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Research Article

Confirmation of Two Undescribed Fungal Species from Dokdo of Korea Based on Current Classification System Using Multi Loci

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Abstract Using dilution plating method, 47 fungal isolates were obtained from a soil sample collected from Dokdo in the East Sea of Korea in 2013. In this study, two fungal isolates, EML-MFS30-1 and EML-DDSF4, were confirmed as undescribed species, *Metarhizium guizhouense* and *Mortierella oligospora* in Korea based on current classification system using multi loci including rDNA internal transcribed spacer, large subunit, small subunit, and β-tubulin (BTUB) genes. Herein, detailed morphological descriptions on characters of the undescribed fungal species as well as their molecular phylogenetic status are provided with comparisons to related species.

Keywords Dokdo, *Metarhizium guizhouense*, *Mortierella oligospora*, Soil fungi

Dokdo is a rocky island located in the northeastern region of Ulleung Island of Korea and consists of two volcanic islands (Dongdo and Seodo) and 89 small islets. It has a variety of environmental conditions, including drought, strong winds, steep inclinations, soil salinity, high uric acid concentrations in the soil, and low organic matter. Thus, it may offer a distinct marine and fungal biodiversity [1].

Few studies have been done on coastal fungal communities. Such unique ecosystems may provide specific habitat and shelter for diverse organisms including fungi [2]. Mandeel [3] has reported the diversity of *Fusarium* species community in saline soil habitats. Some new species of *Fusarium* associated with dieback of *Spartina alterniflora* plant have been isolated from Atlantic salt marshes [2]. A new genus and species *Pileomyces formosanus* has been isolated from a rocky shore of Taiwan [4]. High salt tolerant fungal genera have been isolated from mangroves and solar salterns of Goa, India [5], with main genus of *Aspergillus*, followed by *Penicillium*, *Eurotium*, and *Hortaea*. Rogers [6] has studied fungi from Marshall island of central Pacific ocean. Humber and Rombach [7] have reported a new species of *Torrubiella* (Pyrenomycetes: Clavicipitales) and other fungi from spiders of Solomon islands. Alias *et al.* [8] have studied intertidal fungi from Philippines and reported a new species of *Acrocordiopsis*.

The genus *Metarhizium* was established in 1883 by Sorokin [9]. *Metarhizium* species are frequently found in soil or infected insects. The species belonging to this genus are characterized by the production of conidia in long chains, phialides in dense, parallel arrangement, and conidiophores aggregated in with repeated, verticillate branching [10].

The genus *Mortierella* belongs to the order Mortierellales within the Mortierellaceae family. To date, nearly 100 species has been recognized [11]. *Mortierella* species can be easily isolated from soil, debris or with living plant.

In Korea, You *et al.* [12, 13] have reported the presence of some endophytic fungi isolated from the roots of six native plants in Dokdo. About 30 bacteria species have been reported from that island, including *Virgibacillus*

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dokdonensis [14, 15]. However, few studies on the diversity of mitosporic fungi and microfungi on that unique island have been performed. It is important to investigate the fungal diversity of this solitary area that is so far away from the main land of Korea. The aim of this study was to isolate fungi from Dokdo in the East Sea of Korea, using dilution plating method, and to confirm the first records of two fungal species in Korea based on the current classification system using multi loci including rDNA internal transcribed spacer (ITS), large subunit (LSU), small subunit (SSU), and β-tubulin (BTUB) genes with comparisons to related species.

MATERIALS AND METHODS

Sampling and isolation. Soil samples were collected from Dongdo (eastern part) of Dokdo (37°14′21.3″ N, 131°52′04.4″ E) in the East Sea of Korea in 2013. Three sites of soils were sampled to depths of a couple of cm using a 50-mL conical tube and transferred to the laboratory in sterile plastic containers. In order to isolate fungi from soil samples, serial dilution plating technique was used. Briefly, 1 g of soil sample was mixed with 9 mL of sterile distilled water and vortexed for 15 min followed by serial dilution to 10^{-6} fold of the original suspension. One hundred microliters of each diluted soil solution was then transferred to potato dextrose agar (Difco PDA; 39 g PDA; Becton, Dickinson and Co., Sparks, MD, USA, and 1 L deionized water), and malt extract agar (Difco MEA; 33.6 g MEA; Becton, Dickinson and Co., and 1 L deionized water) plates. Plates were incubated at 25°C for 2 wk to allow fungal growth. Individual colonies of fungi were transferred to different PDA plates to isolate pure cultures. Pure cultures were selected and isolate numbers assumed to be undescribed species in Korea were assigned as EML-MFS30-1 and EML-DDSF4. Cultures were stored in the 20% glycerol at deep freezer of −80°C in the EML (Environmental Microbiology Laboratory Fungal Herbarium, Chonnam National University, Gwangju, Korea). The EML strains, EML-MFS30-1 and EML-DDSF4, were also deposited at the culture collection of National Institute of Biological Sciences (NIBR, Incheon, Korea) as ex-types, KOSPGC 1151 and KOSPGC 1123, respectively.

