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Research Note

First Report and Characterization of *Pestalotiopsis ellipsospora* Causing Canker on *Acanthopanax divaricatus*

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Abstract *Acanthopanax divaricatus*, a member of the Araliaceae family, has been used as an invigorant in traditional Korean medicine. During disease monitoring, a stem with small, irregular, brown lesions was sampled at a farm in Cheonan in 2011. The symptoms seen were sunken cankers and reddish-brown needles on the infected twig. The isolated fungal colonies were whitish, having crenated edges and aerial mycelium on the surface, and with black gregarious fruiting bodies. The reverse plate was creamy white. Conidia were 17~22 × 3.5~4.2 μm, fusiform, 4-septate, and straight to slightly curved. The nucleotide sequence of the partial translation elongation factor 1 alpha gene of the fungal isolate, shares 99% sequence identity with that of known *Pestalotiopsis ellipsospora*. Based on the results of the morphological and molecular analyses, the fungal isolate was identified as *P. ellipsospora*. In Korea, this is the first report of canker on *A. divaricatus*.

Keywords *Acanthopanax divaricatus*, Canker, *Pestalotiopsis ellipsospora*, Translation elongation factor 1 alpha

The genus *Acanthopanax*, a medicinal plant of the Araliaceae family, is widely distributed throughout East and South Asia [1]. Many species of this genus have been used in traditional medicine, and parts of these plants are known to be good as a tonic and in prophylaxis in oriental herbal medication. In China, the major species for medicinal use are *A. gracilistylus* and *A. senticosus* [2]. For commercial use, *A. senticosus* and *A. divaricatus* var. *albeofructus* have recently been cultivated in Korea [3]. Lately, the extracts from the bark, fruit, leaf and twigs of *Acanthopanax* species were reported to have anti-inflammatory, anti-tumor, and anti-oxidant activities [4-6]. Due to its economic values, its cultivation has been increased in several farms.

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Pestalotiopsis is widely distributed throughout the tropical and temperate regions [7]. It is an important phytopathogenic genus [8-10], having more than 235 species. However, there have been no reports of fungal disease being caused by *Pestalotiopsis* in *Acanthopanax* sp. The diseases of *Acanthopanax* sp. reported in Korea include alternaria blight by *Alternaria panax*, and black leaf spot by *Phoma* sp. [11]. The purpose of this study was to identify the causal agent associated with stem canker disease in *Acanthopanax*, based on the morphological and phylogenetic characteristics, and the observed pathogenicity.

Disease symptoms and isolation of fungi. During disease monitoring, a stem with small, irregular, brown lesions was sampled at a farm in Cheonan, Chungnam province, in September of 2011. The infected twig was rotted, and sunken cankers and reddish-brown needles were seen on the infected twig (Fig. 1). After incubation in a humid condition, white mycelia, having dark brown necrosis, developed around the twig cavity. Prior to further analysis, pure cultures of the isolate were obtained from single-spore isolation. These were maintained on potato dextrose agar (PDA). A fungal isolate was coded as DUCC505.

Mycological characterization. The DUCC505 isolate was grown on PDA and maintained at 25°C. A 5-mm diameter mycelial plug was cut from the margin of a 5-day-old culture of the isolate, and was placed centrally in

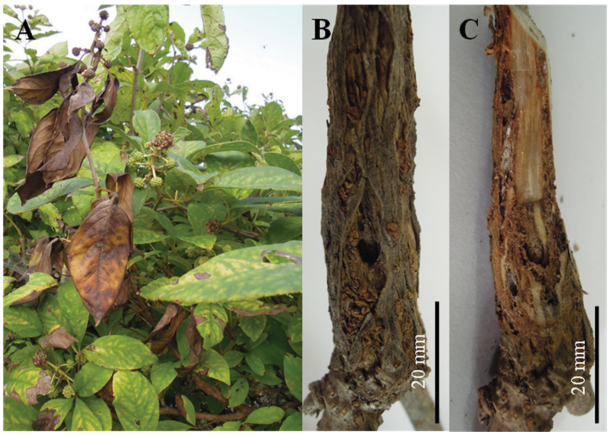

Fig. 1. Typical symptoms of canker on *Acanthopanax divaricatus*, from where the DUCC505 isolate was obtained. A, A wilted twig of *A. divaricatus*; B, C, Discolored and cankered twig by pitch-soaking before and after barking.

