

Alternaria species associated with araliaceous plants in Korea

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Abstract In this work, we assess the taxonomy of *Alternaria* species associated with leaf spot and blight disease on araliaceous plants in Korea. *Alternaria araliae* and *A. panacis* are recognized and a novel species, *A. dendropanacis*, is proposed based on sequence analysis and morphology. They can be differentiated by the combined analysis of Alt a 1, BT1, BT2, EF-1 α , *gpd* and RPB2 gene sequences. Morphologically, the species are distinct by pigment production and conidial characteristics produced under standardized cultural conditions.

Keywords *Alternaria araliae* · *A. dendropanacis* · *A. panacis* · Morphology · Phylogenetic analysis

Introduction

The family Araliaceae includes trees, shrubs, lianas and perennial herbaceous plants distributed over approximately 55 genera and 1,500 species (Wen et al. 2001). Many medicinal plants (e.g., ginseng) and well-known ornamentals (e.g., English ivy and umbrella trees) are members of this family.

Foliar diseases caused by *Alternaria panacis* (as *A. panax*) have been reported from many araliaceous plants worldwide (Deng et al. 2010; Forsberg 1983; Levy et al. 2006; Simmons 2007; Whetzel and Rosenbaum 1912; Yu 2001; Zhang 2003), resulting in considerable economic loss (CSL 2006; Garibaldi et al. 2004; Proctor and Baily 1987; Uchida et al. 1984).

The causal agent of the disease was first described as *Alternaria panacis* (as *A. panax*) from *Panax quinquefolius* (Whetzel and Rosenbaum 1912). Later, some large-spored *Alternaria* species collected from other araliaceous plants were assigned different names based on morphology, such as *A. araliae* (Greene 1953) and *A. actinophylla* (Miller 1969). Simmons (1982) compared the morphology among the *Alternaria* isolates from different araliaceous hosts, and found that conidial morphology was variable depending on age and culturing conditions, producing overlapping characters amongst all isolates. Consequently, he considered *A. araliae* on *Aralia racemosa* and *A. actinophylla* on *Schefflera actinophylla* as synonyms of *A. panacis* (Simmons 1982).

Alternaria is a ubiquitous fungal genus that represents a morphologically diverse assemblage of taxa. Classification of *Alternaria* species has been based mainly on conidial morphology and catenulate spore patterns (Simmons 1982, 2007; Yu 2001; Zhang 2003). However, the taxonomy of the genus has been a subject of controversy because of the high variability of conidial morphology and polymorphism displayed even in pure culture (Rotem 1994; Simmons 1982; Uchida et al. 1984). Simmons (2007) summarized his lifetime study on *Alternaria* taxonomy based on conidial morphology and sporulation patterns, in which 275 species were precisely illustrated. Phylogenetic analyses of *Alternaria* based on multiple genes (e.g., Alt a 1, ATPase, beta-tubulin, *gpd*, ITS, RPB2 and TEF1) have resulted in species groups or sections (Hong et al. 2005; Lawrence et al. 2013, 2014; Pryor and Bigelow 2003; Woudenberg et al. 2013, 2014).

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Recently, two genetic groups were recognized among Korean isolates of *A. panacis* based on molecular methods, morphological characteristics and pathogenicity tests (Deng et al. 2013). An important differentiating character is the production of a diffusible pigment (reddish color) by one genetic group in potato dextrose agar (PDA; Difco) which is lacking in the other. In addition, Korean isolates from *Dendropanax morbifer* produced a yellowish pigment in PDA (Deng et al. 2010), as did isolates collected from *Aralia elata* in Cheongyang (Korea). In the present study, we reassess the *Alternaria* species associated with cultivated araliaceous plants in Korea based on six genes—*Alternaria* allergen a 1 (Alt a 1), two partial genes of β -tubulin (BT1 and BT2), translation elongation factor 1 alpha (EF-1 α), glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and RNA polymerase second largest subunit (RPB2)—and morphological characteristics.

Materials and methods

Fungal isolates

Diseased leaves of araliaceous plants were collected from different locations in Korea from 2007 to 2010. Leaf fragments with necrotic spots and blight symptoms were placed in Petri dishes with moist filter paper and incubated 2–3 days at 25 °C to induce sporulation. Isolates of large-spored *Alternaria* were obtained by extraction of single conidia. Pure cultures of isolates were maintained on PDA (Difco) slants, and deposited in the Culture Collection of Chungnam National University (CNU) Fungal Herbarium and Korean Agricultural Culture Collection (KACC). A total of 14 representatives from araliaceous plants, including ten Korean isolates and four isolates from E. G. Simmons (E.G.S.), Crawfordsville, Indiana, were selected for detailed study (Table 1).

