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Research Note

First Report of *Mortierella alpina* (Mortierellaceae, Zygomycota) Isolated from Crop Field Soil in Korea

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Abstract A new recorded species of *Mortierella* was recovered during the investigation of fungal communities in soil samples collected from different locations of Gangwon-do, Korea. The species was identified and described as *Mortierella alpina* on the basis of phylogenetic analysis of internal transcribed spacer sequences and morphological characteristics. This species has not been officially reported from Korea thus far.

Keywords Morphology, *Mortierella alpina*, Soil fungal community

The *Mortierellales* are a long-known, species-rich order of the basal fungi. With nearly 100 described species, the *Mortierellales* are one of the largest basal fungal orders. However, only 13 genera are described in one family, the Mortierellaceae [1]. The first species of the type genus was described by Coeman in 1863 as *Mortierella polycephala*, originally isolated from a mushroom [2]. The name *Mortierella* was given in tribute to M. Du Mortier, the president of Societe de Botanique de Belgique [2]. *Mortierella alpina* is an oleaginous fungus producing lipids accounting for up to 50% of its dry weight in the form of triacylglycerol, which is used commercially for the production of arachidonic acid [3]. During studies on the diversity of fungal communities in crop field soils of Gangwon-do, Korea, a species of *Mortierella* previously unreported in Korea was encountered. Based on its morphological and molecular characteristics, this species was identified as *M. alpina* and named *M. alpina* KNU13-5.

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Collection of soil samples and fungal isolation. Soil samples were collected from various parts of Taebaek, Gangwon-do, Korea in 2013. Each soil sample was taken from approximately 15-cm depth, air dried, and stored in plastic bags at 4°C until use. The fungi were isolated by conventional dilution technique and cultured on potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA) supplemented with 100 μg chloramphenicol (bacteriostatic agent)/mL PDA for 5 to 7 days at 28°C until growth of fungal colonies was observed.

Sequence analysis of internal transcribed spacers. Genomic DNA of isolate KNU13-5 was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The internal transcribed spacer (ITS1 and ITS2) regions, including the 5.8S rRNA gene were amplified with the primers ITS1 and ITS4 [4]. The amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. The PCR products were sequenced using the ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The sequences were compared with reference ITS1~ITS2 rDNA sequences from NCBI's GenBank using the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/Blast). The nucleotide sequence reported here has been