an 85-mm Petri dish containing PDA. The isolate was cultured at 25°C, and colony characteristics such as color, shape and size were recorded. The colony diameter was measured daily by scoring the average length for a period of 7 days. Colonies were whitish, having crenated edges, aerial mycelium on the surface, and with black gregarious fruiting bodies (Fig. 2A and 2B). The colonies, observed from the reverse of the plate, were creamy white. Light microscopic and scanning electron microscopic images are given in Fig. 2C~2H. Conidia were 17~22 × 3.5~4.2 μm, fusiform, 4-septate, and straight to slightly curved. The basal cell was of conical shape with an obtuse end, and pale brown in color. The three median cells were brown: the second cell from base was pale brown, the third cell was a darker brown, and the fourth cell was brown. The apical cell was conical in shape with a hyaline appearance. There were 2~3 tubular, apical appendages arising from the apex of the apical cell. These morphological properties corresponded to the features of the reference fungus, *Pestalotiopsis ellipsospora* [12]. The DUCC505 isolate grew better on PDA than oat meal agar and malt extract agar (Fig. 3A). The optimum temperature for mycelial growth of the isolate DUCC505 on PDA was 25°C (Fig. 3B). The isolate DUCC505 grew well in a broad range of pH, from 5 to 10 (Fig. 3C). These growth properties could be attributed to overcome the pH and low temperature stress in environment. So far, none of fungicides have been registered for the disease control of *Acanthopanax* sp. in the *Agrochemical Use Guide Book* in Korea [13]. Also, no fungicide has ever been tested for *P. ellipsospora* isolated from *Acanthopanax* sp. We therefore tested five kinds of fungicides for this study that are commercially available for ascomycete plant pathogens in Korea. To understand the agrochemical sensitivity of the DUCC505 isolate, we grew it with different concentration of fungicides, and the results are summarized in Fig. 3D. In the benomyl and tebuconazol supplemented media, the mycelial growth was completely inhibited at 10 μg/mL. This result is similar to *Pestalotiopsis microspora* that is sensitive to tebuconazole [14]. However, the isolate showed relative resistance in all media containing azoxystrobin, dimethomorph and triflumizole. Overall, it is suggested that among the five fungicides, benomyl and tebuconazol are the appropriate choice for the control of *P. ellipsospora*.

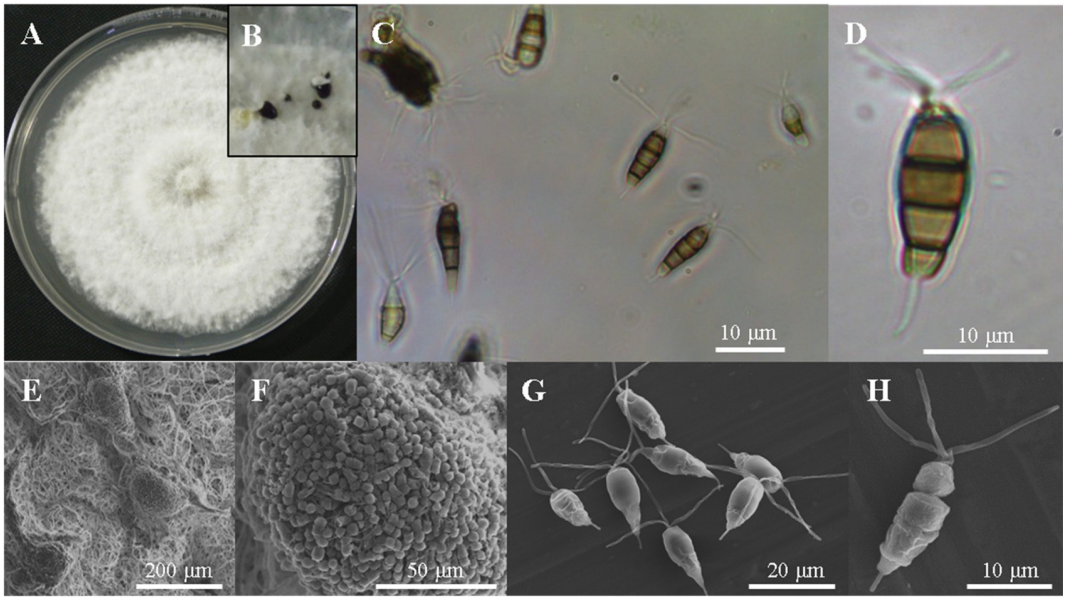

Fig. 2. Morphology of the DUCC505 isolate on potato dextrose agar at 25°C for 10 days. A, Whitish and edge crenate colony; B, Black gregarious fruiting bodies; C, D, Conidia by light microscopy; E, F, Black fruiting bodies observed by scanning electron microscopy (SEM); G, H, Conidia by SEM.

