	HE608639	HE608658	HE608633
Sarocladium bacillisporum	CBS 388.67	HG965003	HG965052	HG964953
Sarocladium bifurcatum	UTHSC 07-3446	HG965010	HG965058	HG964960
Sarocladium bifurcatum	CBS 137658^{T}	HG965009	HG965057	HG964959
Sarocladium gamsii	CBS 707.73^{T}	HG965015	HG965063	HG964965
Sarocladium glaucum	CBS 796.69^{T}	FN691454	HE608657	HE608631
Sarocladium glaucum	CBS 456.74	HG965019	HG965067	HG964969
Sarocladium implicatum	CBS 825.73	HG965022	HG965071	HG964973
Sarocladium implicatum	CBS 959.72^{NT}	HG965023	HG965072	HG964974
Sarocladium subulatum	**KNUF-20-NI016**	**OR794292**	**OR794322**	**OR881962**
Sarocladium subulatum	CBS 217.35^{T}	HG965031	HG965075	HG964984
Sarocladium subulatum	CBS 137661	HG965032	HG965076	HG964985
Sarocladium terricola	CBS 134.71	HG965038	HG965082	HG964990
Sarocladium terricola	CBS 243.59^{T}	FN706553	HE608659	HE608632
Acremonium variecolor	CBS 130360	HE608647	HE608651	HE608622

The strains identified in this study are indicated in bold.
^{T}indicates type strain; ^{NT}indicates neotype.
ITS: Internal transcribed spacer regions; LSU: 28S rDNA large subunit; *ACT1*: Actin gene.

RESULTS AND DISCUSSION

Chaetomella oblonga Fuckel, Fungi Rhenani Exsiccati. Supplementi Fasc. 5: no. 1962 (1867) [MB#163639]

Morphology of strain KNUF-22-003

The colonies achieved a growth with a diameter of 72-81 mm in 14 days at 25°C on PDA. The obverse appeared smooth, with a low aerial, pale vinaceous to vinaceous buff surface. The margin was white and even, having sparse wrinkles (Fig. 1A). On the reverse side, the colony appeared cream in color near the edge and darkened to dark brown in the center (Fig. 1B). The pycnidia were about 180-550×130-260 μm (n=20) and appeared to be elongated, oblong, rarely globose, and dark (Fig. 1C and D). The setae were 106.9-320.0×7.0-10.5 μm (n=30) and dark brown, and appear paler at the apex, with shapes varying from clavate to pointed, found all over the fruiting body and sharp (Fig. 1E and F). The pycnidia developed in the culture were discoid and stalked; the stalks were pale brown and surrounded by setae. The conidia were non-septate, hyaline, and fusiform to falcate with ends that were slightly pointed, straight to curved, and smooth; with a diameter of 7.7-10.9×1.5-2.7 μm (n=50) (Fig. 1G). The cultural and morphological characteristics of strain KNUF-22-003 indicate that it is most closely related to *C. oblonga* 110.78^{T} [4].