DNA extraction and phylogenetic analysis

Alternaria isolates were grown on PDA plates for 5–7 days. Mycelial tufts of each isolate were transferred to microtubes. The mycelia were lyophilized, ground and lysed using an extraction buffer (200 mM Tris-HCl (pH 8.0), 200 mM NaCl, 30 mM EDTA, 0.5 % SDS). Genomic DNA was initially extracted by equilibrated phenol B following extraction with phenol:chloroform:isoamyl alcohol (25:24:1). Ribonuclease (RNase) enzyme was added to each sample and incubated at 37 °C for 1 h to remove RNA. Subsequently, DNA was precipitated by isopropanol, washed with ethanol, and resuspended in sterile distilled water. The resulting product was visualized with LoadingSTAR (Dynebio Inc.) staining solution under ultraviolet illumination.

Six genes—Alt a 1, BT1, BT2, EF-1 α , *gpd* and RPB2—were amplified and sequenced using primers Alt-a1-for/Alt-a1-rev (Hong et al. 2005), Bt-1a/Bt-1b (Glass and Donaldson 1995), Bt-2a/Bt-2b (Glass and Donaldson 1995), EF1-728F/EF1-986R (Carbone and Kohn 1999), *gpd*1/*gpd*2 (Berbee et al. 1999) and RPB2-6F/RPB2-7cR (Liu et al. 1999). PCR amplification was carried out in a GeneAmp PCR System 2700 thermo cycler (Applied Biosystems, Foster City, CA, USA) with a 50- μ L reaction volume containing 1 μ L genomic DNA solution, 5 μ L 10 $\times$ *e-Taq* Reaction buffer (SolGent Co., Ltd., Daejeon, South Korea), 1 μ L of each 10 mM dNTP mix, 1 μ L of each primer, and 1.5 U *Taq*-DNA polymerase. All PCR products were purified with a PCR clean-up system (Promega, Madison, WI, USA) and sequenced using a commercial sequencing service provider (Macrogen Inc., Seoul, Korea).

All nucleotide sequences (Table 1) generated in this study (in bold) and retrieved from GenBank were assembled using the PHYDIT version 3.2 program (Chun 1995) and initially aligned with the Clustal X 2.0 program (Larkin et al. 2007). The sequences were then imported into BioEdit software to manually adjust their nucleotide polymorphisms (Hall 1999). Concatenated sequencing of six genes was used for phylogenetic analysis of a combined dataset. Some gene sequences (‘–’ in Table 1) were treated as missing data. Maximum likelihood analyses including 1000 bootstrap replicates were run using the RAxML program (Stamatakis 2006). The GTRCAT model of nucleotide substitution was assigned to each partition (e.g., Alt a 1, BT1, BT2, EF-1 α , *gpd* and RPB2). The resulting phylogenetic tree was edited using Tree Explorer in MEGA version 5.05 (Tamura et al. 2011). *Stemphylium lycopersici* was used as the outgroup in the phylogeny (Fig. 1).