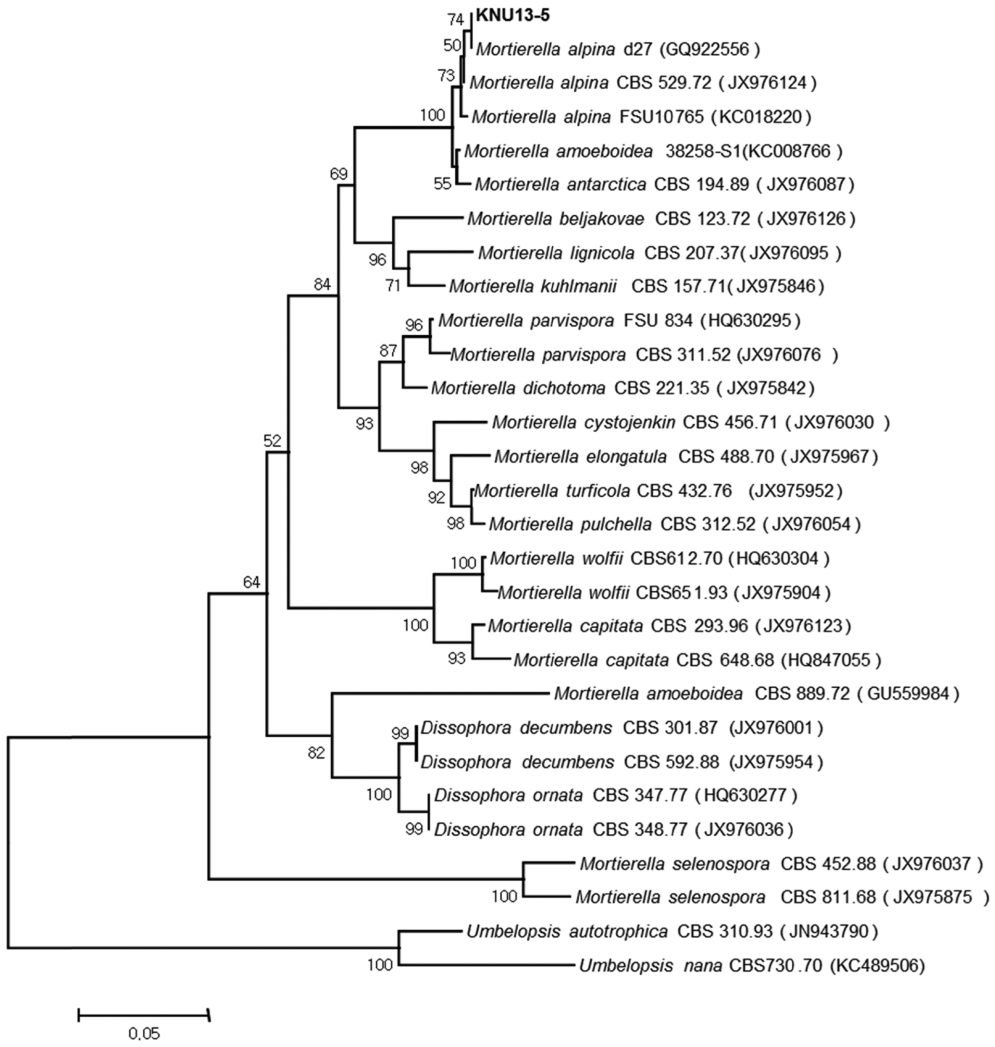

Fig. 1. Neighbor-joining phylogenetic analysis of the partial 18S-ITS1-5.8S-ITS2-28S rDNA sequence of *Mortierella alpina* KNU13-5 obtained from crop field soil in Korea. The phylogenetic tree was constructed using the MEGA 6 program. The sequence obtained in the study is shown in boldface. Numerical values (> 50) on branches are the percentage of 1,000 bootstrap replicates that support the branch. *Umbelopsis nana* and *Umbelopsis autotrophica* were used as outgroups. The scale bar represents the number of substitutions per site.

deposited at GenBank (accession No. KJ921606). The sequences of closely related strains were aligned using the MultAlin program. The DNA sequences were analyzed for phylogenetic relationships using molecular evolutionary genetic analysis (MEGA 6) software [5]. The sequence of isolate KNU13-5 was compared with the sequences in GenBank by using BLAST. The neighbor-joining tree was constructed using the Kimura 2-parameter substitution model [6]. The phylogeny of the tree was inferred using the maximum-likelihood heuristic search option with nearest-neighbor-interchange. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade. The ITS regions of isolate KNU13-5 were identical to those of *M. alpina* (Fig. 1). The ITS sequence (ITS1 and ITS2) of KNU13-5 was 99% identical to that of *M. alpina* d27 (accession No. GQ922556) [7]. These results indicated that isolate KNU13-5 matched with *M. alpina*.

Morphological characteristics and identification. Morphological features of the fungus were observed on PDA medium after three-point inoculations in 9-cm petri dishes and incubation in the dark at 28°C for 7 days. The morphological characteristics were identified with the aid of differential interference contrast microscopy, as detailed by Pitt [8] and Frisvad and Samson [9]. Photomicrographs were taken with a Kodak DCS 14n digital camera (Eastman Kodak Company, Rochester, NY, USA) attached to a compound microscope. Morphological structures of isolate KNU13-5 are shown in Fig. 2.

