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***Armillaria* Root Rot Caused by *Armillaria Tabescens* On *Prunus Salicina* in a Korean Garden**

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The *Armillaria* species causing *Armillaria* root rot on *P. salicina* in Korea was identified as *A. tabescens* based on the morphology of the basidiomata and analysis of DNA sequences. This work reports for the first time in Korea a pathogenic fungus of *A. tabescens* on *P. salicina*. From phylogenetic analysis, *A. tabescens* isolate categorized it as a North American species except for the Czech species. However, the European species from Italy and France were distinctly distinguished from the isolate.

INTRODUCTION

Armillaria species exist as pathogens, saprobes, or necrotrophs on a wide range of hosts (Gregory *et al.*, 1991; Hood *et al.*, 1991; Kile *et al.*, 1991; Fox, 2000). *Armillaria* root rot is a serious disease mainly of woody plants, caused by *Armillaria* (Fr.: Fr.) Staude. *Prunus* spp. hosts are very susceptible to *Armillaria*, especially almond (Guillaumin *et al.*, 1989; Gregory *et al.*, 1991; Tsopelas and Tjamos, 1997) and cherry (Aoshima and Hayashi, 1981; Sahashi, 2004). On the other hand, most reports on infection of almond or cherry come from older literature and refer to *A. mellea* sensu lato (Rhoads, 1965; Aoshima and Hayashi, 1981). Thus, more information is needed to determine which species of *Armillaria* attack these plants.

Armillaria tabescens (Scopoli) Emel (synonym: *Clitocybe tabescens*) has always been distinguished from other *Armillaria* species because its basidiomata do not bear an annulus on the stipe (Tsopelas and Tjamos, 1997). However, basidiomata of the fungus are not very common in nature, and they appear for only short periods of time.

In Korea, *A. ostoyae* has previously been reported as a pathogen on Korean white pine. On the other hand, other basidiomata of *Armillaria* species, *A. tabescens*, have been collected from maple, oak, and red pine (Sung *et al.*, 1994). But their biological and ecological aspects of pathogen or saprophyte including real symptoms were not discussed in detail. Moreover, the symptoms of the disease and the morphology of cultures from vegetative isolates of *A. tabescens* are indistinguishable from those

of other *Armillaria* species. In fungal molecular methods, polymerase chain reaction (PCR) analysis of the internal transcribed spacer (ITS) region of nuclear rDNA has proven to be reliable methods for identification at species level (Burns *et al.*, 1991; Karen *et al.*, 1997).

Here we report on the symptoms of *Armillaria* root rot and basidiomata formation of *A. tabescens* on *P. salicina* planted in a Korean garden. The fungus was identified from the characteristics of the basidiomata, and from the analysis of the rDNA internal transcribed spacer (ITS) region. A phylogenetic analysis including other continental isolates of *A. tabescens* was conducted.

MATERIALS AND METHODS

Sample collection, isolation, and identification

Prunus salicina Lindl planted in Korean garden where is located in 1162, Hojeo-myeon, Wonju-si, Kangwon-do in KOREA was used in this study. Symptoms of *Armillaria* root rot were observed on 2 August 2007, and basidiomata that formed on 23 September 2007 were collected from the stem and butt of a dead tree. Macro-morphological characteristics of the basidiomata were described for species identification of *Armillaria*, and dried specimens were stored in the herbarium of Kangwon National University, Korea. Before the specimens were dried, vegetative isolates from tissue of the basidiomata were cultured on potato dextrose agar (PDA) medium at 25 °C in an incubator. The culture was used for observation of colony morphology and DNA extraction. A Munsell soil color chart (1994) was used to analyze diagnostic basidiomata colors.

DNA extraction and sequencing

Fungal DNA was extracted from 50 to 200 mg of fleshy mycelium using the DNeasy Plant Mini kit (Qiagen, USA) according to the manufacturer's instructions. The internal transcribed spacer (ITS) region, including 5.8 S rDNA, was amplified using primers specific for the higher fungi ITS1-f (Gardes and Bruns, 1993) and ITS4-b (White *et al.*, 1990) regions. Each

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50 μ l mixture contained 5 μ l G-Taq buffer (10X), 4 μ l dNTP (2.5 M), 5 μ l Tuning buffer (Cosmo Genetech Co.) (5X), and 0.5 μ l G-Tag DNA polymerase (Cosmo Genetech Co.). Amplifications were performed using a 2720 Thermal Cycler (Applied Biosystems). Initial denaturation at 94 °C for 3 min was followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using the LaboPass PCR purification kit (COSMO Co. Ltd). Sequencing of the purified products was carried out using a BigDye® Terminator v3.1 Cycle Sequencing Kit and the GeneAmp® PCR System 9700. The BigDye® XTerminator™ Purification Kit was used for purification of the reaction products, and an Applied Biosystems 3730xl DNA Analyzer was used for analysis. All processes were carried out according to the manufacturer's instructions.

