한 국 균 학 회 지 The Korean Journal of Mycology Research Note

Characterization of a Zygomycete Fungus, *Mortierella minutissima* from Freshwater of Yeongsan River in Korea

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ABSTRACT : During a survey of fungal diversity of the order Mortierellales, a zygomycete strain, EML-YS717-1, was isolated from a freshwater sample collected from the Yeongsan River in Gwangju, Korea. Based on its morphological characteristics and a phylogenetic analysis of the internal transcribed spacer (ITS1 and ITS2) and 5.8S rDNA sequences, the strain was identified as *Mortierella minutissima*. To the best of our knowledge, *M. minutissima*, has not previously been authentically reported in Korea.

KEYWORDS : Freshwater, *Mortierella minutissima*, Mortierellales, Undiscovered taxa

The genus *Mortierella* (Mortierellaceae, Mortierellales) was described by Coemans (1863) with the type species *Mortierella polycephala* Coem [1]. To date, nearly 100 species of *Mortierella* have been described [2]. The species belonging to this genus are characterized by the production of primarily coenocytic but irregularly septate mycelium. Sporangiophores are simple or variously branched terminating with sporangia and occasionally with a swelling at the base. Sporangia are globose, multi-, few- or uni-spored. *Mortierella* species typically exhibit rapid growth at temperatures ranging from 15°C to 25°C. They are frequently isolated from the soil and dead or dying plant, and tissues or from animal fecal samples [3, 4]. Many of them show potential as producers of polyunsaturated fatty acids [5, 6]. In addition, several species of *Mortierella* have been used as a pesticide degrading agent, suggesting that they might have potential for the bioremediation of sites contaminated with organochlorine pesticides [7].

Recently, molecular data have been used to evaluate the diversity of the genus *Mortierella* [2, 8]. Six species of the genus *Mortierella* have been reported in Korea [9, 10]. A new *Mortierella* species, *M. fluviae*, was isolated from a freshwater sample from Yeongsan River located in Gwangju, Korea in 2016 [10].

The objective of the present study was to perform the morphological and molecular analyses to characterize an unrecorded zygomycete species–*M. minutissima* in Korea.

Freshwater samples were collected from the Yeongsan River located in Gwangju (35°10'N, 126°55'E), Korea in February 2016. These samples were transported in sterile Falcon tubes, and stored at 4°C until use. A serial dilution technique was used to isolate fungi. In this technique, 1 mL of water sample was mixed with 9 mL of sterilized water. The water sample was serially diluted with sterilized water to obtain a concentration range from 10^{-1} to 10^{-4}. Subsequently, 0.1 mL of each dilution was transferred to potato dextrose agar (PDA; BD Diagnostics, Sparks, MD, USA) and incubated at 25°C for 3~7 days. Individual colonies of fungi that showed varying morphologies were picked up and purely transferred to another PDA plate. All pure isolates, including *M. minutissima* were maintained in PDA slant tubes and stored in 20% glycerol at -80°C at the Environmental Microbiology Laboratory Fungarium, Chonnam National University, Gwangju, Korea.

Genomic DNA was directly extracted from mycelia using the HiGene Genomic DNA prep kit for fungi (BIO-

Kor. J. Mycol. 2016 December, **44**(4): 346-349
https://doi.org/10.4489/KJM.2016.44.4.346
pISSN 0253-651X • eISSN 2383-5249

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Received October 30, 2016
Revised November 16, 2016
Accepted November 18, 2016

