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RESEARCH NOTE

Alternaria brassicifolii sp. nov. Isolated from *Brassica rapa* subsp. *pekinensis* in Korea

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ABSTRACT

A new species belonging to the genus *Alternaria* was isolated from the necrotic leaf spots of *Brassica rapa* subsp. *pekinensis* in Yuseong district, Daejeon, Korea. It is an occasional isolate, not an etiological agent, which is morphologically similar to *A. broccoli-italicae*, but differs in conidial size and conidiophore shape. Phylogenetic analysis using the sequence datasets of the internal transcribed spacer (ITS) region of the rDNA, glyceraldehyde-3-phosphate dehydrogenase (gpd), and plasma membrane ATPase genes showed that it is distantly related to *A. broccoli-italicae* and closely related to *Alternaria* species in the section *Pseudoalternaria*, which belonged to a clade basal to the section *Infectoriae*. Morphologically, the species is unique because it produces solitary conidia or conidial chains (two units), unlike the four members in the section *Pseudoalternaria* that produce conidia as short branched chains. It exhibits weak pathogenicity in the host plant. This report includes the description and illustration of *A. brassicifolii* as a new species.

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The genus *Brassica* is known to include important agricultural and horticultural crops. In Asia, especially in Korea, *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt is one of the most popular leafy vegetables used to prepare kimchi, a traditional Korean food. Three species of *Alternaria*, namely, *A. brassicae* (Berk) Sacc, *A. brassicicola* (Schwein) Wiltshire, and *A. japonica* Yoshii have been isolated from *Brassica rapa* in Korea [1]. *Alternaria* leaf spots caused by these three species is the most common and destructive fungal disease occurring in many cruciferous plants worldwide [2,3]. Other *Alternaria* species are also reported from *Brassica* plants such as *A. brassicinae* Simmons, *A. broccoli-italicae* Simmons, *A. ethzedia* Simmons, and *A. nepalensis* Simmons [4].

Alternaria was originally described by Nees (1816), with *A. tenuis* Nees being the type species. Since then, approximately 280 *Alternaria* species have been reported as plant pathogens and saprophytes, resulting in poor crop yield and spoilage during storage [4,5]. The taxonomy of *Alternaria* species is mainly based on the shape, size, and septation of the conidia, as well as sporulation patterns [1,4,6,7]. Since the 21st century, molecular approaches, especially sequence analyses, have been popularly adopted to identify the *Alternaria* species [8–10]. Both morphological and molecular phylogenetic analyses work in a complementary manner for the classification of this species [11–13]. Recently, *Alternaria* has been classified into 27 sections, and 14 other genera have been synonymized [14,15].

Leaves showing necrotic spots on *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt (Brassicaceae) were collected from Yuseong-gu district, Daejeon, Korea, in June 2011. The samples were processed for sporulation, and *Alternaria* isolates were obtained using the methods described by Deng et al. [12]. Pure cultures were deposited in the Culture Collection center of the Chungnam National University (CNU) in Daejeon, and the ex-type of the species (CNU 111118) was stored in the Korean Agricultural Culture Collection (KACC), Suwon, Korea.

To determine colony characteristics, the isolates were cultured on potato dextrose agar (PDA; Difco, Montreal, Canada) for 7 days at 25 °C under dark condition. To observe sporulation patterns and conidial morphology, the isolates were transferred to potato carrot agar (PCA: 20 g white potato, 20 g carrot, and 20 g agar in 1 L) for 7 days at 22 °C under alternating light and dark conditions (8/16 light/dark) [4].

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Sporulation patterns and conidia (50) were digitally photographed (Figure 1) and measured using an OLYMPUS BX50 light microscope (OLYMPUS, Tokyo, Japan) equipped with an Artcam 300MI digital camera (ARTRAY, Tokyo, Japan).

Taxonomy

Alternaria brassicifolii S. H. Yu and J. X. Deng, sp. nov. (Figure 1).

Mycobank: MB 824688.

Description: Colonies on PDA at 7 days, 52–57 mm diameter in size, cottony, olivaceous buffer (Figure 1(A)); on PCA at 7 days, 65–68 mm diameter in size, velvety, pale gray with conspicuous concentric rings, presenting sectors at the edge (Figure 1(B)). Conidia solitary with a large proportion, some formed chains by means of an apical secondary conidium on the PCA medium (Figure 1(C)). Conidiophores simple, erect or bent, smooth, terminally or laterally from hyphae, septate (1–9), usually only one pigmented terminal