Morphological studies. Agar plugs containing stored fungi including EML-MFS30-1 and EML-DDSF4 were transferred to PDA, MEA, yeast malt extract agar (Difco YMA; 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 2% agar; Becton, Dickinson and Co. and 1 L deionized water), oatmeal agar (OA; 1.5% oatmeal and 1.5% agar; Junsei, Tokyo, Japan, and 1 L deionized water) and water agar plates for morphological analysis. Plates were incubated at 25°C in the dark for 1 mon. Colony characteristics (color, size, and texture) were determined at 4~7 days after inoculation. Samples were mounted in lactophenol solution (Junsei) to observe and measure the size and shape of the conidia and conidiophores using light microscope (Leica DMA light microscope; Leica Micriosystems, Jena, Germany). Fine structures of the fungi were observed by scanning electron microscopy (Hitachi S4700 field emission scanning electron microscope; Hitachi, Tokyo, Japan). Samples were cultured on PDA medium in the dark at 27°C for 7 days. Samples were fixed in 2.5% paraformaldehyde-glutaraldehyde buffer with 0.05 M phosphate (pH 7.2) (Junsei) for 2 hr and washed in carcodylate buffer (Junsei). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (diluted in carcodylate buffer; Electron Microscopy Sciences, Hatfield, PA, USA) for 1 hr, washed again in carcodylate buffer, dehydrated in graded ethanol (Emsure, Darmstadt, Germany) and isoamyl acetate (Junsei), and dried under a fume hood. Finally, these samples were covered with gold in a sputter coater and observed at Korea Basic Science Institute, Gwangju, Korea.

Genomic DNA extraction and PCR. Isolates were cultured on PDA plates overlaid with cellophane at 27°C for 3~5 days. Total genomic DNA was extracted using HiGene Genomic DNA Prep Kit (BIOFACT Corp., Daejeon, Korea). PCR amplification was performed with different primer sets (Table 1) [16-18] in a 20 μL reaction using Accupower PCR Premix (Bioneer Corp., Daejeon, Korea) containing *Taq* DNA polymerase, dNTPs, buffer, and a tracking dye. PCR was conducted using the following conditions: 2 min at 95°C for the initial denaturation step, followed by 35 cycles of 1 min at 94°C for denaturation, 30 sec at 54°C for primer annealing, 1 min at 72°C for

Table 1. List of primers used in this study

Gene	Product name	Primer	Direction	Sequence (5′-3′)	Reference
ITS	ITS rDNA	ITS-1	Forward	TCCGTAGGTGAACCTGCGG	[16, 18]
		ITS-4	Reverse	TCCTCCGCTTATTGATATGC	[16, 18]
18S	SSU rDNA	NS1	Forward	GTAGTCATATGCTTGTCTC	[16, 18]
		NS4	Reverse	CTTCCGTCAATTCCTTTAAG	[16, 18]
28S	LSU rDNA	LROR	Forward	ACCCGCTGAACTTAAGC	[16, 18]
		LR5F	Reverse	GCTATCCTGAGGGAAAC	[16, 18]
BTUB	β-Tubulin	BT2a	Forward	GGTAACCAAATCGGTGCTGCTTTC	[17]
		BT2b	Reverse	ACCCTCAGTGTAGTGACCCTTGGC	[17]

ITS, internal transcribed spacer; SSU, small subunit; LSU, large subunit.

extension, and a final extension at 72°C for 10 min. PCR products were purified using Accuprep PCR Purification Kit (Bioneer Corp.) according to the manufacturer's instructions. Sequencing was performed on an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc., Foster City, CA, USA).

Phylogenetic analysis. Sequences of fungi were submitted for phylogenetic analysis using BioEdit ver. 5.0.9.1 [19], Clustal X ver. 1.83 software [20]. Their phylogenies were assessed using Molecular Evolutionary Genetics Analysis (MEGA) 4 software [21]. Neighbor-joining phylogenetic tree was constructed based on individual ITS, SSU, LSU rDNA, and BTUB sequences as well as their combined sequences. Percent sequence identity (number of matches divided by complete alignment length) was obtained via National Center for Biotechnology Information (NCBI) BLASTn search for each isolate.

Mycelial growth of unrecorded strains. To find the optimum temperature for favorable growth, fungi were incubated for 14 days at three different temperatures (18°C, 27°C, and 32°C). A 6.5 mm diameter plug of inoculum was removed with a cork borer from 7-day-old cultures grown on PDA and placed in the center of OA, MEA, YMA, or PDA media and incubated in the dark for 14 days at 18°C, 27°C, and 32°C. Developing colonies were measured daily for a period of 14 days using a millimeter ruler. Two diameters of the growing colonies were measured at right angles. Growth rates (mm/day) were calculated by linear regression of colony radius against time for each strain grown under each condition.

RESULTS

Using dilution plating method, 47 fungal isolates were obtained from a soil sample collected from Dokdo in the East Sea of Korea in 2013. Out of them, two candidates, EML-MFS30-1 and EML-DDSF4, were primarily identified based on rDNA ITS sequence analysis, and then confirmed as first records in Korea based on the sequence analyses of multi loci including rDNA ITS, LSU, SSU, and β-tubulin (BTUB) genes. The detailed morphological descriptions as well as molecular phylogenetic status of the two undescribed fungal species are

Table 2. Mycological characteristics of *Metarhizium guizhouense* EML-MFS30-1 and its closely related reference species

Characteristics	Present isolate	*Me. guizhouense*[a]
Colony color	Olive	Olive
Phialides (μm)	10.7~13.1 × 2.4~3.2	7.0~12.0 × 2.0~3.0
Conidia (μm)	9.1~10.1 × 2.4~2.9	7.0~10.0 × 2.0~3.0

[a]From description of Bischoff *et al.* [17].