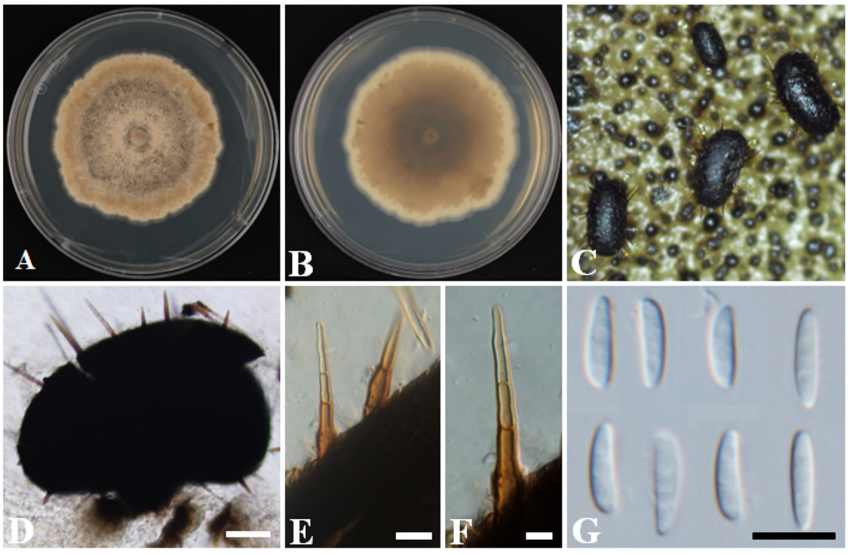

Fig. 1. Cultural and morphological characteristics of strain KNUF-22-003. Cultures were grown at 25°C on potato dextrose agar (PDA). A, B: Front and reverse views of the colony after 14 days; C: Pycnidia in culture; D: Conidia released from pycnidia; E, F: Setae on pycnidia; G: Conidia. Scale bars: D=100 μm; E-G=10 μm.

Molecular phylogeny of strain KNUF-22-003

Based on sequencing analyses, the length of the sequences obtained from the strain KNUF-22-003 for the ITS regions and LSU gene, was 483 and 812 bp, respectively. The ITS region showed 100% similarity

to the *C. oblonga* strains BPI 843552 and BPI 843553. The LSU gene sequences of the strain showed 99.9% similarity to the strains *C. oblonga* BPI 843552 and BPI 843553 and 98.6% similarity to the strain *C. oblonga* CBS 110.78^{T}. The NJ phylogenetic tree (a combination of ITS region and LSU gene sequences; Fig. 2) indicated that strain KNUF-22-003 was clustered with various strains of *C. oblonga* (CBS 110.78^{T}, BPI 843552, and BPI843553). Thus, based on morphological and phylogenetic analyses, strain KNUF-22-003 was identified as *C. oblonga*, a newly described fungus in Korea.

Currently, about 40 species in the genus *Chaetomella* have been reported worldwide. They are saprophytes growing on litter, dead logs, and soil distributed in both temperate and tropical regions [21]. Moreover, *C. raphigera* has been reported as the causal agent of leaf spot disease in various hosts, including cigar flower (*Cuphea* spp.) [22]. Since *C. raphigera* is the only species in the genus *Chaetomella* reported in Korea [23], that this report of *C. oblonga* will contribute to the study of *Chaetomella* taxa in Korea.

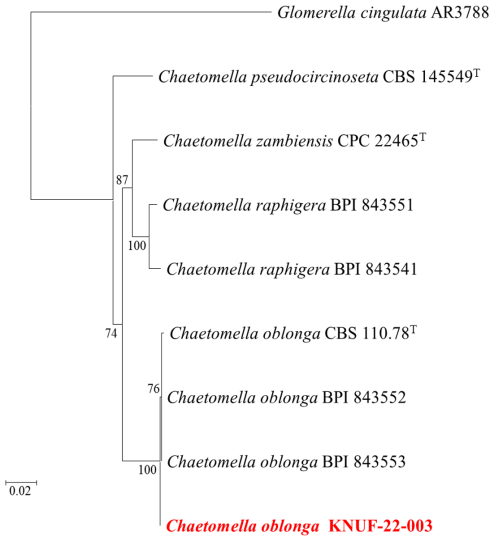

Fig. 2. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions and partial large subunit (LSU) gene sequences showing the phylogenetic position of strain KNUF-22-003 among *Chaetomella* species and its closest relationship with *Chaetomella oblonga*. Bootstrap values greater than 70% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is denoted in bold and red. The tree was rooted using *Glomerella cingulata* AR3788 as an out-group. Bar, 0.02 substitutions per nucleotide position. ^{T} indicates type strain.