Colonies on PDA are fast growing, attaining 35~45-mm diameter after 7 days when grown at 28°C, producing a concentric pattern, sporulating well, and having *Mortierella*-like odor and milky-white color (Fig. 2A and 2B). Mycelia are septate and coenocytic with complex branching (Fig. 2C). Sporangiophores arising from the mycelial substrate

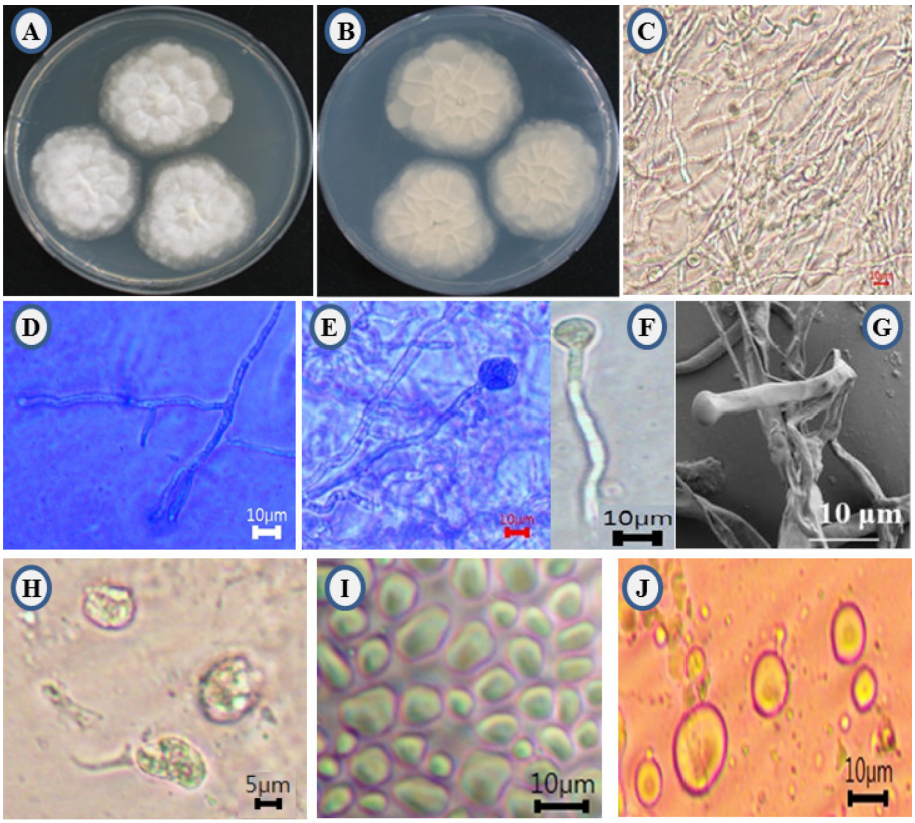

Fig. 2. *Mortierella alpina* KNU13-5. Colonies grown on potato dextrose agar (A, front view; B, back view) for 7 days at 28°C. Mycelial hyphae under a stereo-microscope (C), sporangiophore branching (D), sporangiophore with terminal sporangia (E, F), sporangiophore under scanning electron microscopy (G), sporangia (H), and sporangiospores (I, J).

are 60~110 μm in size, erect, and unbranched (sometimes dichotomously branched) with terminal sporangia (Fig. 2D, 2E, 2F, and 2H). Sporangia are hyaline, obovoid when young and spherical at maturity, 12~15 μm in diameter, and multispored with a deliquescent wall (Fig. 2H). Sporangiospores are cylindrical, 5~7 μm in size, and sometimes curved to irregularly shaped (Fig. 2I and 2J). In conclusion, the phylogenetic analysis and morphological characteristics of strain KNU13-5 indicated it to be *M. alpina*.

Culture examined. KNU13-5, isolated from soil samples, Taebaek, Gangwon-do, Korea.

Note. Shin *et al.* [10] described the morphological characteristics of the species *M. alpina* S49, but the identification was tentative, and they did not perform genetic analysis. There have been limited studies on *M. alpina* in Korea with respect to arachidonic acid production efficiency and its effect on the growth and learning ability in animals; furthermore, the source of the isolates was not indicated [11, 12]. In other cases, *M. alpina* DSA-12 was used for the production of arachidonic acid by using an organic nitrogen source and optimizing the culture conditions [13, 14]. However, strain DSA-12 was obtained from Doosan Biotech, Korea, and the mycological information on the strain has not been officially reported in any publication. Furthermore, in a study on submerged and solid-state fermentations by the species for production of arachidonic acid, the isolate used was obtained from the American Type Culture Collection (ATCC) collection [15]. Therefore, to the best of our knowledge, this is the first authentic, official report of *M. alpina* in Korea.

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