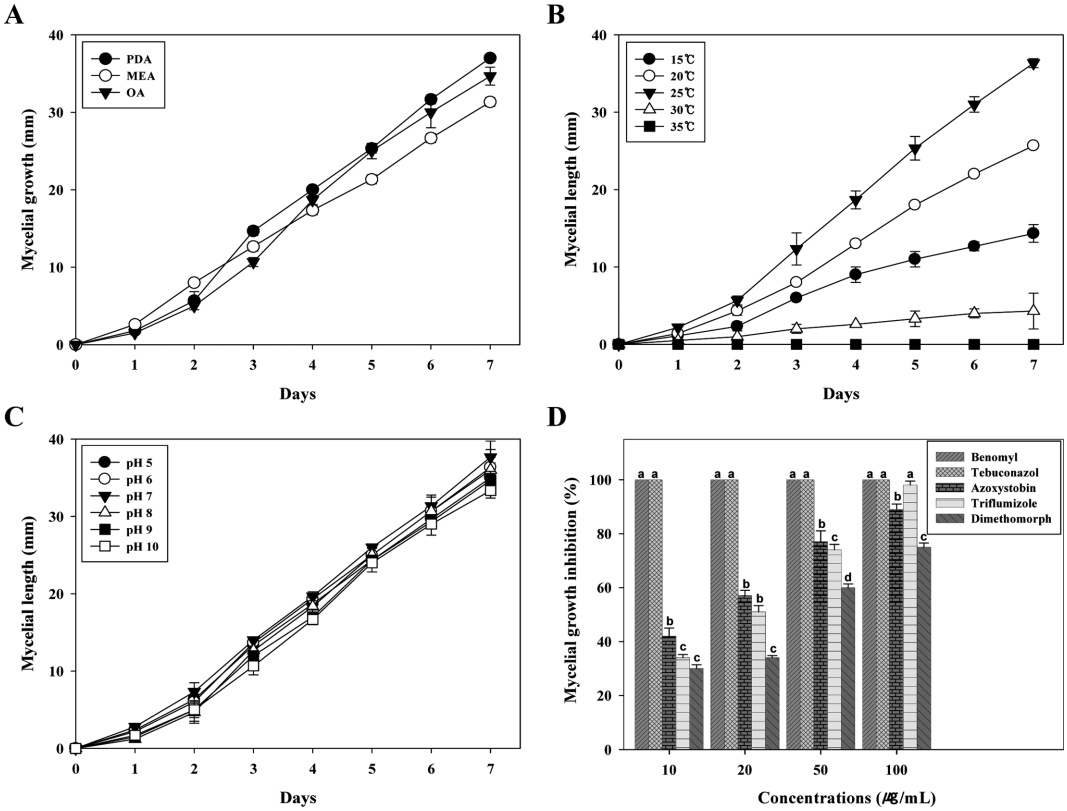

Fig. 3. Mycelial growth of the DUCC505 isolate on different media (A), temperatures (B), and pH (C). D, Mycelial growth inhibition by fungicides. Data shown are an average of three independent cultures for each experiment, and error bars represent the standard deviations. Mean separation by Duncan's multiple range test at $p < 0.05$. The same letter above or near bars (D) represents no significant difference between treatments. PDA, potato dextrose agar; MEA, malt extract agar; OA, oatmeal agar.

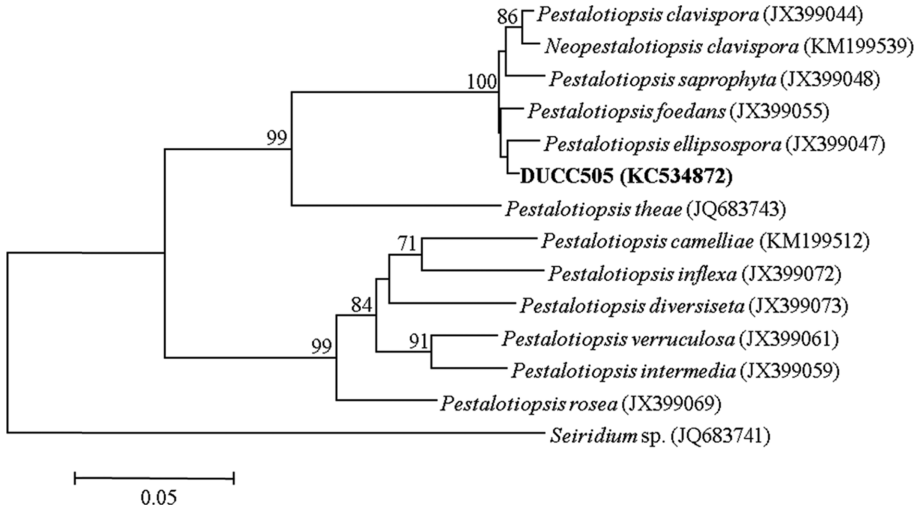

Fig. 4. Phylogenetic tree based on tef1-α gene sequences from the DUCC505 isolate and other species. Phylogram was constructed by the neighbor-joining method using MEGA 5. The numbers above the nodes are supporting percentages by 1,000 bootstrap replicates. *Seiridium* sp. was used as outgroup. NCBI GenBank accession numbers are indicated in parentheses.