Oidiodendron chlamydosporicum Morrall, Canadian Journal of Botany 46: 205 (1968) [MB#335316]

Morphology of strain KNUF-22-005

The colonies achieved growth with a diameter of 29-31 mm in 28 days at 25°C on PDA. The colonies were dark green to black at first but aged to a light olive gray color at the margin (Fig. 3A). The strain

also achieved growth with a diameter of 17-30 mm in 28 days at 25°C on CMA. The colonies on CMA appeared to be cream or light gray to green–gray, or brown; with darker margins and appressed on the obverse side. However, on the reverse side, the colony appeared cream near the edge and darkened to dark brown in the center (Fig. 3B). The chlamydospores were terminal, intercalary, subglobose, and melanized with a diameter of 3.7-7.6×2.6-5.6 μm (n=30) (Fig. 3C). Conidiophores were melanized and bearing chains of hyaline conidia, with the melanized chlamydospores alternating between short, branched, and lightly pigmented to melanized structures and erect structures (Fig. 3D). The conidia were thin-walled, globose, subglobose, or elongated with a diameter of 2.5-5.5×1.4-3.0 μm (n=30) (Fig. 3E). The cultural and morphological characteristics of strain KNUF-22-005 indicate that it is most closely related to *O. chlamydosporicum* CBS 403.69^{T} [7].

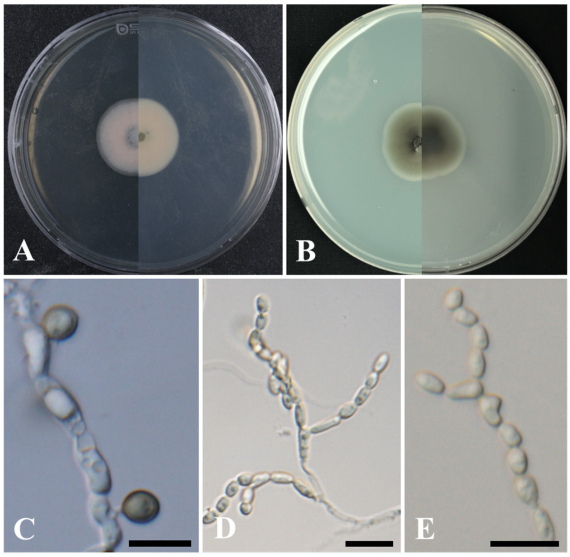

Fig. 3. Cultural and morphological characteristics of strain KNUF-22-005. Cultures were grown at 25°C for 28 days. A, B: Front and reverse views on potato dextrose agar (PDA) and cornmeal agar (CMA), respectively; C: Chlamydospore; D, E: Elongate, hyaline conidia produced in short chains at the apices of short and dark conidiophores. Scale bars: C-E=10 μm.

Molecular phylogeny of strain KNUF-22-005

Based on sequencing analyses, the length of the sequences obtained from the strain KNUF-22-005 for the ITS region and LSU gene, was 459 and 810 bp respectively. The ITS region showed 99.6% and 99.6% similarity to the strains *O. chlamydosporicum* CBS 403.69^{T} and 132.72, respectively. The LSU gene sequences of the strain showed 99.6% similarity to the strains *O. chlamydosporicum* CBS 403.69^{T}, and CBS 132.72. The NJ phylogenetic tree (a combination of ITS regions and LSU gene sequences; Fig. 4) indicated that strain KNUF-22-005 was clustered with previously identified strains of *O. chlamydosporicum* (Fig. 4). Based on morphological and phylogenetic analysis, strain KNUF-22-005 was identified as *O. chlamydosporicum*, a newly described fungus in Korea.

Of the 32 species belonging to the genus *Oidiodendron*, only 5 species (*O. citrinum*, *O. echinulatum*, *O.*

flavum, *O. maius*, and *O. clavatum*) have been reported in Korea [24]. *O. maius* is a mycorrhizal fungus that facilitates nutrient exchange in plants and makes plants tolerant to various metals, such as Zn and Cd, thereby affecting plant growth and nutrient movement [25]. The genus *Oidiodendron*, which can have direct and indirect effects on plants, can also have a significant impact on humans. Chetracin B, a secondary metabolite produced by *O. truncatum*, is cytotoxic against human cancer cells [26]. This suggests that *Oidiodendron* exhibits both medicinal and pathogenic effects and that precautions should be taken against its pathogenicity. The genus *Oidiodendron* is a mycorrhizal fungus that can directly act on plants. It is being studied for its functional diversity and availability for medical purposes; however, there is still a lack of research on *O. chlamydosporicum* and the genus *Oidiodendron* as a whole.