Morphological studies

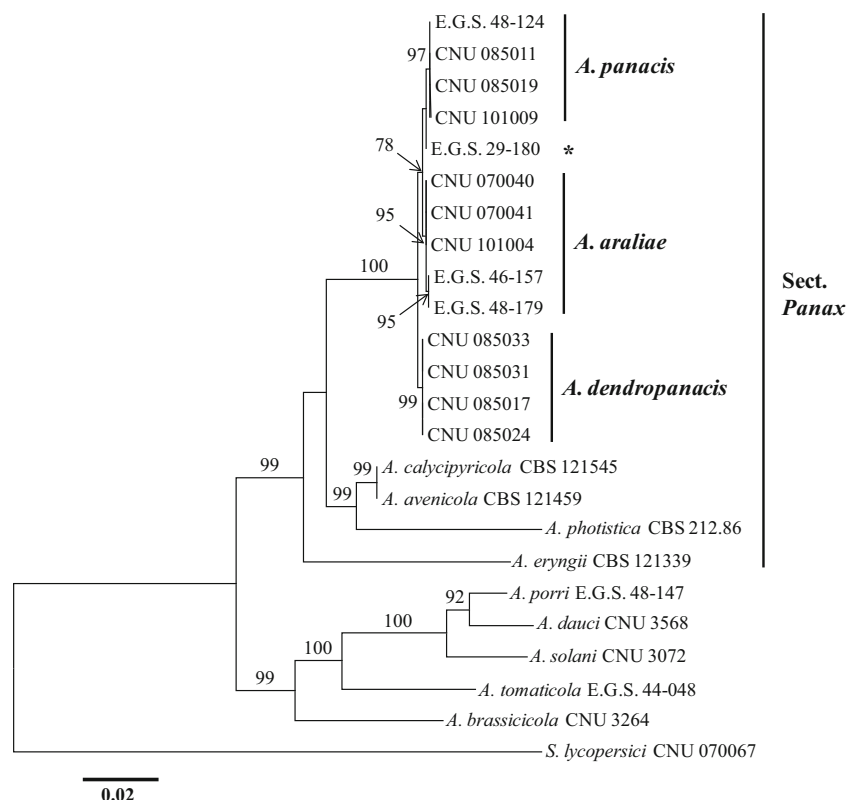
Mycelial plugs (5 mm diam.) of the isolates were taken from the periphery of growing colonies (3–5 days) on PDA (Difco) and transferred to fresh PDA Petri dishes. All cultures were incubated at 25 °C in the dark. Colony characteristics were determined 7 days after inoculation. To characterize conidial morphology, isolates representing each genetic group (CNU 101004, and E.G.S. 46-157; CNU 085031 and CNU 085033; CNU 085019 and E.G.S. 48-124) were grown under standard culture conditions (Simmons 2007). Conidial morphology and sporulation patterns were examined on potato carrot agar (PCA) containing 20 g white potato, 20 g carrot and 20 g agar in 1 l deionized water. Mycelia of each isolate were three-point inoculated in 9-cm plastic Petri dishes. All plates were placed in a chamber maintained without humidity control (a gradually drying atmosphere in unsealed plates) at 22 °C and held under a fluorescent light/dark cycle of 8/16 h for 7 days. Conidia were mounted in lactophenol picric acid solution

Table 1 Sources of *Alternaria* isolates and their GenBank accession numbers for Alt a 1, BT1, BT2, EF-1 α , *gpd* and RPB2 gene sequences used in the study (newly generated sequences marked in bold)