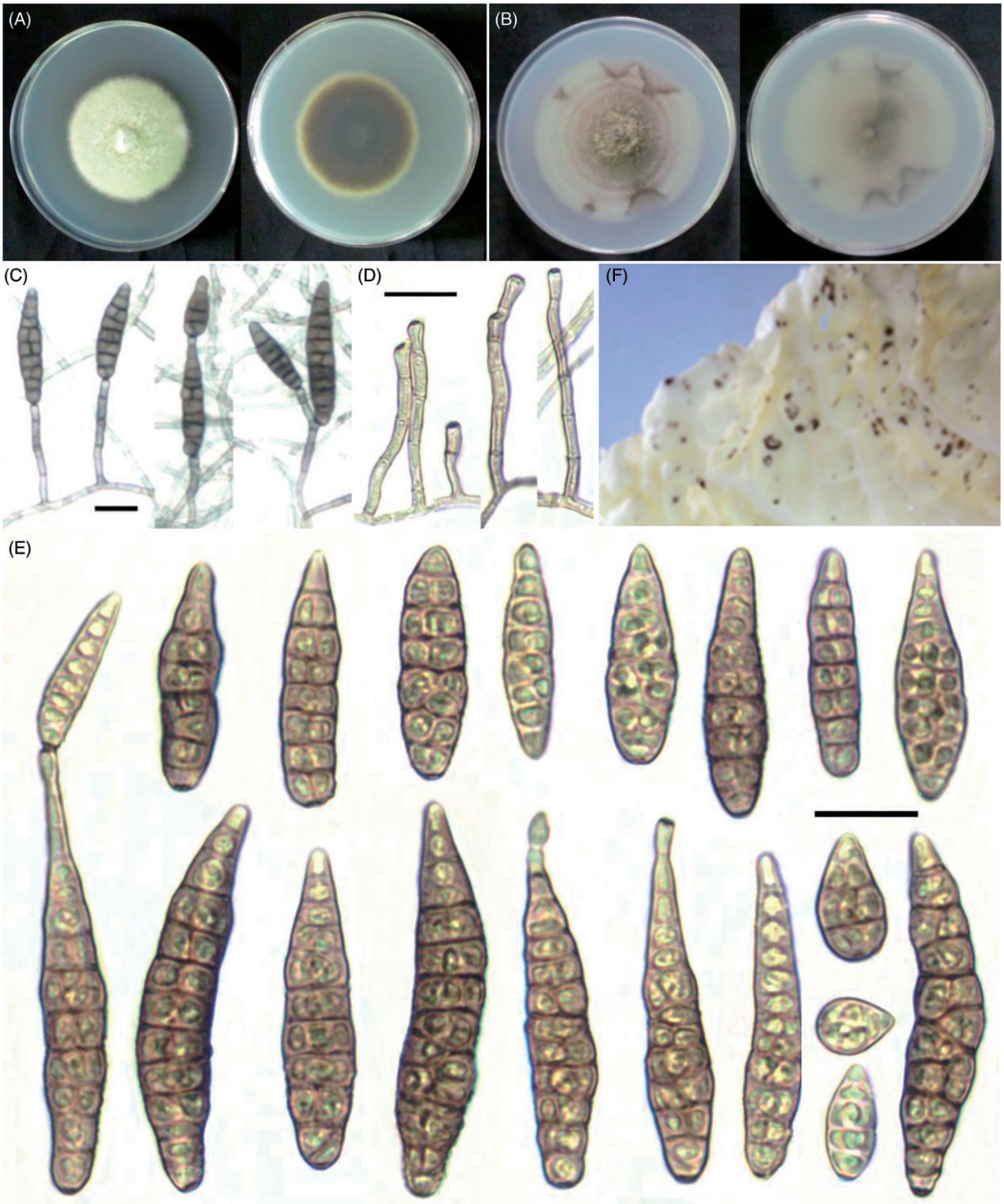

Figure 1. Morphology of *Alternaria brassicifolii* CNU 111118. (A) Colony on PDA; (B) colony on PCA; (C) sporulation pattern; (D) conidiophores; (E) conidia; (F) symptoms on the detached leaves of *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt. Bars = 20 μm.

conidiogenous site, sometimes with one more lateral conidiogenous locus, 15–100 × 2.5–4.5 µm (Figure 1(D)). Juvenile conidia subcylindric, bluntly rounded base and apex, or ovoid with tapered apex containing 1–2 septa, measuring 19–35 × 7–12 µm. Matured conidia long elliptical or obclavate, beakless tapering gradually to a rounded or conical apex, sometimes with a short apical secondary conidiophore, straight or slightly curved, up to 40–80 (~87) × 9–17 µm, dilute tan in color, smoothly or evenly verruculose in the conidial wall with 5–10 transverse septa and 1–2 longitudinal or oblique septum in the transverse segments (Figure 1(E)). Its teleomorph stage was not observed.

