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 Acrociopsis.

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, USA, 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 were also deposited at Microbiology Laboratory Fungal Herbarium, Chonnam National University, Gwangju, Korea. The EML strains, EML-MFS30-1 and EML-DDSF4, were also deposited at 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 Microsystems, 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 carboxylic buffer (Junsei). Cellular membranes were preserved by fixing the samples in 1% osmium tetroxide (diluted in carboxylate buffer; Electron Microscopy Sciences, Hatfield, PA, USA) for 1 hr, washed again in carboxylate 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

| Gene    | Product name | Primer     | Direction | Sequence (5'-3') | Reference |
|---------|--------------|------------|-----------|------------------|-----------|
| ITS     | ITS rDNA     | ITS-1      | Forward   | TCCGTAGGTTGACCTGCGG | [16, 18]  |
|         |              | ITS-4      | Reverse   | TCCCTCGCTTATTGATATGC | [16, 18]  |
| 18S     | SSU rDNA     | NS1        | Forward   | TGAATCATATGCTTGTCTC | [16, 18]  |
|         |              | NS4        | Reverse   | CTTCCGCTAATCTTTAGAG | [16, 18]  |
| 28S     | LSU rDNA     | LROR       | Forward   | ACCGCGCTGACCTTAAAGC | [16, 18]  |
|         |              | LR5F       | Reverse   | GCTATCCTAGGGAGAAC  | [16, 18]  |
| BTUB    | β-Tubulin    | BT2a       | Forward   | GGTAACCAATCGGTGCCTGGTTC | [17]     |
|         |              | BT2b       | Reverse   | ACCCTCAGTGATGACCTTGGC | [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. 5.09.1 [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

| Characteristics               | Present isolate | Me. guizhouense |
|-------------------------------|-----------------|-----------------|
| 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 |

1From 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 forming 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)

**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              | HQ3331444 |
|                   | ARSEF 1914     | Philippines       | Coleoptera     | EU248840              | HQ3331445 |
| *Me. guizhouense*  | ARSEF 4321     | Australia         | Soil           | EU248832              | HQ3331442 |
|                   | ARSEF 6238     | China             | Lepidoptera    | EU248830              | HQ3331447 |
| EML-MFS30-1       | Dokdo, South Korea | Soil   |                | KT374181              | KT374184 |
|                   | CBS 258.90     | China             | Lepidoptera    | EU248834              | HQ3331448 |
| *Me. pingshaense*  | ARSEF 4342     | Solomon Islands   | Coleoptera     | EU248821              | HQ3331454 |
|                   | CBS 257.90     | China             | Coleoptera     | EU248820              | HQ3331450 |
| *Me. robertsii*   | ARSEF 727      | Brazil            | Orthoptera     | EU248816              | HQ3331453 |
| *Me. anisopliae*  | ARSEF 7450     | Australia         | Coleoptera     | EU248823              | HQ3331464 |
|                   | ARSEF 7487     | Eritrea           | Orthoptera     | EU248822              | HQ3331446 |
| *Me. brunneum*    | ARSEF 2107     | USA               | Coleoptera     | EU248826              | KC178691 |
|                   | ARSEF 4152     | Australia         | Soil           | EU248824              | HQ3331452 |
| *Me. lepidotae*   | ARSEF 7488     | Australia         | Coleoptera     | EU248837              | HQ3331456 |
| *Me. acridum*     | ARSEF 324      | Australia         | Orthoptera     | EU248813              | HQ3331458 |
|                   | ARSEF 7486     | Niger             | Orthoptera     | EU248812              | HQ3331457 |
| *Me. globosum*    | ARSEF 2596     | India             | Lepidoptera    | EU248814              | HQ3331459 |
| *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. '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).

| Table 4. Mycological characteristics of *Mortierella oligospora* EML-DDSF4 and its closely related reference species |
|---------------------------------------------------------------|
| Characteristics                                           | Present isolate (WA) | Present isolate (OA) | *M. oligospora* (OA)* |
| Colony color                                                | White               | White                | -                     |
| Sporangiospores (μm)                                        | 8.0–14.3 (avr. 12.6) | -                    | 8.8–17.6 (avr. 14.3) x 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.
*From description of Mehrotra et al. [22].

**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
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

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**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. |
|---------------------|------------|-----------------|-------------------------------------------|-----------------------|
| *Mortierella alpina*| CBS 219.35 | Victoria        | Unknown                                   | JX976018 KC018359    |
|                     | CBS 384.71C| India           | Soil                                      | JX976098 JX976154    |
|                     | CBS 210.32 | Victoria        | Sandy loam soil                          | JX975853 IN940866 JQ040258 |
| *M. bisporalis*      | CBS 145.69 | Italy           | Unknown                                   | JX975857 KC018377    |
|                     | FSU 9675   | Italy           | Unknown                                   | JX975953 JX976176    |
| *M. elongatula*      | CBS 488.70 | Former West Germany | Municipal waste | JX975967 HQ667425 HQ67505 |
| *M. epigama*         | CBS 489.70 | Former West Germany | Municipal waste | JX976057 HQ667367 JQ040250 |
| *M. horticola*       | CBS 305.52 | Former West Germany | Unknown | JX975874 HQ667399 HQ67483 |
| *M. humilis*         | CBS 222.35 | Mexico          | Soil from *Pinus* forest                  | HQ630325 HQ67401 JQ040257 |
| *M. hypsicladia*     | CBS 116202 | Japan           | Bat dung in cave                          | JX975866 HQ667379    |
|                     | CBS 116203 | Japan           | Bat dung in cave                          | JX975872 KC018369    |
| *M. 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    |
| *M. oligospora*      | CBS 101758 | USA             | Supplement to mushroom culture            | JX976032 KC018327    |
|                     | CBS 191.79 | Sudan           | Soil                                      | JX975966 JX76151     |
|                     | CBS 381.71 | India           | Soil                                      | JX976033 KC018368    |
| EML-DDSF4            | Dokdo, South Korea | Soil           |                                           | KT374185 KT374182 KT374183 |
| *M. polycephala*     | CBS 327.72 | England         | Salt-marsh soil under *Spartina townsendii* | JX976085 JX761175   |
|                     | CBS 323.72 | UK              | Soil                                      | JX976102 KC018296    |
|                     | CBS 456.66 | Ukraine         | Dung of wood mouse                        | JX976034 KC018297    |
|                     | FSU 696   | Ukraine         | Unknown                                   | JX976035 HQ667409 HQ67493 |
| *M. polygonia*       | CBS 248.81 | Netherlands     | Clay soil under *Solanum tuberosum*       | JX975891 JX76145     |
|                     | CBS 685.71 | Netherlands     | Agricultural soil                         | JX975900 HQ667378 HQ67463 |
| *M. selenospora*     | CBS 811.68 | Netherlands     | Mushroom compost, together with *Entomophthora coronata* and *Aphanocladium album* | JX975875 HQ667419 HQ67499 |
| *M. 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 bay                               | HQ630304 HQ667381 HQ67465 |
| *Umbelopsis isabellina* | CBS 100559 | Wisconsin      | Soil                                      | IN943789 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-MFS301 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. Classification by Wagner et al. [16] and Petkovits et al. [18].](image-url)
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 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. indolii*, *Mo. polygonia* and *Mo. reticulata* [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 *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].

Mortierellales 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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