Species	Isolate no.	Host	Location	Year	GenBank accession nos.					
					Alt a 1	BT1	BT2	EF-1 α	gpd	RPB2
<i>Alternaria araliae</i>	CNU 070041	<i>Acanthopanax chiisanensis</i>	Geunsan, Korea	2007	JX213288	JF417606	JF417579	JX213324	JF417633	JF417660
	CNU 101004	<i>Aralia continentalis</i>	Eumsung, Korea	2010	KF516487	KP877972	KF516494	KP877987	KF516501	KP877980
	CNU 070040	<i>Aralia elata</i>	Suwon, Korea	2007	JX213289	JF417607	JF417580	JX213325	JF417634	JF417661
	E.G.S. 48-179	<i>Fatsia japonica</i>	Auckland, New Zealand	2000	JX213284	JF417602	JF417575	JX213320	JF417629	JF417656
<i>A. araliae</i>	E.G.S. 46–157, CBS 116532	<i>Meryta sinclairii</i>	Auckland, New Zealand	1998	JX213285	JF417603	JF417576	JX213321	JF417630	JF417657
<i>A. araliae</i>	E.G.S. 29–180, CBS 482.81	<i>Aralia racemosa</i>	Indiana, USA	1970	JQ646382	KP877979	JQ672009	KC584675	JQ646299	KC584417
<i>A. dendropanacis</i>	CNU 085024	<i>Aralia elata</i>	Cheongyang, Korea	2008	KF516491	KP877976	KF516498	KP877991	KF516505	KP877984
<i>A. dendropanacis</i>	CNU 085033	<i>Aralia elata</i>	Cheongyang, Korea	2008	KF516493	KP877978	KF516500	KP877993	KF516507	KP877986
<i>A. dendropanacis</i>	CNU 085017	<i>Dendropanax moribifer</i>	Jindo, Korea	2008	KF516490	KP877975	KF516497	KP877990	KF516504	KP877983
<i>A. dendropanacis</i>	CNU 085031	<i>Dendropanax moribifer</i>	Jindo, Korea	2008	KF516492	KP877977	KF516499	KP877992	KF516506	KP877985
<i>A. panacis</i>	CNU 101009	<i>Panax ginseng</i>	Eumsung, Korea	2010	KF516489	KP877974	KF516496	KP877989	KF516503	KP877982
<i>A. panacis</i>	CNU 085019	<i>Panax ginseng</i>	Suwon, Korea	2008	KF516488	KP877973	KF516495	KP877988	KF516502	KP877981
<i>A. panacis</i>	CNU 085011	<i>Panax quinquefolius</i>	Suwon, Korea	2008	JX213304	JF417622	JF417595	JX213340	JF417649	JF417676
<i>A. panacis</i>	E.G.S. 48–124, CBS 116535	<i>Panax quinquefolius</i>	Oregon, USA	1998	JX213298	JF417616	JF417589	JX213334	JF417643	JF417670
<i>A. avenicola</i>	E.G.S. 50-185	<i>Avena</i> sp.	Volden, Norway	1988	^a	–	–	KC584639	KC584100	KC584380
<i>A. brassicicola</i>	CNU 3264	<i>Armoracia rusticana</i>	Buyeo, Korea	2000	JX213314	JF417706	JF417691	JX213350	JF417696	JF417701
<i>A. calyciprlicola</i>	CBS 121545, E.G.S. 52-071	<i>Pyrus communis</i>	China	2007	JQ646381	–	JQ672008	KC584643	JQ646298	KC584384
<i>A. dauci</i>	CNU 3568	<i>Daucus carota</i>	Bongpyong, Korea	2000	JX213313	JF417705	JF417690	JX213349	JF417695	JF417700
<i>A. eryngii</i>	CBS 121339, E.G.S. 41-005	<i>Eryngium</i> sp.	Unknown	Unknown	AY563313	–	JQ672007	KC584656	AY562416	KC584397
<i>A. photistica</i>	CBS 212.86	<i>Digitalis purpurea</i>	Cambridge, UK	1982	AY563282	–	JQ671973	KC584678	KC584131	KC584420
<i>A. porri</i>	E.G.S. 35-172	<i>Allium fistulosum</i>	Ithaca, New York	1996	JF331538	JF331581	JF331566	KC584679	KC584132	KC584421
<i>A. solani</i>	E.G.S. 48–147, CBS 116698	<i>Lycopersicon esculatum</i>	Buyeo, Korea	2000	JX213315	JF417707	JF417692	JX213351	JF417697	JF417702
<i>A. tomaticola</i>	CNU 3072	<i>L. esculentum</i>	Florida, USA	1996	KP877996	KP877997	KP877998	KP877994	KP877999	KP877995
<i>Semphylium lycopersici</i>	E.G.S. 44–048, CBS 118814	<i>Capsicum annuum</i>	Suwon, Korea	2007	JX213311	JF417703	JF417688	JX213347	JF417693	JF417698

^a Sequences not obtained in this study were treated as missing data.

Fig. 1 Maximum likelihood tree inferred from analysis of combined Alt a 1, BT1, BT2, EF-1 α , *gpd* and RPB2 gene sequences of *Alternaria* isolates associated with araliaceous plants. Bootstrap support values above 70 % from 1000 replicates are shown at the nodes. The tree is rooted to *Stemphylium lycopersici*



and photographed with an Artray Artcam-300MI digital camera (Japan) under a BX50 Olympus light microscope. Fifty conidia were measured.

Results

Phylogenetic analysis

A total of 2673 characters (446 for Alt a 1, 401 for BT2, 273 for BT2, 299 for EF-1 α , 535 for *gpd*, and 719 for RPB2), including alignment gaps, were used in the phylogenetic analysis. The combined phylogenetic tree showed that 14 representative *Alternaria* isolates from araliaceous plants clustered together with 100 % bootstrap support and were divided into three distinct groups (Fig. 1). The phylogenetic relationship between two groups (herein named *A. panacis* and *A. araliae*), well supported with bootstrap values of 97 and 95 %, respectively, was similar to that reported by Deng et al. (2013). The only difference was that the *A. panacis* group (isolates from ginseng plants *Panax ginseng* and *P. quinquefolius*) was closely related to a representative isolate, E.G.S. 29-180, from *Aralia racemosa*. The other group (herein named *A. dendropanacis*) with 99 % bootstrap support included four isolates from *Aralia elata* and *Dendropanax morbifer*. In addition, two groups (*A. panacis* and *A. araliae*) comprising the isolate E.G.S. 29-180 were grouped together with moderate bootstrap support (78 %).

