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RESEARCH NOTE

Occurrence of *Sporendocladia bactrospora* on *Quercus variabilis* in Korea

Dong-Hyeon Lee, Sang-Tae Seo, Sang-Hyun Lee, Seung Kyu Lee, Sun Keun Lee*

Division of Forest Diseases and Insect Pests, National Institute of Forest Science, Seoul 02455, Korea

*Corresponding author: lskyou@korea.kr

Abstract

A survey to assess the diversity of wound-associated Ophiostomatales and Microascales, the so-called ophiostomatoid fungi, on Korean native trees, was undertaken in 2017. Wounds were artificially created, and a fungus resembling a species of *Sporendocladia* was consistently isolated from the exposed cambium and inner bark of artificially induced wounds on *Quercus variabilis*. Morphological examination and DNA sequence comparisons based on the internal transcribed spacer (ITS) and 5.8S regions of the rDNA confirmed the identity of the fungus as *Sporendocladia bactrospora*. This is the first report on *S. bactrospora* occurring on *Q. variabilis* in Korea.

Keywords: *Leptographium*, Ophiostomatoid fungi, *Phialocephala*, Wound

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The genus *Phialocephala* was first established by Kendrick [1] to accommodate fungi that produce a dark mononematous conidiophore and a conidiogenous head consisting of one to several series of penicillate branches terminating in metulae and phialides. In this regard, some *Phialocephala* species were originally treated as a species of *Leptographium* [1-4]. However, due to the different type of conidiogenesis in *Sporendocladia bactrospora* (W.B. Kendr.) M.J. Wingf., which was treated in the *Leptographium* complex of asexual fungi [1, 3, 4], and other species of *Phialocephala* and *Leptographium*, Wingfield et al. [3] transferred five *Phialocephala* species to *Sporendocladia*.

Sporendocladia bactrospora is a wood-inhabiting ascomycete that is generally regarded as a saprophytic fungus associated with decayed plant materials [1] or trunks of living trees [5]. Although species of this group have not been studied in detail, various tree species were reported as host for *S. bactrospora* in many countries. These include *S. bactrospora* from *Quercus suber* L. in Spain [5], *Tilia* sp. in England [1], *Populus trichocarpa* Torr. & A. Gray and *P. tremuloides* Michx. in Canada [1, 6], and *Betula* spp., *P. tremula* L., and *Quercus* sp. in Norway and Sweden [7].

Recent surveys aiming to identify ophiostomatoid fungi on oak trees in Korea consistently recovered a species of *Sporendocladia* from artificially induced wounds on *Q. variabilis* Bl. in Gangneung, South Korea (Fig. 1A). To ensure the correct identity of the

fungus isolated in this study, its morphological characters were examined, and DNA sequence comparisons based on the ITS and 5.8S regions of the rDNA were performed. All isolates obtained in this study were deposited at the National Institute of Forest Science (CDH003, CDH004 and CDH005).

To examine the morphological characteristics of the fungal structures, single spore isolates, CDH003, were made on malt extract agar (MEA; 20 g Bacto malt extract and 20 g Bacto Agar) and incubated at 25°C for two weeks in the dark condition (Fig. 1B). A detailed microscopic examination was performed using a Zeiss AX10 microscope, and bright-field and differential-interference contrast micrographs were captured using an AxioCam MRc5 camera (Carl Zeiss, Oberkochen, Germany). At least 30 measurements were made for each structure.

The conidiophore was macronematous, mononematous, smooth, multiseptate, solitary, erect, straight, thick-wall, 70~190 μm high, 3.5~5 μm wide at the first septum, dark brown becoming light toward the apex, tapering toward the end or the middle, and arising vertically or laterally from the mycelium (Fig. 1C). The conidiogenous head at the apex of the conidiophore consisted of branches with 1~9 series of terminal phialides. Phialides were lageniform, straight to slightly curved, thick-walled, light brown, 7~9.5 μm long, and 3~5 wide (Fig. 1C). Conidia arranged in false chains were oblong, non-septate, smooth, and 2.1 × 3.9 μm (Fig. 1C, 1D).