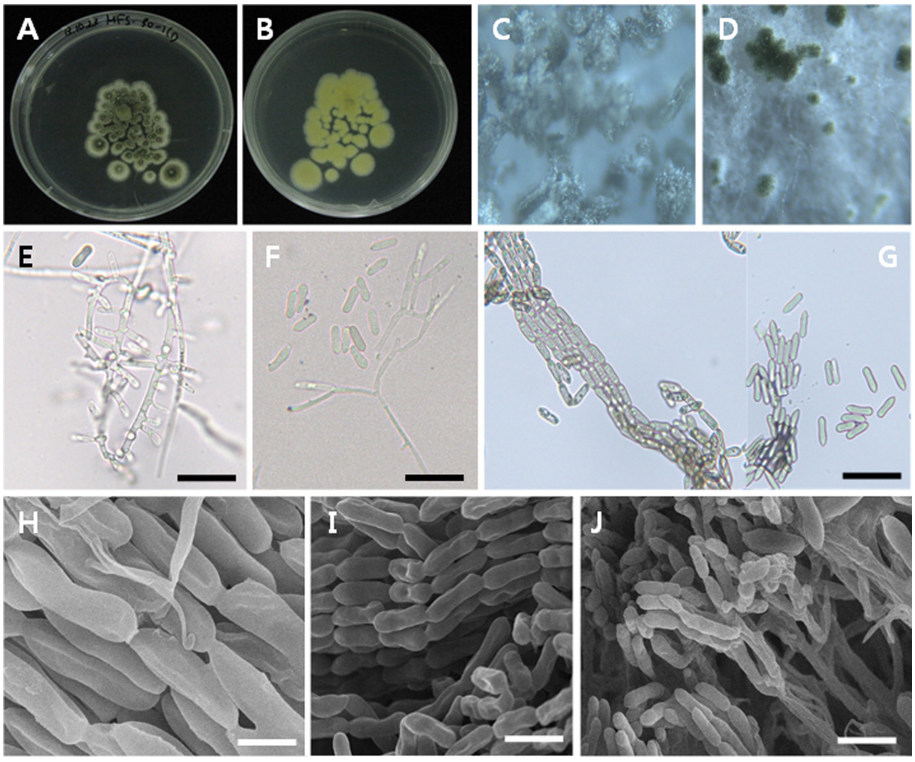

Fig. 1. Morphology of *Metarhizium guizhouense* EML-MFS30-1. A, B, Colony on potato dextrose agar (PDA) medium for 5 days at 27°C; C, D, Pustules on PDA under the stereo-microscope (magnification); E, F, Conidiophores, hyphae, phialides and conidia; G, H, Basipetal chains comprising conidia and conidiophores formimg a sporulating layer as conidial columns; I, J, Conidia with basipetal chains under scanning electron microscopy (scale bars: E~I = 20 μm, J = 5 μm).

presented with comparisons to related species as follows.

Taxonomy of EML-MFS30-1. *Metarhizium guizhouense* Q. T. Chen & H. L. Guo, Acta Mycol. Sin.: 181 (1986) [MB#130206].

Etymology: This species was isolated from soil sample, Dokdo in the East Sea of Korea.

Description: Colonies exhibited slow growth on PDA,

Table 3. List of species within the genus *Metarhizium* used for molecular phylogenetic analysis

Original name	Strain numbers	Locality	Host	GenBank accession No.	
				BTUB	ITS
Metarhizium majus	ARSEF 1015	Japan	Lepidoptera	EU248838	HQ331444
	ARSEF 1914	Philippines	Coleoptera	EU248840	HQ331445
Me. guizhouense	ARSEF 4321	Australia	Soil	EU248832	HQ331442
	ARSEF 6238	China	Lepidoptera	EU248830	HQ331447
	EML-MFS30-1	**Dokdo, South Korea**	**Soil**	**KT374181**	**KT374184**
	CBS 258.90	China	Lepidoptera	EU248834	HQ331448
Me. pingshaense	ARSEF 4342	Solomon Islands	Coleoptera	EU248821	HQ331454
	CBS 257.90	China	Coleoptera	EU248820	HQ331450
Me. robertsii	ARSEF 727	Brazil	Orthoptera	EU248816	HQ331453
Me. anisopliae	ARSEF 7450	Australia	Coleoptera	EU248823	HQ331464
	ARSEF 7487	Eritrea	Orthoptera	EU248822	HQ331446
Me. brunneum	ARSEF 2107	USA	Coleoptera	EU248826	KC178691
	ARSEF 4152	Australia	Soil	EU248824	HQ331452
Me. lepidiotae	ARSEF 7488	Australia	Coleoptera	EU248837	HQ331456
Me. acridum	ARSEF 324	Australia	Orthoptera	EU248812	HQ331457
	ARSEF 7486	Niger	Orthoptera	EU248813	HQ331458
Me. globosum	ARSEF 2596	India	Lepidoptera	EU248814	HQ331459
Me. flavoviride	ARSEF 2133	Czech Rep.	Coleoptera	EU248827	AY375449
Me. frigidum	ARSEF 4124	Australia	Coleoptera	EU248828	HM055448
Nomuraea rileyi	CBS 806.71	USA	Lepidoptera	AY624250	AY624205

BTUB, β-tubulin; ITS, internal transcribed spacer; ARSEF, The United States Department of Agriculture Agricultural Research Service (USDA-ARS) Collection of Entomopathogenic Fungi, U.S; EML, Environmental Microbiology Lab Fungal Herbarium, Chonnam National University, Gwangju, South Korea; CBS, Centraalbureau Voor Schimmel cultures, Utrecht, The Netherlands.