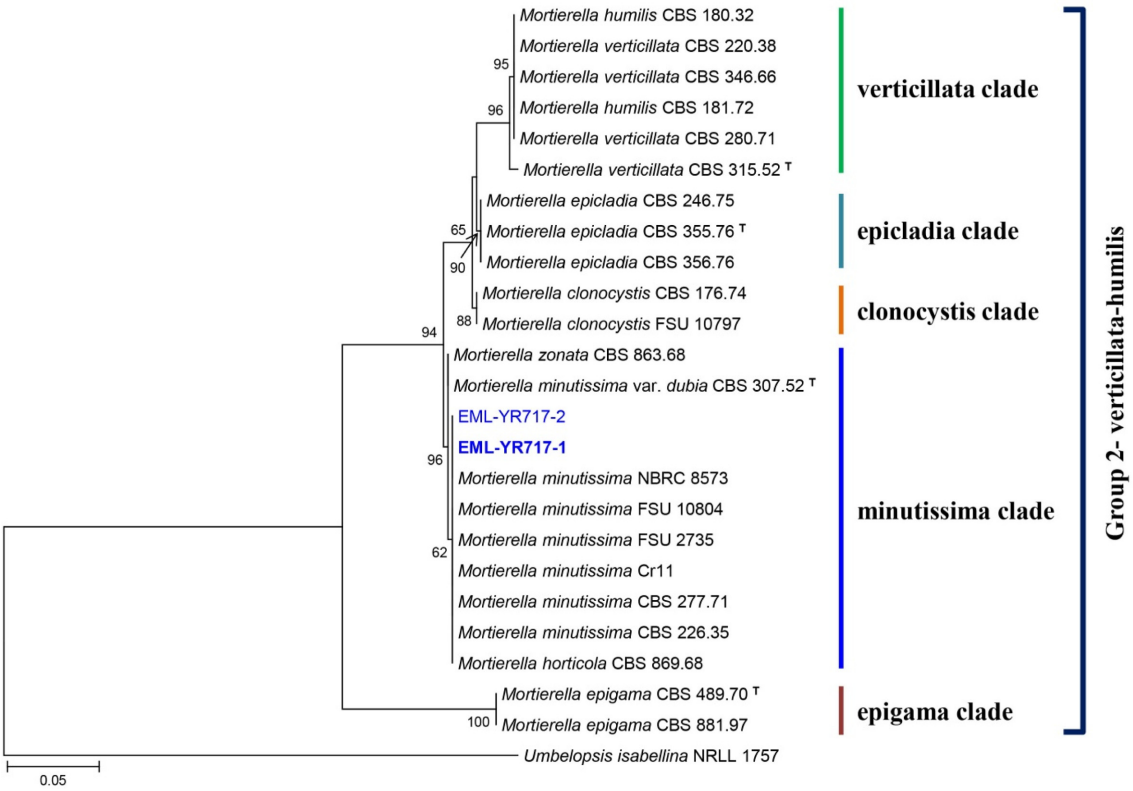

Fig. 1. Phylogenetic tree of *Mortierella minutissima* EML-YS717-1 and EML-YS717-2 and related species based on maximum likelihood analysis of internal transcribed spacer (ITS) rDNA sequences. Sequence of *Umbelopsis isabellina* was used as the outgroup. Bootstrap support values of ≥50% are indicated at the nodes. The bar indicates the number of substitutions per position. The group and clades on tree was named based on the classification system constructed by Wagner et al. [2].

FACT, Daejeon, Korea). The internal transcribed spacers (ITS1 and ITS2) and 5.8S gene were amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') following the method described by White et al. [11]. The sequences were initially aligned using CLUSTAL X [12], and edited manually [13]. Phylogenetic analyses were performed using MEGA 6 [14] with the default settings. Phylogenetic trees were constructed from the data using maximum likelihood (ML). The ITS sequences of EML-YR717-1 and EML-YR717-2 were deposited in the GenBank database with the accession numbers (KY056587 and KY056588, respectively). A BLASTN search revealed that the rDNA ITS homology of EML-YR717-1 and EML-YR717-2 represented 100% (584/584 bp) sequence identity value with *M. minutissima* (GenBank accession no. AB476417). Based on analysis of the ITS region, isolates were identified as *M. minutissima* (Fig. 1). To confirm the molecular species identification, we examined morphological features of the isolate EML-YR717-1. Cultural features were observed on PDA and water agar (WA). The plates were incubated at 20°C in the dark for 7 days. Samples were mounted on lactophenol solution (Junsei Chemical, Tokyo, Japan) and observed under a light microscope (DFC 290; Leica

Table 1. Morphological characteristics of the *Mortierella minutissima* EML-YS717-1 and other closely related species on water agar medium at 20°C

Characteristics	Present isolate	*Mortierella minutissima* [a]
Colony	White	White
Sporangia	Globose, 13.5~20.5 long × 13.0~5.0 wide μm	Globose, 11~24 μm in diameter
Sporangiospores	Globose to subglobose, 4.0~5.5 μm × 3.5~5.0 μm	Globose, 3.5~7 μm in diameter
Zygospores	Not observed	Present

[a]From description of Van Tieghem [15].