Etymology: *Brassicifolii*, refers to the genus of the host plant (*Brassica*) and the leaf (folium) from it was collected.

Type: Korea, Daejeon, Yuseong-gu, from the leaves of *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt, June 2011, by S. H. Yu and J. X. Deng, cultures: CNU 111118 and CNU 111116.

The morphological characteristics of *A. brassicifolii* were different from those of any other *Alternaria* species described, except *A. broccoli-italicae*, which produces solitary and long-ovoid conidia, and reported, for the first time, from *Brassica oleracea* L. var. *italica* Plenck (Brassicaceae) [4]. However, *A. broccoli-italicae* grows slowly on the PCA medium (2–3 cm diameter) in 5–7 days and produce smaller conidia (35–60 × 8–12 µm) with respect to the *Alternaria* species described in this study. The most obvious difference between these two species is found in the conidiophores. The conidiophores of *A. broccoli-italicae* frequently extended into twisted, branching elements that are conidiogenous at several successive loci on the PCA medium [4]. This type of conidiophore was not observed among the isolates of *A. brassicifolii*.

To perform molecular analysis, genomic DNA of the isolate CNU 111118 and the ex-type strain E.G.S. 40-134 of *A. broccoli-italicae* was extracted using a method described by Park et al. [16] with some modifications. PCR amplification of ITS, gpd, and ATPase genes was performed using the primer pairs of ITS1/ITS4 [17], gpd1/gpd2 [18], and ATPDF1/ATPDR1 [19], respectively. PCR was performed as described by Lawrence et al. [19]. The products were purified using a Wizard PCR prep kit (Promega, Madison, WI) and sequenced by a commercial sequencing service provider (Macrogen, Daejeon, Korea). Each gene sequence was deposited in GenBank and assigned an accession number (Table 1).

The sequences obtained in this work and the sequences of related species from the sections of *Alternaria* phylogeny [14,19,20] were used for phylogenetic analysis. Sequence alignment was generated with the Clustal X program [21] and manually adjusted.

Table 1. NCBI GenBank accession numbers of the *Alternaria* isolates used in the study.

		GenBank accession No.		
Species	Isolate	ITS	gpd	ATPse
A. abundans	CBS 534.83	JN383485	FJ214852	JQ671802
A. arrhenatheria	BMP 1942	JQ693677	JQ693635	JQ693603
A. arbusti	E.G.S. 91-136	JQ693644	JQ646365	JQ671940
A. brassicifolii	**CNU 111118**	**JQ317188**	**KM821537**	**KY412558**
A. broccoli-italicae	**E.G.S. 40-134**	**KM821536**	**KM821538**	**KY412557**
A. caricis	E.G.S. 13-094	AY278839	AY278826	JQ671780
A. cetera	E.G.S. 41-072	JN383482	AY562398	JQ671801
A. conjuncta	E.G.S. 37-139	AF392988	AY562401	JQ671824
A. didymospora	CBS 766.79	FJ357312	FJ357300	JQ671796
A. frumenti	E.G.S. 44-001	JQ693654	JQ646295	JQ671823
A. gossypii	CBS 135.31	JQ693638	JQ646278	JQ671800
A. graminicola	E.G.S. 41-139	JQ693650	JQ646291	JQ671819
A. humuli	E.G.S. 47-140	JQ693652	JQ646293	JQ671821
A. incomplexa	E.G.S. 17-103	JQ693658	JQ646287	JQ671815
A. infectoria	E.G.S. 27-193	AF347034	AY278793	JQ671804
A. intercepta	E.G.S. 49-137	JQ693656	JQ646297	JQ671826
A. kordkuyana	IRAN 2764C	MF033843	MF033826	MF033860
A. limaciformis	CBS 481.81	KC584203	KC584123	JQ671798
A. oregonensis	E.G.S. 29-194	FJ266478	FJ266491	JQ671827
A. parvicaespitosa	LEP 014858	MF033859	MF033842	KJ908217
A. photistica	E.G.S. 35-172	JQ693659	AY562402	JQ671807
A. phragmospora	E.G.S. 27-098	JN383493	FJ357302	JQ671797
A. rosea	E.G.S. 41-130	JQ693639	JQ646279	JQ671803
A. scirpicola	CBS 481.90	KC584237	KC584163	JQ671781
A. telluster	CBS 538.83	FJ357316	FJ357304	JQ671794
A. ventricosa	E.G.S. 52-075	JQ693649	JQ646290	JQ671818

Bold letters are designated in this study.