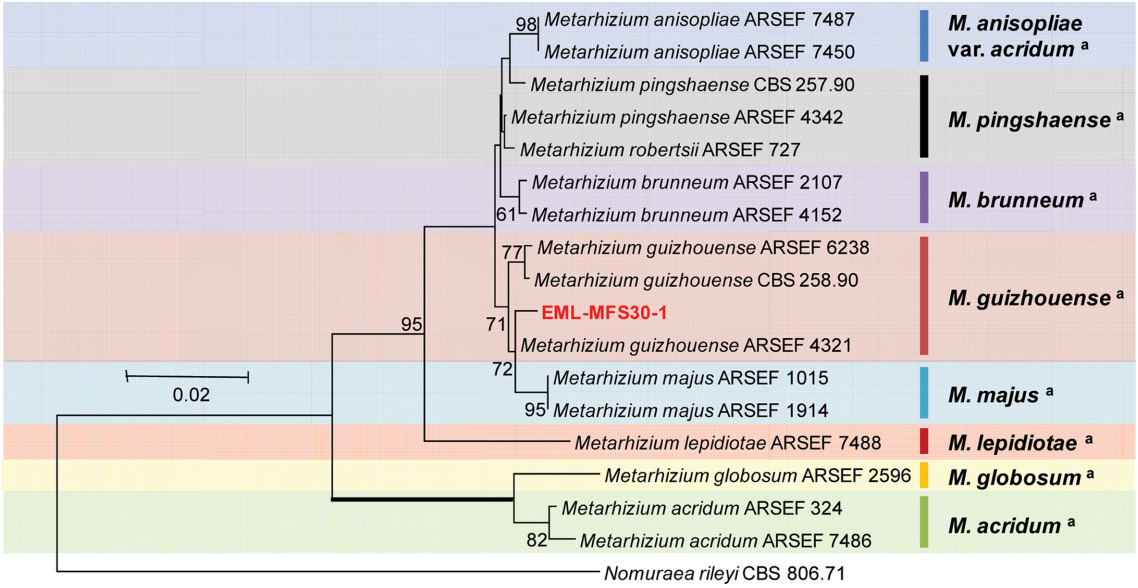

Fig. 2. Neighbor-joining tree of alignment based on combined data set of internal transcribed spacer rDNA (GenBank accession No. KT374184) and BTUB (GenBank accession No. KT374181) sequences from *Metarhizium guizhouense* EML-MFS30-1 and its related species from GenBank database. *Nomuraea rileyi* CBS 806.71 (GenBank accession No. AY624205) was used as outgroup. Bootstrap values over 50% are shown above the branches supported by 1,000 replications. The tree shows that EML-MFS30-1 strain belongs to *guizhouense* clade. [a]Classification by Bischoff *et al.* [17].

attaining a diameter of 10 mm after 5 days at 27°C. The color of colonies was dark green in the center with a lighter margin. The reverse of colony was yellow. A large number of conidia were produced on this medium forming conidial columns. Conidiophores formed a sporulating layer as conidial columns. Basipetal chains were formed in conidiation. The conidia were uninucleate, ovoid to cylindrical, and some more slender in body center. The conidia measured 9.1~10.1 μm wide × 2.4~2.9 μm long (Table 2, Fig. 1).

Molecular phylogeny. The sequences of EML- MFS30-1 were deposited in the NCBI database under accession numbers KT374181 and KT374184 (Table 3). NCBI BLAST search revealed that EML-MFS30-1 had 99.1% identity (467/471 bp) with *Me. guizhouense* M335-11 (GenBank accession No. KJ542166, ITS) and 98.8% identity (336/340 bp) with *Me. guizhouense* ARSEF 4321 (GenBank accession No. EU248832, BTUB). Combined sequence analysis of ITS and BTUB genes produced a *guizhouense* clade showing that EML-MFS30-1 was closely

Table 4. Mycological characteristics of *Mortierella oligospora* EML-DDSF4 and its closely related reference species

Characteristics	Present isolate (WA)	Present isolate (OA)	*Mo. oligospora* (OA)[a]
Colony color	White	White	-
Sporangiospores (μm)	8.0~14.3 (avr. 12.6) × 8.8~16.0 (avr. 13.4)	-	8.8~17.6 (avr. 14.3) × 8.0~14.3 (avr. 13.2)
Sporangia (μm)	17.4~19.1 × 18.0~21.6	-	15.2~22.5 (avr. 17.5) × 10.0~20.0 (avr. 15.0)

WA, water agar; OA, oatmeal agar; –, not produced.
[a]From description of Mehrotra *et al.* [22].

