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. albofructus 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.

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
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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 tebuconazole 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 tebuconazole are the appropriate choice for the control of *P. ellipsospora*.

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**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.

**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.
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.
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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**REFERENCES**

1. Ohwi J. Flora of Japan. Tokyo: Shibundo; 1972.
2. Li XY. Immunomodulating Chinese herbal medicines. Mem Inst Oswaldo Cruz 1991;86 Suppl 2:559-64.
3. Jung BS, Shin MK. Hyang Yak Dae Sa Jeon. 3rd ed. Seoul: Young Lim Sa Publisher; 2003.
4. Hong SS, Hwang JY, Lee SA, Hwang BY, Ha KW, Ze KR, Seung RS, Ro JS, Lee KS. Isolation and quantitative analysis of acanthoside D from *Acanthopanax* cortex. Korean J Pharmacogn 2001;32:316-21.
5. Lee S, Son D, Ryu J, Lee YS, Jung SH, Kang J, Lee SY, Kim HS, Shin KH. Anti-oxidant activities of *Acanthopanax* seneciosus stems and their lignan components. Arch Pharm Res 2004;27:106-10.
6. Niihmi NX, Kim PV, Minh CV, Tai BH, Quang TH, Soung KS, Koo JH, Koh YS, Kim YH. Anti-inflammatory activity on LPS-stimulated dendritic cells of lupane-type triterpenoids from the leaves of *Acanthopanax koreanum*. Arch Pharm Res 2011;34:1593-8.
7. Bate-Smith EC, Metcalle CR. Leuco-anthocyanins. 3. The nature and systematic distribution of tannins in dicotyledonous plants. J Linn Soc Lond Bot 1957;55:669-705.
8. Yasuda F, Kobayashi T, Watanabe H, Izawa H. Addition of *Pestalotiopsis* spp. to leaf spot pathogens of Japanese persimmon. J Gen Plant Pathol 2003;69:29-32.
9. Das R, Chutia M, Das K, Jha DK. Factors affecting sporulation of *Pestalotiopsis disseminata* causing grey blight disease of *Persea bombycina* Kost., the primary food plant of muga silkworm. Crop Prot 2010;29:963-83.
10. Maharachchikumbura SS, Guo LD, Chukeatirote E, Bahkali AH, Hyde KD. *Pestalotiopsis* morphology, phylogeny, biochemistry and diversity. Fungal Divers 2011;50:167-87.
11. Korean Society of Plant Pathology. List of plant disease in Korea, 5th ed. Seoul: Korean Society of Plant Pathology; 2009.
12. Maharachchikumbura SS, Guo LD, Cai L, Chukeatirote E, Wu WP, Sun X, Crous PW, Bhat DJ, McKenzie EH, Bahkali AH, et al. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Divers 2012;56:95-129.
13. Korean Crop Protection Association. Agrochemical use guide book. Seoul: Korea Crop Protection Association; 2007.
14. Kim SH, Uzunovic A, Breuil C. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood by PCR. Appl Environ Microbiol 1999;65:287-90.
15. Carbone I, Kohn LM. A method for designing primer sets for
speciation studies in filamentous ascomycetes. Mycologia 1999;91:553-6.

16. Samuels GJ, Dodd SL, Gams W, Castlebury LA, Petrini O. *Trichoderma* species associated with the green mold epidemic of commercially grown *Agaricus bisporus*. Mycologia 2002;94:146-70.

17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28:2731-9.