Analysis of DNA sequence data

ITS sequences were compared to the GenBank database at the DNA Data Bank of Japan (DDBJ) using the basic local alignment search tool (BLAST) program, and the homology for *A. tabescens* and other *Armillaria* species was calculated. All sequences used in the analysis, except the isolate used in this study, have been deposited into GenBank (Table 1).

Phylogenetic analysis was performed for each data set using neighbor-joining (Saitou and Nei, 1987). The ITS sequences were initially aligned using CLUSTAL W in DDBJ. Neighbor-joining analysis was performed using the Tamura-Nei model for estimation of evolutionary

distance, and relative support for nodes in resulting trees was generated using 1000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Sample collection, isolation, and identification

On 2 August 2007, an approximately 15-year old *P. salicina* tree, 15 cm diameter at breast height (DBH) and 5 m in height, was observed with the entire canopy collapsed with leaves attached, after looking healthy the previous year (Fig. 1 A). The dying tree was excavated to a depth of about 10 cm, and the butt and root collar were examined for the presence of mycelial fans under the bark (Fig. 1 B). Rhizomorphs were observed on the surface of the roots. Basidiomata of *Armillaria* that had developed on the stems were collected on 23 September 2007 (Fig. 1 C).

In culture, the *Armillaria* isolate produced a sparse flat whitish to brownish mycelium with abundant cylindrical rhizomorphs (Fig. 1 D). Macro-morphological features of the basidiomata are described in detail as follows: pileus 8.4–86.8 mm diameter, when young, convex, sometimes conical then plano-convex and finally planar and uplifted to depressed, sometimes irregularly undulating with age. Surface dry, when young, reddish yellow (7.5YR–7/6), strong brown (7.5YR–5/6), and reddish brown (7.5YR–8/6) at center and reddish yellow (5YR–6/8) to yellowish red (5YR–5/6) toward the margin, usually covered with distinct brownish scales more densely concentrated toward the center. Lamellae white when young, to light reddish brown (5YR–6/4) later,

Table 1. *Armillaria tabescens* collected in this study and best BLAST match

Species*	Origin**	Gen Bank accession no.	Similarity (%)
<i>Armillaria tabescens</i>	USA/GA	AY213590	98
<i>Armillaria tabescens</i>	USA/GA	AY213589	98
<i>Armillaria tabescens</i>	USA/SC	AY695409	98
<i>Armillaria tabescens</i>	USA/SC	AY213588	97
<i>Armillaria socialis</i>	Czech	DQ784799	97
<i>Armillaria socialis</i>	Czech	DQ784800	97
<i>Armillaria tabescens</i>	Czech	AY175806	96
<i>Armillaria tabescens</i>	USA/SC	DQ109806	96
<i>Armillaria ostoyae</i>	USA/NH	AY213552	93
<i>Armillaria gemina</i>	USA/NY	AY213556	92
<i>Armillaria sinapina</i>	USA/WA	AY213565	92
<i>Armillaria gallica</i>	USA/MI	AY213570	92
<i>Armillaria clavescens</i>	USA/MI	AY213561	92
<i>Armillaria cepistipes</i>	USA/WA	AY213583	92
<i>Armillaria nabsnona</i>	USA/ID	AY213572	92

*: *Armillaria socialis* (DC: Fr.) Herink is synonym to *A. tabescens* (David, 2000)

** : GA, Georgia; SC, South Carolina; NH New Hampshire; NY, New York State; WA, Washington State; MI, Michigan; ID, Idaho



Fig. 1. Dying *P. salicina* (A), mycelial fan under the bark (red arrow, B), basidioma formation (C), and culture on PDA medium (D).

crowded, sinuate, subdecurrent. Stipe central, 33.6–140 mm in length, 4.2–11.2 mm thick at the apex, tapering below, whitish, bruising brown with fibrillose surface without annulus. Habitat stem and root collar, densely caespitose.

DNA sequencing

The ITS *Armillaria* isolate from *P. salicina* was 886 bp long. A BLAST search using the ITS 1 and 5.8S gene sequences for this isolate against sequences in DDBJ showed that the DNA sequences of the *Armillaria* isolate were most similar to those identified as *A. tabescens* (Table 1). On the other hand, the similarity between the isolate and other *Armillaria* species except for *A. tabescens* was less than 93%.

Analysis of DNA sequence data

Twelve *A. tabescens* including the *P. salicina* isolate (KOREA/Kangwon) used in this study formed two main groups in a neighbor-joining tree with bootstrap support of 1000. Group I sequences originated from Italy and France in Europe. The other group was from Korea (KORE/Kangwon), and South Carolina and Georgia in the USA and the Czech Republic in Europe (Fig. 2).

