

Ophiostoma ips Isolated from Reddish Brown Stained Japanese Red Pine Wood

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Wood discoloration caused by sapstain fungi is one of the problematic issues in the forest products industry. Sapstain fungi include tree pathogens belonging to *Ophiostoma*, *Ceratocystis*, and *Grosmannia* genera. During a recent survey on sapstain fungi occurring in Japanese red pine (*Pinus densiflora*) logs and lumber in Korea, deep reddish brown stain that had never been reported in wood stain damage was observed. We isolated and identified *Ophiostoma* fungus from the region of reddish brown stain (Fig. 1). A voucher specimen was deposited at Korean Agriculture Culture Collection, Suwon, Korea (KACC 43403).

The fungus grew optimally at 30°C on 2% MEA reaching up to 13 mm in diameter in one day. No growth was found at either below 5°C or above 45°C. The fungus was able to grow on MEA containing high concentrations of cycloheximide (250 µg/ml). On MEA, colony color was pale brown to hyaline and hyphae of the fungus were immersed (Fig. 2). Inoculation test results showed that the fungus has the ability to stain Japanese red pine sapwood wafers causing discoloration on wood surfaces (Fig. 3). However, the color produced on the wafers was closer to dark-brown rather than reddish brown. From a single spore, the fungus developed perithecia with long neck on MEA (Fig. 4), indicating that it is a homothallic *Ophiostomatoid* species. Perithecia were black to light brown at the apex and (150–)550(–900) × (10–)35(–60) mm long. Perithecial bases were globose, dark brown to black, and 150–200 µm in diameter and had dark-brown ornamenting hyphal elements. Ostiolar hyphae were absent in the neck (Figs. 5, 6). Perithecial necks were straight (Figs. 4, 7) or curved (Fig. 8), tapering towards the tip. Ascospores had hyaline gelatinous sheaths (Fig. 9) and were one-celled, rectangular, pillow-shaped in side or plain view, quadrangular in end view and (3–)4.2(–5) × (2–)2.8(–3.5) µm long (Fig. 10). The ascospores of the fungus resembled those of *Ophiostoma ips*,

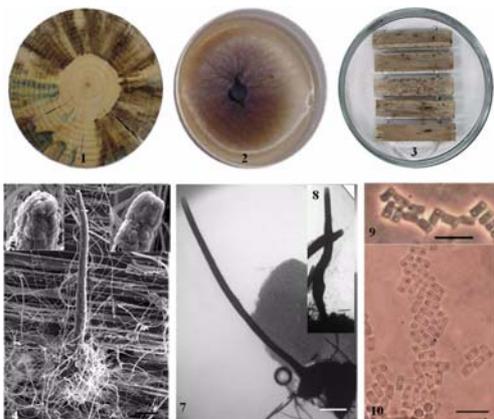


Fig. 1. A cross sectioned Japanese red pine disk with reddish-brown stain. **Fig. 2.** Colony characteristics of *Ophiostoma ips* grown on 2% MEA at 25°C for 5 days. **Fig. 3.** Inoculation test of *O. ips* on wood wafers. Discolored stain was formed on the surface of all the wood wafers. **Figs. 4–10.** *O. ips* teleomorph. **Figs. 4–6.** SEM micrograph of perithecia and ostiolar hyphae. **Figs. 7–10.** Light microscopic micrograph of perithecia and ascospores. Scale bars: 10 µm for **Figs. 4, 6–10.**

O. montium, and *O. bicolor* group (Upadhyay, 1981).

To identify the fungus at a molecular level, 772 bp size of partial β -tubulin gene was PCR amplified with primers T10 and BT12 (Kim et al., 2010) and sequenced. Similarity search through GenBank database showed the determined sequence shares 100% sequence identity with that of known *Ophiostoma ips* (AY194952). A phylogenetic tree was constructed using Phylogenetic Analysis Using Parsimony (PAUP*) by the neighbor-joining method with the heuristic search option (Swofford, 2002). The fungus positioned with *O. ips* (Fig. 11). This molecular data confirmed the fungus is *O. ips*. This is first report of *O. ips* in Korea.

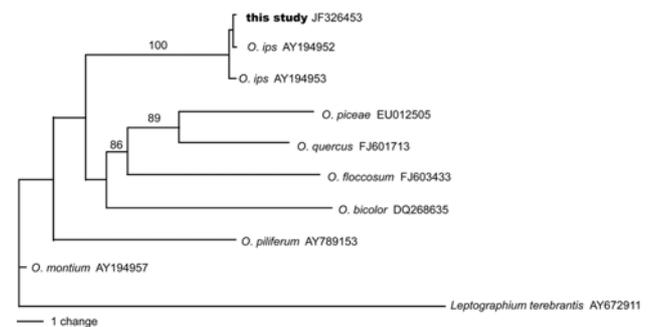


Fig. 11. Phylogenetic position of the *Ophiostoma* species isolated from reddish brown stain. Phylogenetic tree was constructed based on β -tubulin gene sequences by the neighbor joining method. Numbers above the node represent the bootstrap values.

With the occurrence of reddish brown stain, the cosmetic damages of pinewood products could be more serious in Korea. Thus, understanding of the spreading mechanism of *O. ips* is of great significance. *O. ips* is known as a bark beetle-associated fungus. Since we isolated the *O. ips* fungus from *Tomicus piniperda* beetle-infested Japanese red pinewood, *T. piniperda* would be an *O. ips* vector. Further works are needed to verify this surmise.

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