Taxonomy

Distinct cultural and conidial characteristics of the three *Alternaria* groups are summarized in Table 2. *Alternaria* isolates from the *A. panacis* group produced thinner conidia in longer chains (1–6 conidia per chain) than isolates from the other two groups, which produced larger, more bulging conidia (1–3 conidia per chain). *Alternaria* isolates from the *A. dendropanacis* group differed from *A. araliae* group isolates in producing larger and more inflated conidia. One distinguishable characteristic among the three groups was the color of colony reverse, which was caused by the distinct pigment production in PDA (Figs. 2, 3 and 4). The pigment production was also readily observed when growing in PDB (potato dextrose broth) liquid (data not shown). We conclude that the large-spored *Alternaria* species from araliaceous plants represent three different species.

Alternaria araliae H.C. Greene, *Transactions of the Wisconsin Academy of Science* 42: 80, 1953. (Fig. 2)

Mycobank: MB 292406

Colonies on PDA white to pale gray, velvety, effuse, with pale orange-red colony reverse, secreting a reddish pigment into the medium, 53–59 mm diam. after 7 days at 25 °C. Conidiophores arising singly, terminally or laterally from hyphae, straight or curved, smooth-walled, pale brown, with one darker terminal conidiogenous locus, 20–150(>200) μ m long, 4–7.5(–9) μ m wide, with 0–9 septa. Conidia solitary or 2–3 in

Table 2 Morphological comparison of *Alternaria* associated with araliaceous plants in Korea

Groups	Conidial size	No. of conidia per chain	Pigment in colony reverse on PDA
<i>A. araliae</i>	35–110(–133)×10–45(–60)	1–3	Reddish
<i>A. dendropanacis</i>	30–160(–180)×10–70(–75)	1–3	Yellowish
<i>A. panacis</i>	30–90(–110)×10–30(–32)	1–6	None

a chain, straight or slightly curved, long-ellipsoidal or obclavate, golden-brown to dark brown, smooth-walled at first, later becoming verruculose to verrucose, with some bulging cells; conidium body 35–110(–133)×10–45(–60) µm, with 4–11 transverse, and 1 to several longitudinal or oblique septa; beak 10–45(–60) µm long, 3–8 µm wide.

Specimens examined: CNU 101004 (Eumsung, 2010) from *Aralia continentalis* in Korea; E.G.S. 46-157 from *Meryta sinclairii* in New Zealand, 1998, obtained from E.G. Simmons.

Hosts and distribution: *Acanthopanax chiisanensis*, *Acanthopanax koreanus*, *Acanthopanax senticosus*, *Aralia elata*, *Aralia continentalis* and *Fatsia japonica* in Korea; *Echinopanax horridus* and *Meryta sinclairii* in New Zealand.

Notes: This species is distinct from the other two in the production of a reddish pigment in PDA which causes a distinguishable color of colony reverse, and having smaller conidia than *A. dendropanacis* and more distorted conidia than *A. panacis*.

Alternaria dendropanacis S.H. Yu and J.X. Deng **sp. nov.** (Fig. 3)

Mycobank: MB 805082

Etymology: *dendropanacis* refers to the host genus *Dendropanax*.

Colonies on PDA white to pale gray, velvety, effuse, with dark orange-brown colony reverse, secreting a yellowish pigment into the medium, 44–54 mm diam. after 7 days at 25 °C. Conidiophores arising singly, terminally or laterally from hyphae, straight or curved, smooth-walled, pale brown, 25–150(–>200) µm long 6–9(–10) µm wide, with 0–10 septa. Conidia solitary or 2–3 in a chain; conidium body 30–160(–180)×10–70(–75) µm, with 4–11 transverse and 1 to several longitudinal or oblique septa; beak variable in length 10–50(–63) µm long, 3–8(–11) µm wide, some cells bulging strongly in the lower part.

Holotype: on leaf blight of *Dendropanax morbifer* H. Lév. in Jindo, Republic of Korea, in August, 2008, deposited in the Chungnam National University Fungi Herbarium, CNUMH 08073 (dried cultures) (ex-type culture: CNU 085031; other isolate CNU 085017).