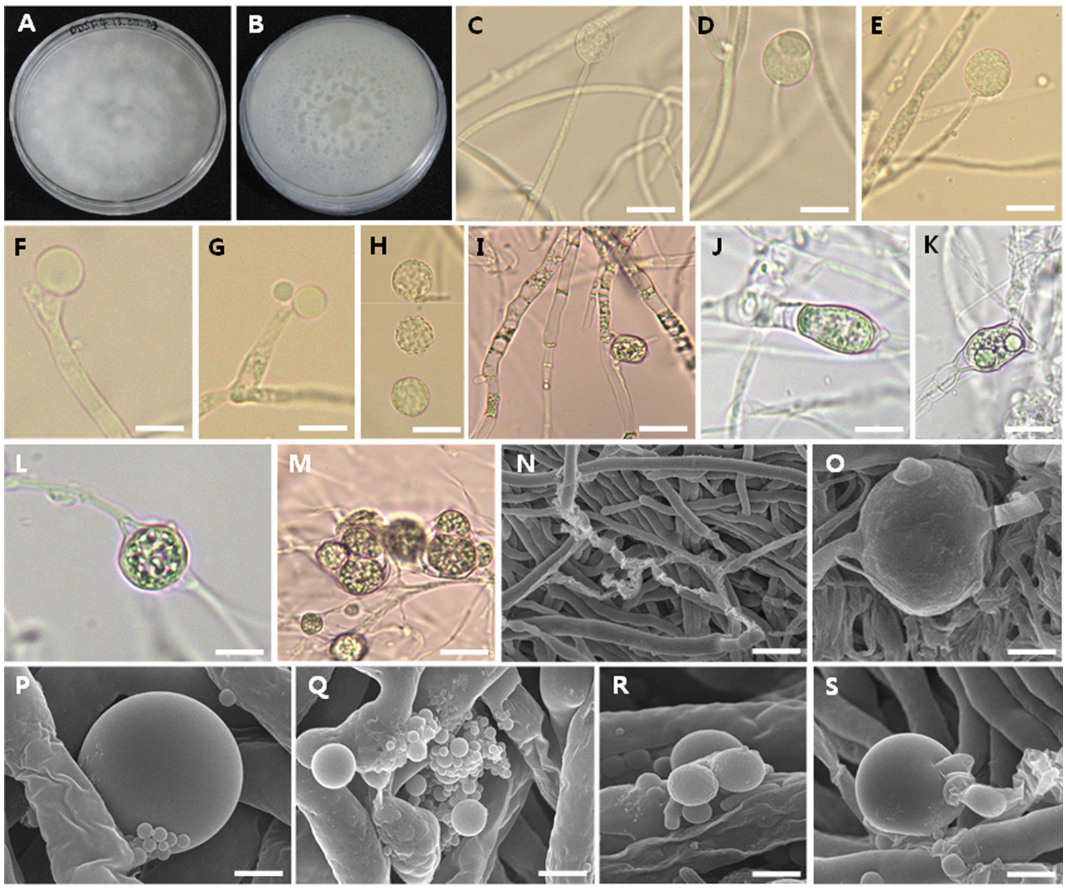

Fig. 3. Morphology of *Mortierella oligospora* EML-DDSF4. A, B, Culture on potato dextrose agar (PDA) for 7 days at 27°C; C~E, Different stage of sporangia on sporangiophores developed on water agar (WA) medium; F, G, Simple sporangiophore on WA; H, Sporangiospores on WA; I, Irregular and transparent hyphae with septa on PDA; J~M, Chlamydospore structures with intercalary or terminal patterns on PDA; N, Branched hyphae on PDA; O, Magnified chlamydospore structures with intercalary patterns on PDA; P, Globose chlamydospore or chlamydospore-like structure on PDA; Q~S, Different sized vesicles or spores derived from the chlamydospore or chlamydospore-like structures on PDA (scale bars: C~M = 20 μm, N = 30 μm, O~Q = 5 μm, R = 3 μm, S = 10 μm).

related to *Me. guizhouense* ARSEF 4321 (GenBank accession No. EU248832) with a 72% bootstrap value (Fig. 2). Thus, the EML-MFS30-1 isolate was confirmed as *Me. guizhouense* that has not been previously reported in Korea.

Taxonomy of EML-DDSF4. *Mortierella oligospora* Björl., Bot. Not. 1936: 121 (1936) [MB#272844].

Etymology: This species was isolated from soil sample, Dokdo in the East Sea of Korea.

Description: Colonies exhibited fast growth on PDA, attaining a diameter of 70 mm after 7 days at 27°C. The color of colonies was cotton white. The reverse of colony was also white with irregularly zonate. On artificial media, typical sporangia and sporangiospores were not observed although the PDA medium showed good mycelial growth. However, sporangia were produced only on water agar medium. Sporangia were globose to oval, measured 17.4~19.1 × 18.0~21.6 μm. The sporangiospores were globose, roughened, and measured 8.0~14.3 (avr. 12.6) × 8.8~16.0 (avr. 13.4) μm. Intercalary (commonly) or terminal chlamydospores with smooth surface were produced on PDA medium. The structures were mostly globose or subglobose containing different sized vesicles (or spores). Few-spored to 3-spored sporangioles were unclearly observed on PDA (Table 4, Fig. 3).