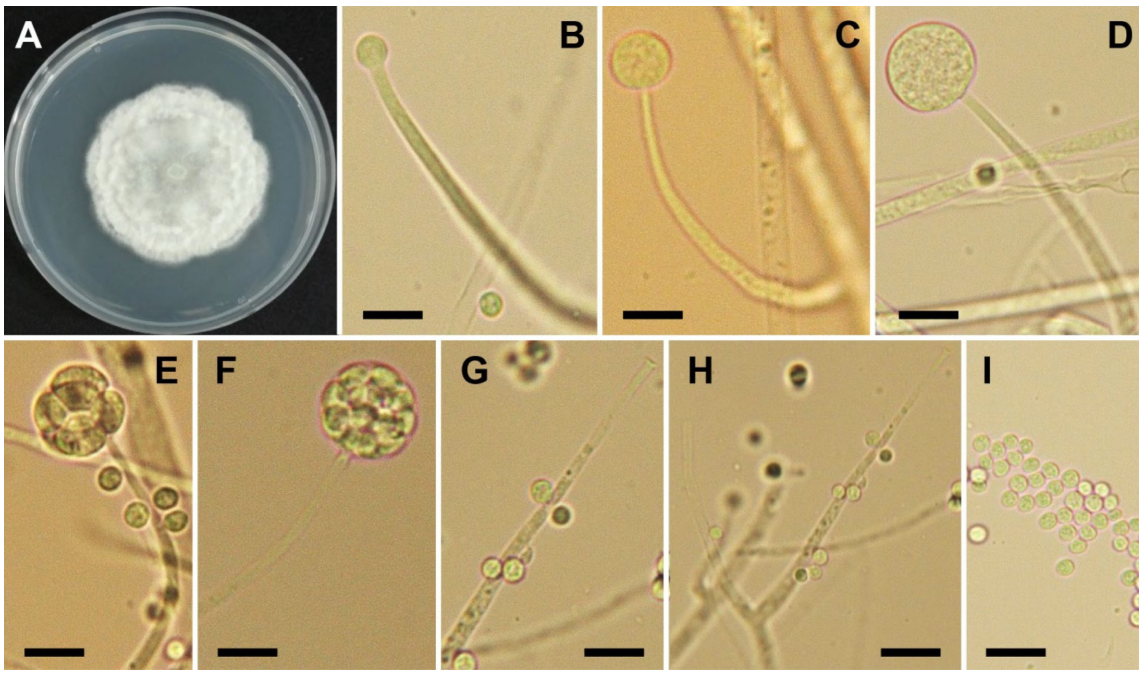

Fig. 2. Morphological characteristics of *Mortierella minutissima* EML-YS717-1. A, Colony in potato dextrose agar; B~F, Young and mature sporangia on sporangiophores; G, H, Single sporangiophore; I, Spores on water agar (scale bars = 20 μm).

Microsystems, Wetzlar, Germany).

Mortierella minutissima Tiegh., Annales des Sciences Naturelles Botanique 4: 385 (1878) (Table 1, Fig. 2).

Description: Colonies grew fast on PDA, cotton in the center with a white margin, the reverse white and irregularly zonate, reaching 56~59 mm diameter after 7 days of incubation at 20°C. For colonies grown on WA, aerial hyphae were dispersed on the agar surface. Sporangiophores were short and grew to a width of 2.0~3.5 μm. Sporangia measured 13.5~20.5 × 13.0~5.0 μm and were globose, having deliquescent walls. Spores were globose to subglobose and measured 4.0~5.5 × 3.5~5.0 μm. Zygospores were not observed. The isolate showed the best growth and abundant sporulation when grown on WA agar. Morphology of the present isolate was most similar to that of the previously described of *M. minutissima* [15].

Currently, Mortierellales contains only one family (Mortierellaceae) and six genera: *Aquamortierella*, *Dissophora*, *Modicella*, *Lobosporangium*, *Gamsiella*, and *Mortierella* [2]. The sporangial morphology of *Mortierella* is quite variable. Based on the morphological characteristics, Gams divided the subgenus *Mortierella* into nine sections: *Actinomortierella*, *Alpina*, *Haplosporangium*, *Hygrophila*, *Mortierella*, *Schmuckeri*, *Simplex*, *Spinosa*, and *Stylospora* [3]. Based on the sequences of the ITS rDNA regions, Wagner et al. [2] demonstrated that the genus *Mortierella* contains 7 groups: *selenospora* and *parvispora*; *verticillata-humilis*; *lignicola*; *mutabilis*, *globulifera* and *angusta*; *strangulata* and *wolfii*; *alpina* and *polycephala*; and *gamsii*. In the ITS tree, our strains, EML-YR717-1 and EML-YR717-2, belonged to group 2 (*verticillata-humilis*) as presented by Wagner et al. [2]. Furthermore, this fungus is morphologically most similar to *M. minutissima* placed in the *minutissima* clade. *M. minutissima* has been reported as a novel psychrotrophic fungus for biotransformation D-limonene and as a producer of arachidonic acid and dihomo-gamma-linolenic acid [16, 17]. This finding suggests that the strain EML-YR717-1 may be a useful source for biotransformations and biotechnological applications. Thus, potential application of *M. minutissima* EML-YR717-1 should be studied further.

Acknowledgements

This work was supported by the Project on Discovery of Fungi from Freshwater and Collection of Fungarium funded by Nakdonggang National Institute of Biological Resources (NNIBR) of the Ministry of Environment (MOE), and in part by a fund from National Institute of Animal Science under Rural Development Administration, Republic of Korea.

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