Specimens examined: CNU 085031 (Jindo, 2008) from *Dendropanax morbifer*; CNU 085033 (Cheongyang, 2008) from *Aralia elata* in Korea.

Hosts and distribution: *Dendropanax morbifer* and *Aralia elata* in Korea.

Notes: The species can be readily differentiated from the other two in the color of colony reverse due to the secretion of a yellowish pigment in PDA, and producing the largest and most distorted conidia among the three species.

Fig. 2 Morphology of *Alternaria araliae*: E.G.S. 46-157 (left) from *Meryta sinclairii* and CNU 101004 (right) from *Aralia continentalis*. Colonies on PDA; conidia, conidiophores, and sporulation patterns on PCA. Bar=50 µm

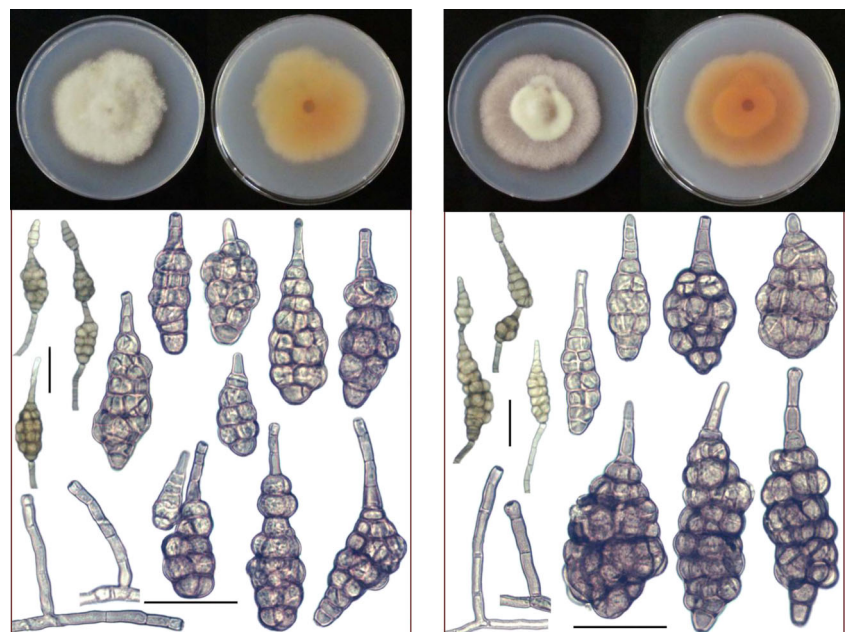
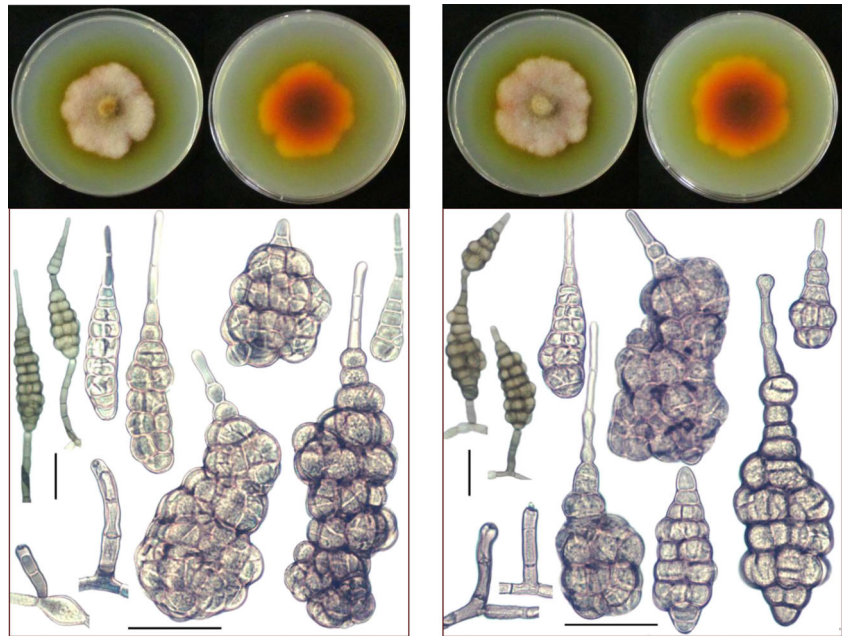


Fig. 3 Morphology of *Alternaria dendropanacis*: CNU 085031 (left) from *Dendropanax morbifer* and CNU 085033 (right) from *Aralia elata*. Colonies on PDA; conidia, conidiophores, and sporulation patterns on PCA. Bar=50 μ m



Alternaria panacis Whetzel, *Bulletin of the U.S. Department of Agriculture* 250: 11, 1912 (as '*A. panax*'), spelling corrected in J. Agric. Res. 5: 181. 1915.