Molecular phylogeny. To determine the phylogenetic relationship between EML-DDSF4 isolated from the soil

Table 5. List of species within the genus *Mortierella* and *Umbelopsis* used for the molecular phylogenetic analysis

Original name	Strain No.	Locality	Host	GenBank accession No.		
				ITS	28S	18S
Mortierella alpina	CBS 219.35	Victoria	Unknown	JX976018	KC018359	-
	CBS 384.71C	India	Soil	JX976098	JX976154	-
	CBS 210.32	Victoria	Sandy loam soil	JX975853	JN940866	JQ040258
Mo. bisporalis	CBS 145.69	Italy	Unknown	JX975857	KC018377	-
	FSU 9675	Italy	Unknown	JX975953	JX976176	-
Mo. elongatula	CBS 488.70	Former West Germany	Municipal waste	JX975967	HQ667425	HQ667505
Mo. epigama	CBS 489.70	Former West Germany	Municipal waste	JX976057	HQ667367	JQ040250
Mo. horticola	CBS 305.52	Former West Germany	Unknown	JX975874	HQ667399	HQ667483
Mo. humilis	CBS 222.35	Mexico	Soil from *Pinus* forest	HQ630325	HQ667401	JQ040257
Mo. hypsicladia	CBS 116202	Japan	Bat dung in cave	JX975866	HQ667379	-
	CBS 116203	Japan	Bat dung in cave	JX975872	KC018369	-
Mo. indohii	CBS 331.74	Netherlands	Root	JX975860	KC018292	-
	CBS 460.75	Georgia	Dung of animal	JX975878	HQ667438	-
	CBS 665.70	Netherlands	Agricultural soil	JX975956	KC018357	-
Mo. oligospora	CBS 101758	USA	Supplement to mushroom culture	JX976032	KC018327	-
	CBS 191.79	Sudan	Soil	JX975966	JX976151	-
	CBS 381.71	India	Soil	JX976033	KC018368	-
	EML-DDSF4	**Dokdo, South Korea**	**Soil**	**KT374185**	**KT374182**	**KT374183**
Mo. polycephala	CBS 327.72	England	Salt-marsh soil under *Spartina townsendii*	JX976085	JX976175	-
	CBS 328.72	UK	Soil	JX976102	KC018296	-
	CBS 456.66	Ukraine	Dung of wood mouse	JX976034	KC018297	-
	FSU 696	Ukraine	Unknown	JX976035	HQ667409	HQ667493
Mo. polygonia	CBS 248.81	Netherlands	Clay soil under *Solanum tuberosum*	JX975891	JX976145	-
	CBS 685.71	Netherlands	Agricultural soil	JX975900	HQ667378	HQ667463
Mo. selenospora	CBS 811.68	Netherlands	Mushroom compost, together with *Entomophthora coronata* and *Aphanocladium album*	JX975875	HQ667419	HQ667499
Mo. wolfii	CBS 209.69	England	Coal spoil tip soil	HQ630303	HQ667380	JQ040256
	CBS 611.70	New Zealand	Lung, dying from mycotic pneumonia	HQ630306	HQ667383	JQ040255
	CBS 612.70	New Zealand	Decayed hay	HQ630304	HQ667381	HQ667465
	CBS 651.93	Netherlands	Compost for mushrooms	JX975904	JN940865	JQ040254
Umbelopsis isabellina	CBS 100559	Wisconsin	Soil	JN943789	AF157220	AF157166

FSU, Friedrich Schiller University, Jena, Germany; EML, Environmental Microbiology Lab Fungal Herbarium, Chonnam National University, Gwangju, South Korea.

of Ulleung island and its related species, 18S (SSU), ITS, and 28S (LSU) regions were analyzed. The sequences of EML-DDSF4 were deposited in the National Center for Biotechnology Information (NCBI) database under accession numbers KT374185, KT374182, and KT374183 (Table 5). NCBI BLASTn search revealed that EML-DDSF4 shared 100% sequence identity (350/350 bp) with *Mo. oligospora* CBS 101758 (GenBank accession No. JX976032, ITS), 99.8% identity (810/812 bp) with *Mo. polygonia* CBS685.71 (GenBank accession No. HQ667463, SSU), and 99.6% identity (967/971 bp) with *Mo. oligospora* CBS 101758 (GenBank accession No. KC018327, LSU). Sequence analysis revealed 98~100% sequence similarity between EML-DDSF4 isolate and its relevant sequences in GenBank based on NCBI BLASTn searches. The isolate and its related species in GenBank formed a single clade based on combined sequence analysis of the three sequences (ITS, 18S, and 28S). EML-DDSF4, *Mo. oligospora* CBS 101758 (accession No. JX976032), *Mo. oligospora* CBS 381.71 (accession No. JX976033), and *Mo. oligospora* CBS 191.79 (accession No. JX975966) clustered together in a group. The phylogenetic tree shows EML-DDSF4 strain belongs to *oligospora* subclade (arrow in Fig. 4) within *polycephala* clade. EML-DDSF4 matched *Mo. oligospora* CBS 101758 (accession No. JX976032) with 85% bootstrap value (Fig. 4). Based on morphological evaluation and molecular phylogenetic analysis, the isolate EML-DDSF4 was confirmed as *Mo. oligospora*.

