A New Report on *Oidiodendron flavum* Isolated from Field Soil in Korea

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**ABSTRACT :** *Oidiodendron flavum* KNU13-6 was isolated for the first time from field soil in Korea and identified based on the internal transcribed spacer region (ITS) of rDNA and morphological characteristics. Based on phylogenetic analysis of ITS and morphological characteristics, the species has not been previously reported in Korea.

**KEYWORDS :** Molecular identification, Morphology, *Oidiodendron flavum*, Proteolytic activity

*Oidiodendron flavum* Svilv., Lent. Bakto. belongs to the genus *Oidiodendron*, a cosmopolitan genus whose members can usually be found in a wide range of habitats, including soils, different cellulose substrates (litter, wood pulp, bark, mosses, paper), and occasionally from lichens or from air [1]. Some *Oidiodendron* species have been reported as ericoid mycorrhizal fungi [2]. Plants in the Ericaceae have a distinctive ericoid mycorrhizal association, which plays important roles in plant growth, nutrient uptake, and soil mineralization [3]. The ability of some ericoid mycorrhizal fungi (*Oidiodendron maius*) to dissolve Zn oxide has been reported [6]. In addition, ericoid mycorrhizal fungus, *Oidiodendron cf. truncatum* have medical value as novel antifungal agent producers used in treatment of life-threatening fungal infections in immunocompromised hosts such as human immunodeficiency virus (HIV) infected persons and cancer patients [7]. Among the species of *Oidiodendron*, *O. flavum* is a thermophillic fungus with the capacity for production of thrombolytic agents utilized in treatment of thrombosis [8,9]. The fibrinolytic enzyme obtained from thermophilus fungus *O. flavum*, exhibits a profound fibrinolytic activity and also exhibits relatively high pH and temperature stabilities [10]. Tahany *et al.*, [8] also reported that a *O. flavum* released maximum amounts of either ammonia, peptides, or total soluble nitrogen. Thus, there has been considerable recent interest of mycologists in working with *O. flavum*.

During the studies of fungal diversity in agricultural soils in Korea, a species of *Oidiodendron* was discovered that was not previously reported in Korea. Based on morphological and molecular characteristics, this species was identified as *O. flavum*.

**Collection of soil samples and fungal isolation.** Soil samples were collected from different locations in Taebaek city, Korea in 2013. Soil from (0-15) depth, air dried and stored in plastic bags at 4°C until used. The fungi were isolated by conventional dilution and supplemented with 100 µg chloramphenicol per mL potato dextrose agar (PDA; Difco Laboratories, Detroit, USA) and grown for 7 d at 28°C until the growth of colonies was observed.

**ITS sequencing analysis.** Genomic DNA of the strain was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The ITS regions, including the 5.8S were amplified with the primers ITS and ITS4 [11] The amplified PCR product was purified using a QIA quick PCR purification Kit (Qiagen, Valencia, CA, USA) following the manufac-
turer’s recommendations. The PCR product was sequenced using an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster city, CA, USA). The sequence was compared with reference ITS1-ITS4 rDNA sequences in GenBank using BLAST analysis (http://www.ncbi.nlm.nih.gov/blast). The sequences of closely related strains were aligned using the MultAlin program. The DNA sequences were analyzed for phylogenetic relationship using Molecular Evolutionary Genetics Analysis (MEGA 5) software [12] The sequence of present isolate, KNU13-6, was compared with the sequences in GenBank using Basic Local Alignment Search Tool (BLAST). Neighbor-Joining tree was constructed using Kimmura 2-parameter substitution model bootstrap analysis was performed with 1,000 replications in order to determine the support for each clade. ITS regions of the KNU13-6 were 100% identical to the culture collection of O. flavum (accession no. KJ921607) [13] (Fig. 1). Phylogenetic tree of the ITS regions of the isolate (KNU13-6) was identical to O. flavum with 97% bootstrap value support (Fig. 1). The results strongly suggest that the isolate is O. flavum. Consequently, the nucleotide sequence of the isolate reported here has been registered in the NCBI GenBank (Accession no. KJ921607).

Morphological characteristics and identification. Morphological features were observed on potato dextrose agar

Table 1. Morphological characteristics of *Oidiodendron flavum* isolated in this study

| Characteristics |  *O. flavum* isolated in this study |  *O. flavum* isolate reported here |
|-----------------|------------------------------------|----------------------------------|
| Colony Texture  | Colonies on PDA are limited in growth, non-aerial and radially sulcate | Colonies on PDA are limited in growth, non-aerial and partially ropy |
| Colony Color    | Pale brown and cream               | Yellowish brown                  |
| Conidiophores   | Conidiophores hyaline, branched oppositely and verticillately in the median, bearing conidia apically and denticulate after detachment of conidia and | Conidiophores brown, erect branched alternately, oppositely or rarely verticillately in the median, bearing conidia apically, apparently dendroid like |
| Size (µm)       | 60.0–90.0                          | 62.5–87.5                        |
| Conidia Shape and position | Hyaline, irregular, one celled. Conidial chains straight and readily detached | Hyaline, globose or irregular, occasionally with a fragment of conidiophores, one celled and readily detached |
| Size (µm in diam.) | 2.0–3.8 × 2.5–3.5 | 1.8–3.8 |

Source of description (Barron, 1962; Watanabe et al., 1986a).

Fig. 1. Neighbor-joining phylogenetic analysis of *Oidiodendron flavum* KNU13-6 partial 18S-ITS1-5.8S-ITS2-28S rDNA region sequence obtained from crop field soil in Korea. The sequence obtained in the study is shown in boldface. Numerical values (>50) on branches are the bootstrap values as percentage of bootstrap replication from 1,000 replicate analysis. *Leotiomycetes* sp. (JF273533) was used as the outgroup.
(PDA) by doing three point inoculations in 9 cm petri plates which were incubated in the dark at 28°C for 7 days. The morphological characteristics were identified with the aid of differential interference contrast microscopy. Photomicrographs were taken with a Kodak14n digital camera attached to the microscope. Slide material was mounted in water and sometimes with aniline blue staining. Colonies on PDA were slow growing, pale brown, cream, attaining 20–30 mm after growing for 10 days at 28°C. Conidiophores were hyaline, branched alternately, oppositely or rarely verticillately in the medium (Fig. 2). Conidiophores arising from the mycelial substrate, 60-90 µm tall from base to branching site. Conidia 1.8-3.8 µm in diameter. Conidia arthrospores, hyaline, globose or irregular, occasionally with a fragment of conidiophore, one celled readily detached (Fig. 2). Conidia were hyaline, irregular, 2-3.8 × 2.5-3.5 µm in diameter. Morphological characteristics of the isolate agreed with the description of *O. flavum* [9,15]. Based on the phylogenetic analysis and morphological characteristics of strain KNU 13-6 was *O. flavum*.

In conclusion, we identified and described *Oidiodendron flavum* KNU13-6 as an unrecorded species in Korea. The species of *Oidiodendron* have the ability to produce phytohormones, solubilize insoluble phosphate and convert complex organic substances to simple forms and fibrinolytic enzymes. Thus, in the future, further investigation in this respect would be worthwhile.

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**Fig. 2.** Morphological characterization of *Oidiodendron flavum* KNU13-6 observed using a compound microscope and scanning electron microscope (SEM). A, Colony in front; B, Colony in reverse; C, Conidiophores (Compound microscope image; bar = 10 µm). D and E, Conidiophores and conidia (SEM micrograph; bar = 2 and 10 µm), and F, Conidia (SEM micrograph; bar = 20 µm).
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