Molecular identification of *P. ellipsospora*. The DUCC505 fungal isolate was grown on PDA plates for 5 days at 25°C. Mycelia were harvested by scraping the fungal colonies with a sterile blade. Genomic DNA was extracted as described by Kim *et al.* [14], with modifications. From the extracted genomic DNA, partial translation elongation factor 1 alpha (tef1-α) gene sequence was analyzed. Polymerase chain reaction (PCR) was performed

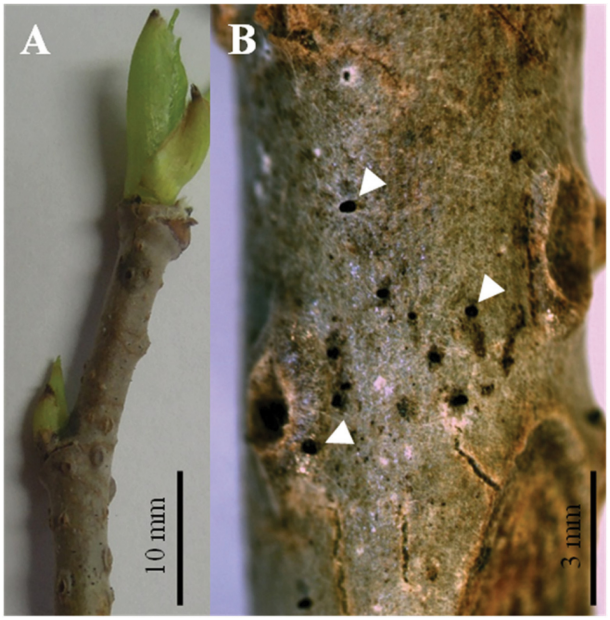

Fig. 5. Pathogenecity test of *Pestalotiopsis ellipsospora* DUCC505 on young twigs of *Acanthopanax divaricatus*. The white mycelia infested on a young twig (A) and black gregarious fruiting bodies (B) were formed on the surface. Conidial suspension of the isolate was artificially infected. The white arrowheads indicates black gregarious fruiting bodies.

as described previously using the universal primers TEF728 (5′-CAT CGA GAA GTT CGA GAA GG-3′) [15] and TEF1 (5′-GCC ATC CTT GGA GAT ACC AGC-3′) [16]. The PCR products were purified with a High Pure PCR Purification Kit (Roche, Basel, Swiss) and sequencing was carried out by Macrogen Inc. (Seoul, Korea). The nucleotide sequence of partial tef1-α gene of the fungal isolate shares 99% (531/536) sequence identity with that of known *Pestalotiopsis ellipsospora*. The tef1-α gene sequence of the DUCC505 was deposited in GenBank DNA database under accession number KC534872. Phylogenetic analyses were done by the neighbor-joining method using the MEGA ver. 5 [17]. Bootstrap values were generated with 1,000 replicates. A phylogenetic tree showed that the isolate DUCC505 positioned with *P. ellipsospora* (Fig. 4). Thus, based on the molecular and morphological data generated, the isolate was identified as *P. ellipsospora*.

Pathogenecity test. Pathogenicity test was conducted on young twigs of *A. divaricatus*. The young twigs were inoculated with a droplet of the DUCC505 conidia suspension (1.3×10^6 conidia/mL), which was prepared from the fungal cultures grown on PDA. Control inoculation was done with sterile water. The inoculated young twigs were incubated in a humid chamber at 25°C for 7 days. As rotting progressed, superficial white mycelium and small black acervuli were observed (Fig. 5). *P. ellipsospora* was re-isolated from the rotted twig lesion, thus fulfilling Koch's postulates. These results demonstrated that *P. ellipsospora* DUCC505 was able to infect a twig of *A. divaricatus*.

This is the first detailed report describing *P. ellipsospora* isolated from *Acanthopanax* in Korea, and it appears to be the first confirmation proving its pathogenicity on *Acanthopanax* twigs.

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