Mycelial growth of unrecorded strains. Mycelial growth varied depending on environmental factors such as temperature and growth medium. *Mo. oligospora* EML-DDSF4 grew fast (avr. 15 mm/day) while *Me. guizhouense* EML-MFS30-1 grew slowly (avr. 3 mm/day) on MEA medium at 18°C. Fungal growth was the slowest in MEA medium (Fig. 5).

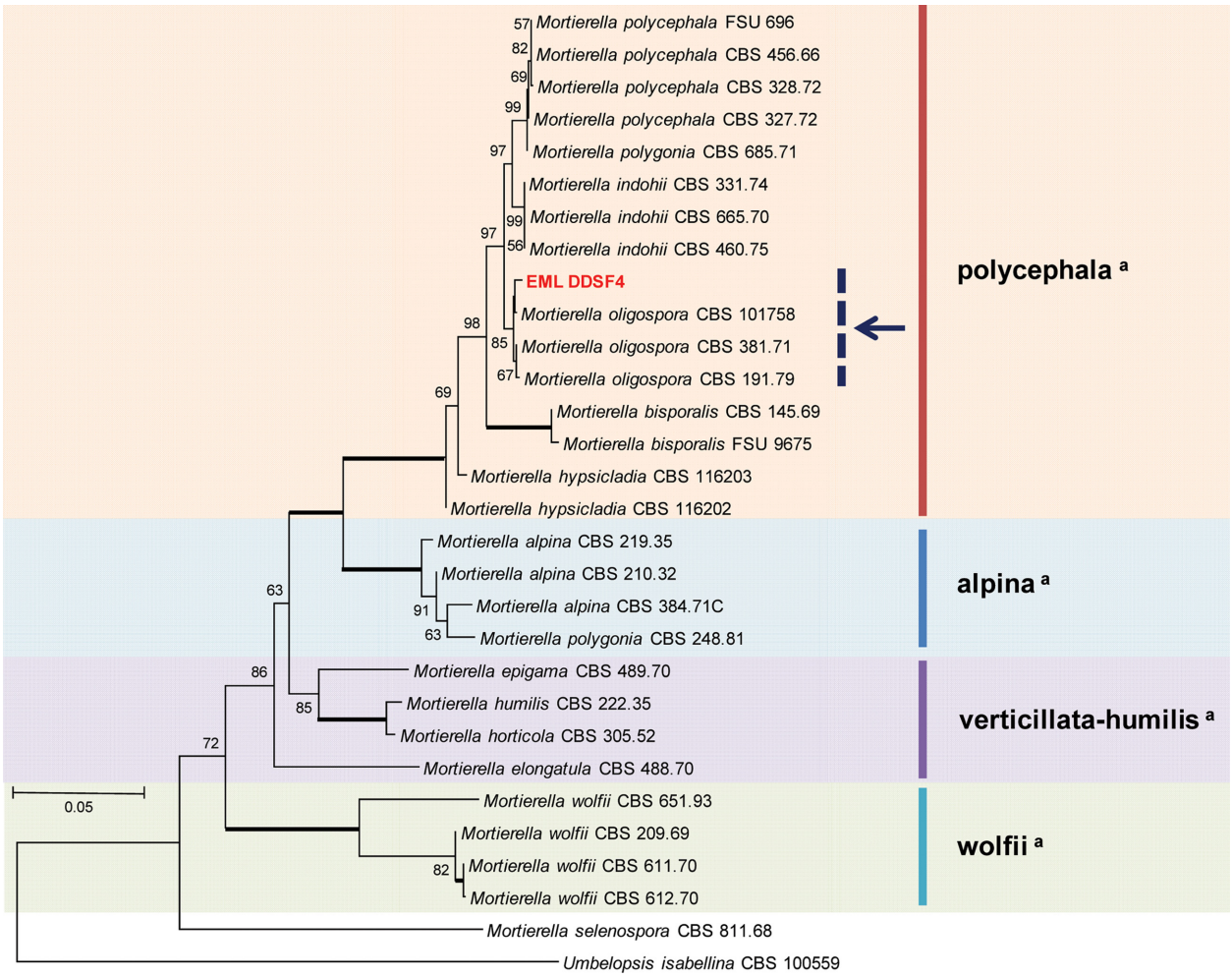

Fig. 4. Neighbor-joining tree of combined data set of small subunit rDNA (GenBank accession No. KT374183), internal transcribed spacer rDNA (GenBank accession No. KT374185), and large subunit rDNA (GenBank accession No. KT374182) sequences of *Mortierella oligospora* EML-DDSF4 and its related species from GenBank databases. *Umbelopsis isabellina* CBS 100559 (GenBank accession No. JN943789) was used as outgroup. Bootstrap values over 50% are shown above the branches supported by 1,000 replications. The tree shows that EML-DDSF4 strain belongs to *oligospora* subclade (arrow) within *polycephala* clade. [a]Classification by Wagner *et al.* [16] and Petkovits *et al.* [18].