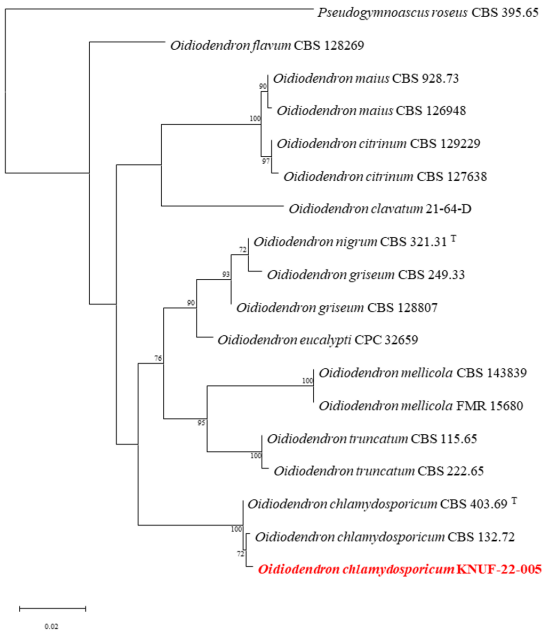

Fig. 4. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions and partial large subunit (LSU) gene sequences showing the phylogenetic position of strain KNUF-22-005 among *Oidiodendron* species. Bootstrap values greater than 70% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is denoted in bold and red. The tree was rooted using *Pseudogymnoascus roseus* CBS 395.65 as an out-group. Bar, 0.02 substitutions per nucleotide position. T indicates type strain.

Sarocladium subulatum Giraldo, Gené & Guarro, Persoonia 34: 20 (2015) [MB#807948]

Morphology of strain KNUF-20-NI016

The colonies achieved growth with a diameter of 23-26 mm in 14 days at 25℃ on PDA. The obverse side appeared to be yellowish white and membranous. It became velvety and formed a crateriform shape over time (Fig. 5A). The colonies grew to 28-32 mm in 14 days at 25℃ on OA (Fig. 5B). The colonies on CMA appeared flat with a diffuse margin and were powdery with a yellowish white color. The hyphae

of strain KNUF-20-NI016 were thin and transparent and had a smooth surface with septae observed. The conidiophores were erect, mostly unbranched, and simple, and were observed to be smooth and transparent (Fig. 5D). The phialides arising directly from vegetative hyphae were straight or slightly flexuous and exhibited an awl-like shape with a slender, elongated, pointed tip; 14-23 μm long and 1.4-2.6 μm wide at the base (Fig. 5C). Similar to the conidia, the hyphae were transparent and had a thin, smooth surface (Fig. 5D and E). The cultural and morphological characteristics of strain KNUF-20-NI016 indicate that it is most closely related to *S. subulatum* CBS 217.35^{T} [8].

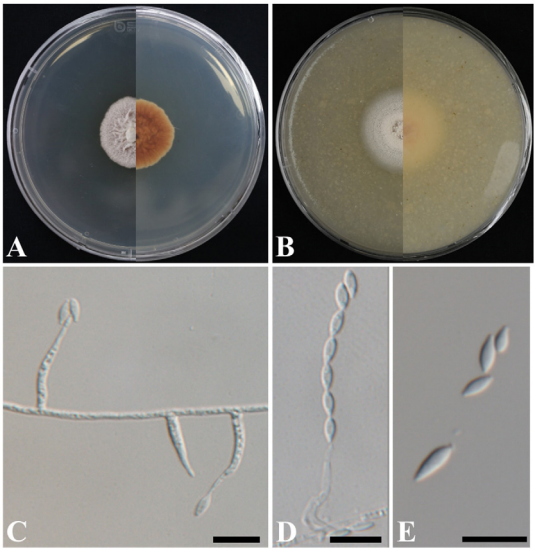

Fig. 5. Cultural and morphological characteristics of strain KNUF-20-NI016. Cultures were grown at 25°C for 14 days. A, B: Front and reverse views on potato dextrose agar (PDA) and oatmeal agar (OA), respectively; C: Phialides arising directly from vegetative hyphae; D: Conidia arranged in chains; E: Conidia. Scale bars=10 μm.