Mycobank: MB 215071

Colonies on PDA white to pale gray, velvety or cottony, effuse, with pale gray or dark gray colony reverse, 56–67 mm diam. after 7 days at 25 °C. Conidiophores arising singly, terminally or laterally from hyphae, straight or curved, smooth-walled, pale brown, 15–150(–>200) μ m long, 4–8(–10) μ m wide, with 0–9 septa. Conidia mostly in chains of 2–6 or solitary, straight or slightly curved, long-ellipsoidal or obclavate, golden-brown to dark brown, smooth-walled at first, later

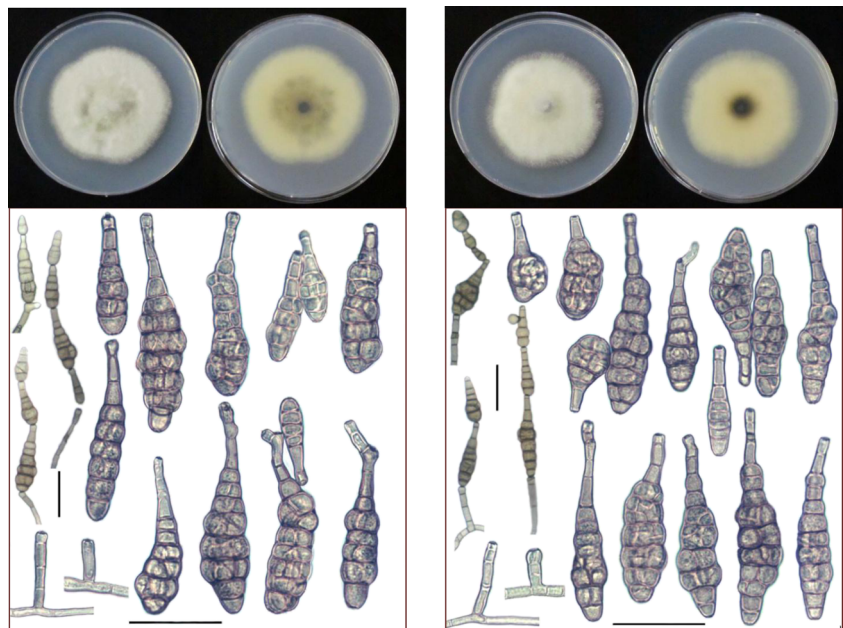
becoming verruculose to verrucose, conidium body 30–90(–110) $\times$ 10–30(–32) μ m, with 3–11 transverse and 1 to several longitudinal or oblique septa; beak 5–35(–58) μ m long, 4–6.5(–7) μ m wide, some cells bulging strongly in the lower part.

Specimens examined: CNU 085019 (Suwon, 2008) from *Panax ginseng* in Korea; E.G.S. 48-124 from *Panax quinquefolius*, USA, 2000, obtained from E.G. Simmons.

Hosts and distribution: *Panax ginseng* in Korea and *Panax quinquefolius* in Korea and the USA.

Notes: The species differs from the other two in the absence of pigment in PDA (colony reverse color), and producing smaller and less distorted conidia, in longer chains.

Fig. 4 Morphology of *Alternaria panacis*: E.G.S. 48-124 (left) from *Panax quinquefolius* and CNU 085019 (right) from *Panax ginseng*. Colonies on PDA; conidia, conidiophores, and sporulation patterns on PCA. Bar=50 μ m



Discussion

In this study, three species of *Alternaria*—*A. araliae*, *A. dendropanacis* and *A. panacis*—were recognized among *Alternaria* isolates from araliaceous plants on the basis of morphological and molecular characteristics. They form three distinct phylogenetic groups based on a combined sequence analysis of *Alt a 1*, *BT1*, *BT2*, *EF-1 α* , *gpd*, and *RPB2* genes (Fig. 1), and differ in pigment production, conidia characteristics and sporulation patterns (Table 2). In addition, the representative isolate, E.G.S. 29-180, collected from *Aralia racemosa* is phylogenetically close to *A. panacis*, but conidial morphology of the isolate illustrated by Simmons (1982) is similar to that of *A. araliae* in this study. The isolate (E.G.S. 29-180) considered as *A. araliae* is excluded for morphological comparison, as conidia were not produced in the present study. Re-collection of *Alternaria* isolates from *Aralia racemosa* is necessary for future study.

