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Research Note

Ophiostoma ips from Pinewood Nematode Vector, Japanese Pine Sawyer Beetle (*Monochamus alternatus*), in Korea

Dong Yeon Suh[1], Min Woo Hyun[1], Jae Jin Kim[2], Seung Yeol Son[1] and Seong Hwan Kim[1,*]

[1]*Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan 330-714, Korea*
[2]*Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Korea*

Abstract Japanese pine sawyer beetle (*Monochamus alternatus*) is an economically important pest in coniferous trees. *Ophiostoma ips* was isolated from the beetle and identified based on analysis of morphological properties and the β-tubulin gene sequence. The fungus easily produced perithecia with a long neck on malt extract agar and its ascospores were rectangular shaped. This is first report of *Ophiostoma* species associated with the pinewood nematode vector beetle in Korea.

Keywords Japanese pine sawyer beetle, *Ophiostoma ips,* Pinewood nematode disease, Sapstaining fungus

The pinewood nematode (PWN), *Bursaphelenchus xylophilus* [1], is a causative agent of pine wilt disease and a major forestry pest in China, Japan, Korea and Europe. It is well known that fungi is involved in the life cycle of PWN disease. Fungi associated with the Japanese pine sawyer beetle (JPS, *Monochamus alternatus*), the insect vector of PWN [2], are known to be the food source for JPS. In addition, JPS is known to be the primary vector of PWN in Korea [3]. However, it is not yet known which fungal species are associated with the ecosystem of PWN in Korea. In a recent survey of fungi associated with the PWN life cycle eight genera, including *Ophiostoma*, were isolated from JPS [4]. This study was undertaken to identify one of the Ophiostoma species associated with JPS. Here, we report that *Ophiostoma ips* is one of the fungal associates of the PWN vectoring cerambycid beetle in Korea.

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***Corresponding author**
E-mail: piceae@dankook.ac.kr

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Initially, three *Ophiostoma* isolates with very similar colony morphology were selected for this study from the fungal cultures isolated from JPS in the previous study [4]. When we observed their mycelial and spore structures in detail using microscopes, the three isolates were almost the same. Thus, one of the isolates was selected at random, denoted as DUCC1302 and used for identification. To accomplish this, micromorphological characteristics of the isolate DUCC1302 were observed using a phase-contrast microscope (Axioskop 40; Carl Zeiss, Jena, Germany), a dissecting microscope (SZ2-ILST; Olympus, Tokyo, Japan)

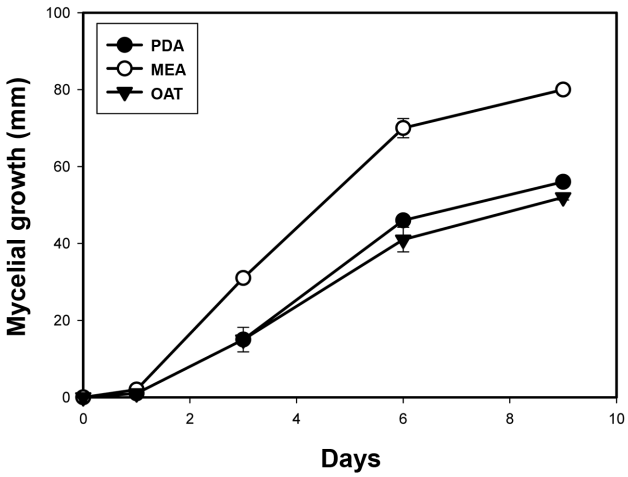

Fig. 1. Mycelial growth of *Ophiostoma ips* DUCC1302 on different nutrient media. The fungus was grown on each media at 25°C for 9 days, PDA, potato dextrose agar; MEA, malt extract agar; OAT, oat meal agar.