DISCUSSION

The *Armillaria* species causing *Armillaria* root rot on *P. salicina* in Korea was identified as *A. tabescens* based on the morphology of the basidiomata and analysis of DNA sequences. *Armillaria tabescens* is considered a significant pathogen of orchid trees and ornamental

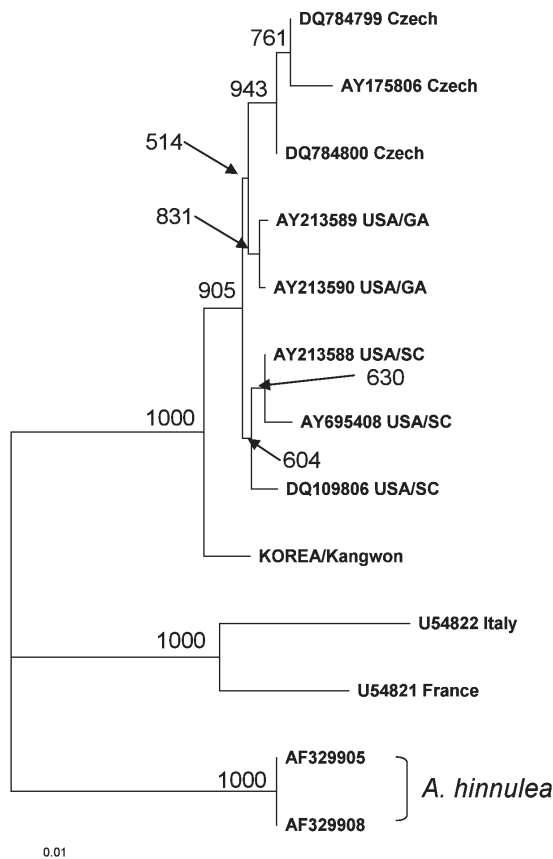


Fig. 2. Phylogram generated from Neighbor-joining analysis of the ITS sequence data used in this study. Bootstrap values are shown on the branch. *Armillaria hinnulea* is used as out group.

plants in southern USA (Gregory *et al.*, 1991) Although the occurrence of the fungus in Europe on cultivated plants are rare, *A. tabescens* on citrus trees in Corsica (Laville and Vogei, 1984) and on pear tree (*Pyrus communis*) in Portugal (Rishbeth, 1985). However, Tsopelas and Tjamos (1997) reported on the attack of almond (*P. amygdalus*) in Greece. Although Sung *et al.* (1994) reported that *A. ostoyae* occur a serious disease of pine (*Pinus koraiensis*) in plantation after clear cut of forest, the studies on the *Armillaria* root rot are rare in Korea. This work reports for the first time in Korea a pathogenic fungus of *A. tabescens* on *P. salicina*.

In morphology, the macro- and micro-scopical characteristics were not distinguish with other continental fungus, *A. tabescens*. It is easy to distinguish *A. tabescens*, the non-annulus *Armillaria*, from other species based on the morphology of basidiomata. Although another *Armillaria* species without an annual ring on the stipe, *A. ectypa* (Fr.: Fr.) D. Lam, has been reported in Europe (Chillai *et al.*, 1998) and Japan (Imazeki and Hongo, 1995), it is found in very different habitat from that of *A. tabescens*, which is ligneous, while *A. ectypa* is terrestrial. Moreover, *A. ectypa* has not been reported in Korea yet.

Additionally, a BLAST search in DDBJ of the ITS region identified it as *A. tabescens* with 96% to 98%

sequence homology, strongly supporting the identity of this species. Phylogenic analysis of the *A. tabescens* isolate categorized it as a Northern American species except for the Czech species. However, the European species from Italy and France were distinctly distinguished from the isolate. The bootstrap support for a continental origin of the ITS region was strong with bootstrap support of 1000. Several researchers doubt exists whether *A. tabescens* found in Europe is the same species as the one reported in North America and other places in the world (Tsopelas and Tjamos, 1997). Because the compatibility tests between isolates from Europe and USA showed them to belong to different species (Guillaumin *et al.*, 1989, 1993). However, Darmono *et al.* (1993) reported that haploid isolates from Europe were compatible with haploid isolates from USA, and that isolates from different areas of North America all belong to the same species.

As mentioned above, among the continental isolates are slightly complex in mating behavior and some aspect of the pathogenicity to plants. From this study, although the Czech isolates was included in the same group of Korean and USA, the Korean group including USA was clearly separated in genetic. But, it is considered that the diversity from the mating behaviors and DNA analysis is originated from not the continent but environmental condition such as temperature in habitat. Because *A. tabescens* is thermophilic. So, the geographical distribution in the northern temperate hemisphere will be appeared to increase dramatically depending upon continental climatic conditions. Therefore, it can be expected that severe tree diseases such as *Armillaria* root rot caused in *A. tabescens* will increase with global warming and abrupt climatic changes in forest ecosystems. Therefore, the diversity in taxonomy and heredity, and ecology of *Armillaria* spp. including *A. tabescens* will be clarified in regional in the future.

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