In our previous report (Deng et al. 2013), two genetic groups (A and B) of *Alternaria panacis* from araliaceous plants were recognized based on sequence analysis of four genes (*BT1*, *BT2*, *gpd* and *RPB2*) and amplified fragment length polymorphism (AFLP) analysis. All isolates belonging to genetic group B were from ginseng plants (*Panax ginseng* and *P. quinquefolius*), whereas isolates belonging to group A were from other araliaceous plants such as *Aralia* and *Acanthopanax*. Conidial morphology was also distinguishable between the two genetic groups, although some overlap was observed. Based on the results of phylogenetic analysis, morphological characteristics and pathogenicity testing, Deng et al. (2013) considered the two genetic groups of *Alternaria* from araliaceous plants as two separate species.

Alternaria from *Aralia racemosa* was first reported as *Macrosporium araliae* by Dearness and House (1940), without a Latin description and diagnosis. Greene (1953) later compared the fungus from *Aralia* with authentic material of *A. panacis*, and cited differences in characteristics of its conidia and conidiophores from *A. panacis* as the basis for describing the fungus as *A. araliae*. However, Simmons (1982) regarded the species as a synonym of *A. panacis* after examining 25 specimens of *Alternaria* species associated with araliaceous plants. According to Simmons (1982), Greene's observations were not critically capable of identifying the considerable overlap in conidial morphology in the two species. In the present study, we compared sequences of six genes and cultural characteristics of *Alternaria* isolates from different hosts of the family Araliaceae, and also found that *Alternaria* isolates from plants in the genera *Aralia*, *Acanthopanax*, *Echinopanax*, and *Kalopanax* differed from *Alternaria* isolates from ginseng hosts (*Panax ginseng* and *P. quinquefolius*). We assign the former *Alternaria* species (genetic group A) as *A. araliae* H.C. Greene, as *A. araliae* (1953) meets all standards of nomenclatorial legitimacy and is properly typified (Simmons

1982), and we assign the latter *Alternaria* species (genetic group B) as *A. panacis* Whetzel (syn. *A. panax* Whetzel).

The proposed new species, *A. dendropanacis*, differs phylogenetically from the other two species by forming an individual group (Fig. 1). The fungus was previously misidentified as *A. panacis* (*A. panax*) based on ITS sequence analysis (Deng et al. 2010), as the ITS sequence of the fungus is 100 % identical to that of *A. panacis*. In this study, we found ITS sequence of *A. dendropanacis* to be consistent with the other two species (data not shown). However, it differs from the other two species in the gene sequences of *Alt a 1* and *EF-1 α* . Based on *gpd* sequences, *A. panacis* and *A. dendropanacis* cannot be differentiated, while *A. dendropanacis* and *A. araliae* have identical *BT2* gene sequences. However, the three species can be separated by *BT1* and *RPB2* gene sequences and by the combined analysis of six genes. Morphologically, the species is characterized by the production of yellowish pigment and the largest and most inflated conidia among the three species.

The morphology of three species examined on standard culture conditions (Table 2) is quite different from that described by Deng et al. (2010, 2013). The results reconfirm that the morphology of *Alternaria* is viable based on culture conditions and substrate (Rotem 1994; Simmons 1982), and the precise definition of a cultural regime is useful for the delimitation of *Alternaria* (Pryor and Michailides 2002; Roberts et al. 2000). Recently, Woudenberg et al. (2013) delineated phylogenetic lineages within *Alternaria* and allied genera based on nucleotide sequence data of six gene regions. They found that the *Alternaria* clade contained 24 sections (internal clades) and six monotypic lineages. *Alternaria* associated with araliaceous plants (as *A. panax*) belongs to the section *Panax*, which also consists of *A. calycipyricola*, *A. eryngii*, *A. avenicola* and *A. photistica* (Woudenberg et al. 2013). In this study, *A. araliae*, *A. dendropanacis*, *A. panacis* and those four species consistently clustered together, supported with high bootstrap value (99 %) (Fig. 1).

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