and a scanning electron microscope (Hitachi S-4300; Hitachi, Tokyo, Japan). Examination of the fungal structures was conducted using fresh materials prepared on malt extract agar (Oxoid, Hampshire, UK) at 25°C for 7~14 days. The isolate grew better on malt extract agar (MEA) than potato dextrose agar (Difco, Detroit, MI, USA) or oatmeal agar (Fig. 1). Moreover, the isolates were able to grow in the presence of high concentrations of cycloheximide (250 μg/mL) at 25°C in the dark. The colony color was dark to hyaline on MEA (Fig. 2A), and the fungus could easily produce perithecia with a long neck from a single spore (Fig. 2B), indicating that it is a homothallic species. Perithecial bases were globose, dark brown to black, and 150~200 mm in diameter. Perithecial necks were dark-brown with ornamental hyphal elements, tapering towards the tip and (135~) 520 (~850) × (9~) 33 (~63) mm long. Ascospores were one-celled, rectangular, pillow-shaped in side or plain view, quadrangular in end view and (3~) 3.9 (~4) × (2~) 2.4 (~3.2) mm long (Fig. 2C). The ascospores of the fungus resembled those of *Ophiostoma bicolor*, *O. ips*, and *O. montium* [5].

Conidia were enteroblastic or phialidic. Each conidium was single-celled, oblong with obtuse to truncate ends or obovoid, (4~) 9 (~20) × (1~) 2.5 (~4) mm long, and accumulated in a mucilaginous head (Fig. 2D and 2E). Conidiophores were synnematous, sporodochial, or absent (Fig. 2F). Synnemata were hyaline to cream-colored or pale brown, occasionally with synnematous-like groups of