Molecular phylogeny of strain KNUF-20-NI016

Based on sequencing analyses, the length of the sequences obtained from the strain KNUF-20-NI016 for ITS regions, LSU, and *ACT1* genes, was 600, 900, and 780 bp, respectively. The ITS region showed 100% similarity to the strains *S. subulatum* CBS 217.35^{T} and CBS 137661. The LSU gene sequences of the strain showed 99.9% similarity to various *Sarocladium* strains, such as *S. bacillisporum* CBS 425.67^{T}, *S. terricola* CBS 243.59^{T}, and *S. subulatum* CBS 217.35^{T}. Moreover, the *ACT1* gene sequences showed the highest similarity of 98.1% to *S. subulatum* 217.35^{T}, followed by *S. subulatum* CBS 137661 (96.8% similarity) and *S. bacillisporum* CBS 485.67^{T} (93.1% similarity). The NJ phylogenetic tree (a combination of ITS regions, LSU region, and *ACT1* gene sequences; Fig. 6) indicated that strain KNUF-20-NI016 was clustered with previously identified strains of *S. subulatum* (Fig. 6). Based on morphological and phylogenetic analysis, strain KNUF-20-NI016 was identified as *S. subulatum*, a newly described fungus in Korea.

Several species belonging to the genus *Sarocladium* are traditionally known as plant pathogens [11]. For example, *S. oryzae* has been reported as a pathogen causing leaf blight in rice [9]. Although the

pathogenicity of *S. subulatum* was not assessed in this study, further research is necessary considering that several fungi belonging to the genus *Sarocladium* act as plant pathogens and mycorrhizal fungi have also been reported as plant pathogens.

According to the effectuation of the Nagoya Protocol in 2014, knowledge of biodiversity is becoming increasingly important [27]. Accordingly, to obtain domestic biological resources and increase our knowledge of fungal diversity, 3 newly discovered species, *C. oblonga*, *O. chlamydosporicum*, and *S. subulatum*, were isolated from soil samples collected from Korea. The three genera are well-known causal agents of various plant pathogens on a wide range of host plants. Therefore, further research is needed for a better understanding of their diversity, geographical distribution, pathogenicity, and ecological and biological roles, particularly under Korean environmental conditions. Through this research, we believe that we can discover biological resources that can become the mainstay of the Korean bio-industry and contribute to the promotion of biodiversity and the growth of the Korean bio-industry.

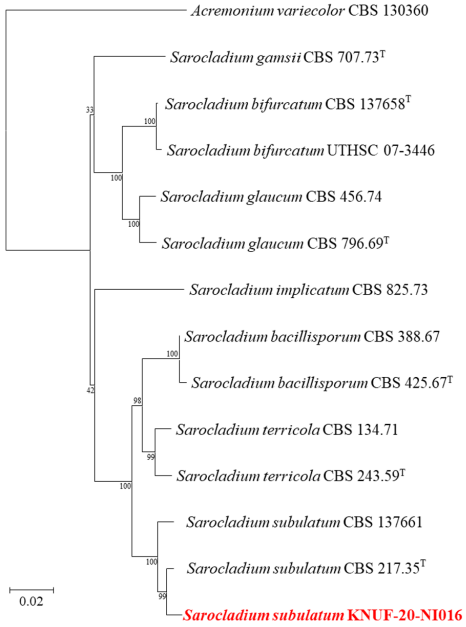

Fig. 6. Neighbor-joining phylogenetic tree based on a combined dataset of partial sequences of internal transcribed spacer (ITS) regions, partial large subunit (LSU) sequences, and actin (*ACT1*) gene sequences showing the phylogenetic position of strain KNUF-20-NI016 among *Sarocladium* species and its closest relationship with *Sarocladium subulatum*. Bootstrap values greater than 99% (percentage of 1,000 replications) are shown at branching points. The strain isolated in this study is denoted in bold and red. The tree was rooted using *Acremonium variecolor* CBS 130360 as an out-group. Bar, 0.02 substitutions per nucleotide position. ^{T}indicates type strain.

CONFLICT OF INTERESTS

No potential conflict of interest was reported by the author(s).

ACKNOWLEDGEMENTS

This research was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202002104 and NIBR202203112).

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