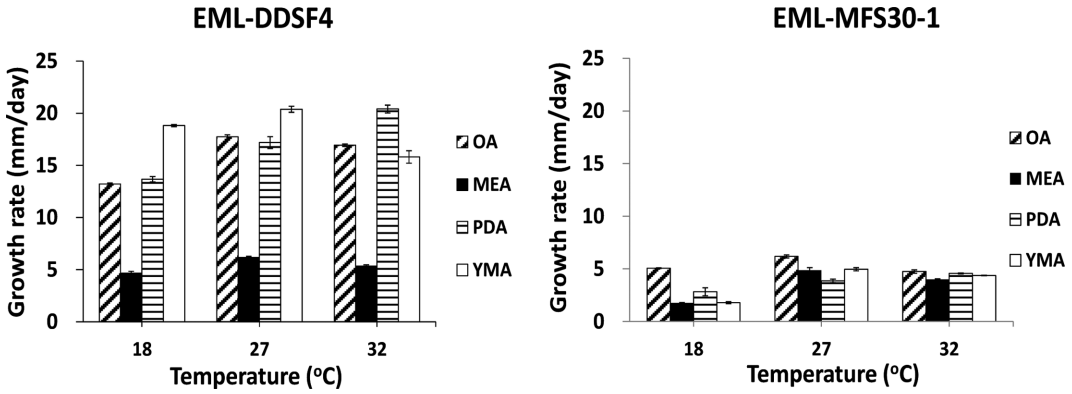

Fig. 5. Mycelial growth rates for the newly recorded fungi EML-DDSF4 and EML-MFS30-1. Colonies were grown on oatmeal agar (OA), malt extract agar (MEA), yeast malt extract agar (YMA), and potato dextrose agar (PDA) at different temperatures for 14 days.

DISCUSSION

Most fungal species isolated from Dokdo soil samples belonged to ascomycetes. However, some isolates were classified as zygomycetous fungi including *Mo. oligospora* and *Absidia* sp. It was difficult to identify the zygomycetous fungi at the level of species because their detailed descriptions lack in literature. Interestingly, in the case of the species of *Mo. oligospora* EML-DDSF4, typical sporangia and sporangiospores were produced only on water agar medium. Only different shapes of chlamydospores, sporangiole-like structures and various stages for zygospore formation in EML-DDSF4 strain were observed on PDA medium.

Mortierellales constitutes one of the largest orders in the basal lineages. This group consists of one family and six genera. However, the phylogenetic position of Mortierellales is still controversial. Recently, only one species of *Mortierella alpina* was reported as new record in Korea [23]. Based on LSU polygram, *Mo. oligospora* belonged to group 6 including *alpina* and *polycephala*. *Mo. oligospora*, *Mo. bisporalis*, *Mo. hypsicladia*, *Mo. indohii*, *Mo. polygonia* and *Mo. reticulate* [22], which was well supported by our SSU, LSU, and ITS rDNA analysis. Many mortierellean species showed potential as very interesting fungi for biotransformations and biotechnological applications [24]. Kataoka *et al.* [25] have isolated endosulfan-degrading *Mortierella* species from a soil contaminated with organochlorine pesticides.

On the other hand, *Metarhizium* is a genus of entomopathogenic fungi in the Clavicipitaceae family in the phylum Ascomycota. The teleomorphs of *Metarhizium* species appear to be members of the genus *Metacordyceps* [26]. *Metarhizium anisopliae*, the type species of the anamorph of genus *Metarhizium*, contains four varieties, and is closely related to *Me. taii*, *Me. pingshaense*, and *Me. guizhouense* Bischoff *et al.* [17] has proposed to recognize *Me. guizhouense* as anamorph of *Metacordyceps taii*. As in many hyphomycetous genera, it may be still difficult to distinguish them. In this study, we could see that EML-MFS30-1 strain belongs to a *guizhouense* clade in the phylogenetic tree based on beta-tubulin gene and ITS rDNA sequences. Maniania *et al.* [27] have studied the potential of *Me. anisopliae* for the control of *Thrips tabaci* in onion agroecosystem. New source of chitosanase has been studied from *Me. guizhouense* under solid state fermentation conditions [28]. Temperature plays an important role in the physiological characteristics of entomopathogenic fungi. In our study, *Me. guizhouense* EML-MFS30-1 exhibited optimum growth on OA at 27°C and was unable to grow at 37°C. The results were similar to those by Ouedraogo *et al.* [29] who studied the optimal temperature for species of *Metarhizium*. Several species of *Metarhizium* have shown great potential for the management, as they produce variety of compounds including cyclic peptide, destruxins with insecticidal activity [30, 31].

Mortierellean fungi have important biotechnological applications as producers of polyunsaturated fatty acids, lipoxygenase enzyme [32, 33]. In particular, polyunsaturated fatty acids as arachidonic acid (5,8,11,14-ciseicosatetraenoic acid) is very important for maintaining biofunctions in mammalians [34]. However, the genus *Mortierella* also contains an animal pathogen, *Mo. wolfii* [35].

Fungal diversity may be affected by different climate conditions, soil conditions, and collection seasons. Thus, it is important to conduct more studies on seasonal distribution of fungi in Korea. The weather on the island is characterized by mostly snow in winter season and an oceanic climate that is affected by warm ocean currents. It is foggy and cloudy for more than 160 days a year [36]. Our soil samples were collected in August. However, the distribution of fungi may be different in other months. More studies on seasonal diversity of (dominant) fungi, their ecophysiological characteristics and bioactivities are merited for future studies.

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