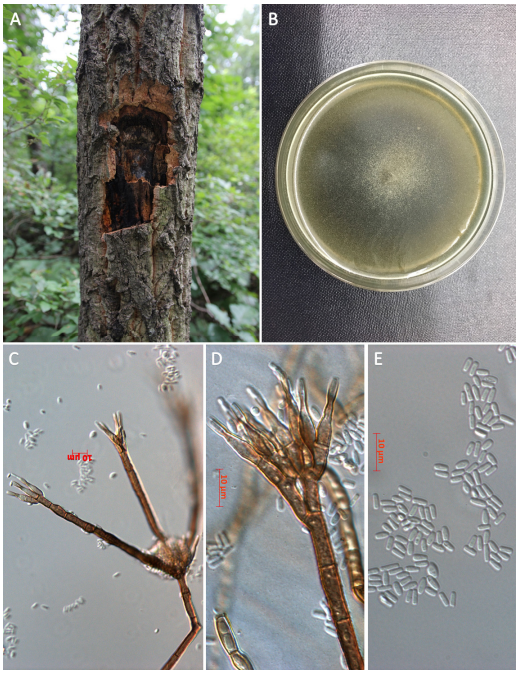

Fig. 1. Artificially induced wound on *Quercus variabilis* colonized with *Sporendocladia bactrospora*. A, Wound created on *Q. variabilis*; B, Colony of *S. bactrospora* on MEA; C, D, Conidiophore of *S. bactrospora* and conidia in germination; E, Oblong conidia of *S. bactrospora*; MEA, malt extract agar.

In addition to the morphological identification, DNA sequence comparisons were conducted to ensure the correct identity of the fungus. DNA sequence analysis was carried out following the techniques described by Lee et al. [8] based on the ITS1 and ITS2 and the 5.8S rDNA [9, 10]. The identification based on the morphology was supported by the ITS sequencing data, which showed that the maximum sequence identity percentage of the isolates was identical (100%) when BLASTn searched against the nucleotide database of the National Center for Biotechnology Information (NCBI, http://blast.st27va.ncbi.nlm.nih.gov/Blast.cgi), and against the nucleotides of *S. bactrospora* strains deposited at the NCBI. All sequence data obtained in this study have been deposited in the NCBI database (accession numbers: MF967564 and MF967565).

To construct a phylogenetic tree, the obtained sequences were aligned with ten published sequences of *Sporendocladia* species retrieved from GenBank using the online version of MAFFT ver.7.215 (http://mafft.cbrc.jp/alignment/server/) [11]. Maximum likelihood (ML) analysis was performed using RAxML HPC BlackBox ver.8.1.11 [12, 13] using default options with the general time reversible (GTR) substitution model implemented in the CIPRES cluster server (https://www.phylo.org/) at the San Diego Supercomputing Center. ML analysis resulted in a well-supported placement of the isolates obtained in this study (CDH003, CDH004) with the authenticated *S. bactrospora* isolates retrieved from GenBank (Fig. 2).

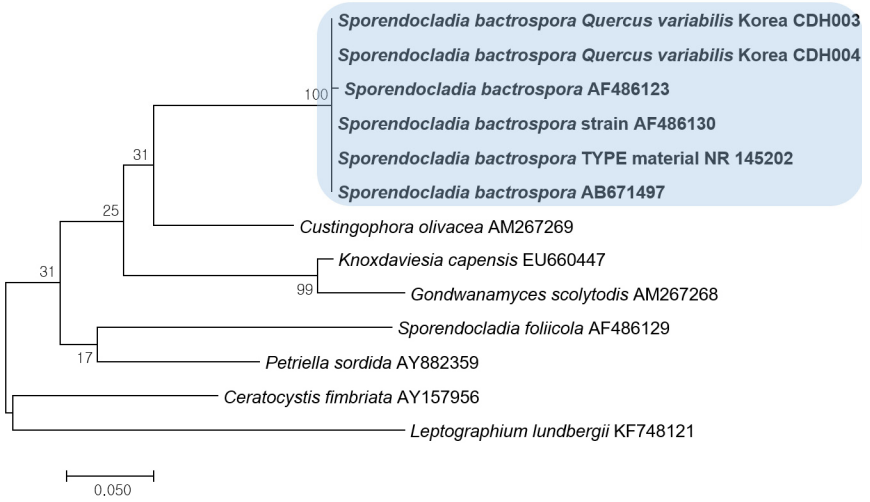

Fig. 2. Phylogenetic relationship between *Sporendocladia bactrospora* isolates and some reference isolates retrieved from the NCBI database, inferred by the maximum likelihood method using the rDNA internal transcribed spacer regions. Bootstrap values (≥50%) based on 1,000 replications are indicated. The scale bar represents 0.05 nucleotide substitutions per site. The isolates used in this work are indicated in bold.

The results showed clearly that *S. bactrospora* is occurring as a colonist of fresh wounds on oak trees, *Q. variabilis* in Korea, on which a new host range and a new niche of the

fungus were discovered. Although *S. bactrospora* is generally regarded as a saprophyte associated with dead plant material, it was shown that the fungus could be recovered from fresh wounds on living trees, on which it showed the ability to produce lesions in the artificial inoculation trials. [5, 7]. Since the taxonomic placement of *S. bactrospora* remains to be clarified [14], additional studies are needed to determine a reasonable ordinal placement of the fungus.

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