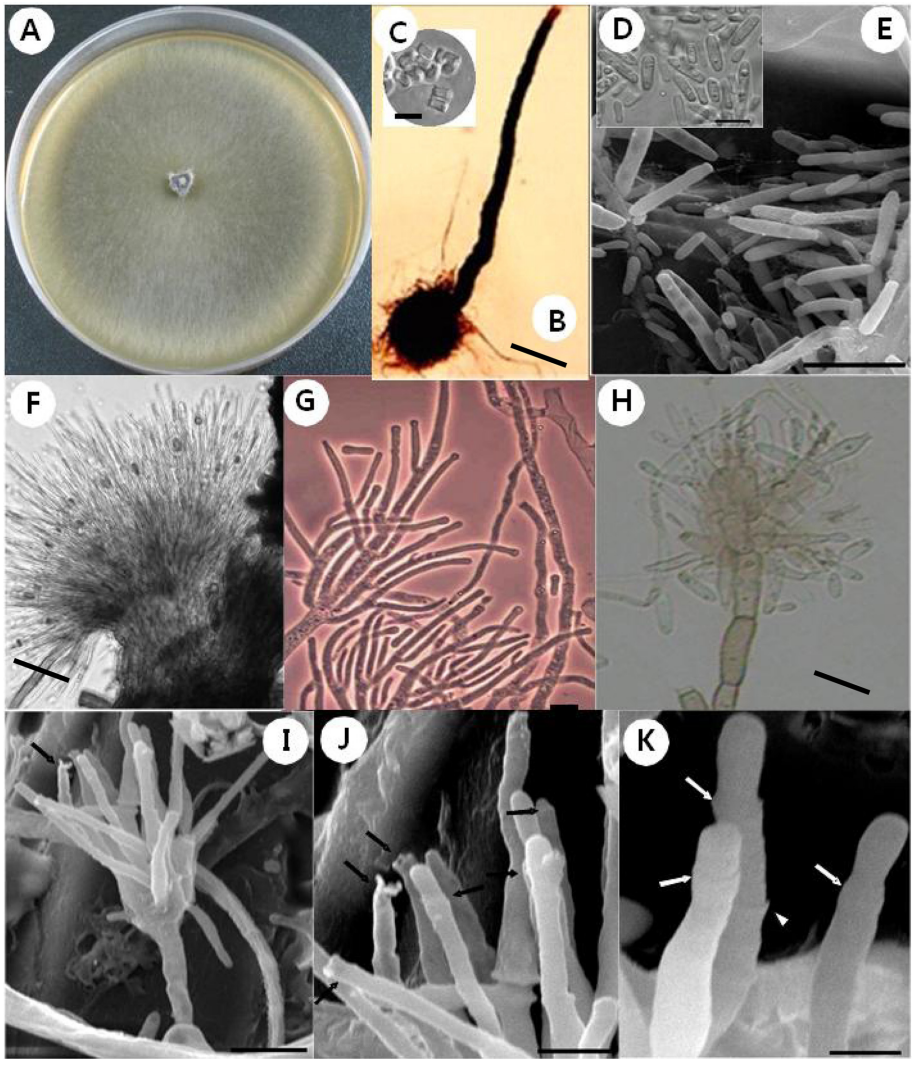

Fig. 2. Morphology of *Ophiostoma ips* DUCC1302. A, Colony characteristics of samples grown on 2% malt extract agar at 25°C for 9 days; B, Light micrograph of a perithecium; C, Scanning electron micrograph of ascospores; D, Light micrograph of conidia; E, Scanning electron micrograph of conidia; F, Scanning electron micrograph of *Graphilbum synanamorph*; G, Light micrograph of conidiogenous cells showing straight, elongate phialides and phialidic conidiogenesis; H, Light micrograph of conidiophore and conidiogenous cells showing relatively short phialides with collarettes; I~K, Scanning electron micrograph of conidiophore and conidiogenous cells showing phialides with broadly flared collarettes and phialidic conidiogenesis (arrows), annellations (arrowhead) and usually long proliferations (scale bars: B = 100 μm, C, J = 5 μm, D~I = 10 μm, K = 2 μm).

conidiophores having a curled base area. Sporodochial conidiophores were produced in an inverted conical shape from a small aggregation of prostrate hyphae embedded in the agar (Fig. 2G~2I) [6]. The conidiophores were erect or lax, up to (30~) 55 (70) mm long including the conidiogenous apparatus and had branches in 1~4 series. Conidiogenous cells were phialidic and annellidic, usually percurrent, cylindrical, ampulliform to lageniform, sometimes subulate in old culture, with collarettes (Fig. 2H~2K). These anamorphic structures look like mixed features of two different types including *Graphilbum* synanamorph. Among *Ophiostoma bicolor*, *O. ips*, and *O. montium*, which have sexual spores resembling DUCC1302, only *O. ips* has been reported to have different anamorphic structures such as *Graphilbum*, and *Acremonium* [5, 6] or *Hyalorhinocladiella* [7].

To identify the fungus at the molecular level, genomic DNA of DUCC1302 was extracted using the method described by Kim *et al.* [8] and the β-tubulin gene was amplified by PCR using primers T10 and BT12 [9] and sequenced. A BLAST search of the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) using the determined sequence revealed that the highest similarity (98%) was with that of *Ophiostoma ips* (Rumbold) Nannf; therefore, we identified DUCC1302 as *Ophiostoma ips*. The determined β-tubulin gene sequence was deposited in the GenBank database under accession number KC588951 and a voucher specimen was deposited in the Dankook University Culture Collection (DUCC 1302).

O. ips is well known not only as a sapstainer, but also as a tree pathogen [10, 11]. Recently, *O. ips* has been reported in Korea from Japanese red pinewood [12]. However, the report only described telemorphic features of the species, even though it has two or three anamorphic features. In this study, we described the anamorphic features of *O. ips* in detail to help understand its fungal structure. In general, Ophiostoma are insect vectored fungal groups. Previously reported isolates showed a reddish brown colony color on MEA plates. Since the reddish brown colored *O. ips* was isolated from *Tomicus piniperda* beetle-infested wood, *T. piniperda* was assumed to be a vector of *O. ips* in Korea. In contrast to the reddish isolate, *O. ips* DUCC1302 showed dark brown to blackish colony color and was isolated from *M. alternatus*. Due to identification of *O. ips* from *M. alternatus* in this study, we could easily identify *O. ips* from Ophiostomatoid fungi obtained from PWN diseased Japanese black pine, Japanese red pine, and Korean pine (unpublished data). Since *M. alternatus* is the vector of nematode disease, we are certain that *O. ips* is a fungal associate of *M. alternatus* in Korea. Our certainty is supported by the fact that *O. ips* was identified from pupal chambers of the beetle from *Bursaphelenchus xylophilus*-infested Masson pine in Zhejiang in 2007, China [13]. Meanwhile, in Japan, *O. minus* has been reported as a fungal associate of *M. alternatus* and a food source for the beetle and pine wood nematode [14]. Currently, not much information is available regarding the association of *O. ips* with *M. alternatus* and its role(s) in the PWN disease cycle in nature. Further work is needed to explore this association. We expect that this report will be valuable to individuals involved in forest pathology and the wood products industry domestically and internationally.

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