The three gene sequences were assembled in a single sequence resulting in a 2460 characters. Maximum likelihood analysis was performed using the GTRCAT model in RAxML program [22]. Branch support measures were calculated with 1000 bootstrap replicates. The resulting phylogenetic tree (Figure 2) was constructed with Mega v5.05 [23]. In the phylogram, *A. brassicifolii* fell into a clade (100% bootstrap values) comparing species of the sections *Infectoriae* and *Pseudoalternaria*. Moreover, the species was closely related to four members in the section *Pseudoalternaria* (*A. arrhenatheria*, *A. kordkuyana*, *A. parvicaespitosa,* and *A. rosea*) and they all gathered in a clade (81% bootstrap values) basal to the section *Infectoriae*. Meanwhile, *A. brassicifolii*, which is phylogenetically distant to *A. broccoli-italicae*, fell into the section *Infectoria*. Morphologically, the species in the section *Pseudoalternaria* commonly produced conidia in short branched chains; however, *A. brassicifolii* is unique and mostly produces solitary conidia, at times with simple chains of up to two conidia (Table 2). The molecular phylogenetic data available and the unique morphology of this fungus further confirms that it is a new species belonging to *Alternaria*, proposed to be named as *A. brassicifolii* sp. nov.

The pathogenicity of *A. brassicifolii* sp. nov. was evaluated. Spore suspension (10^6 spores/mL) was sprayed on the detached leaves of *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt and kept in a clean moistened box at 25 °C for 5 days. Small necrotic spots (1–2 mm) were observed on the leaves, which

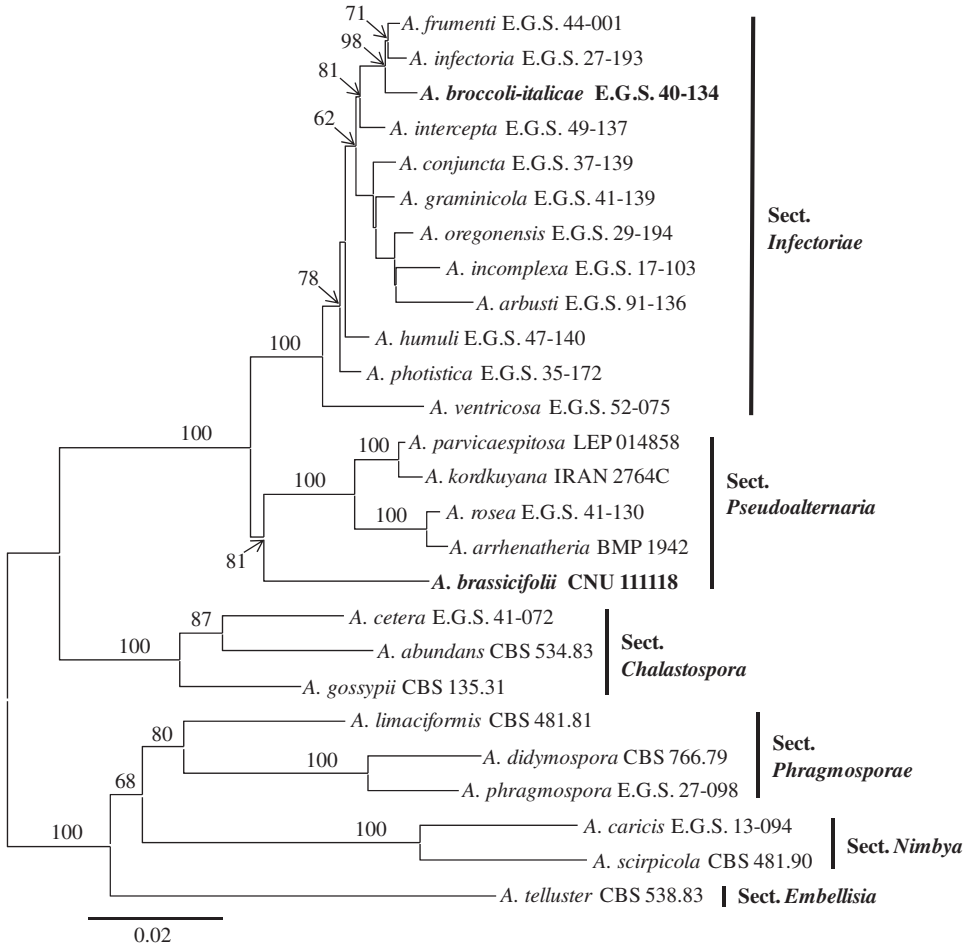

Figure 2. Maximum likelihood tree obtained from the combined datasets of ITS, gpd, and ATPase gene sequences of *Alternaria brassicifolii* and other related species. Bootstrap values (>60%) calculated for 1000 replicates are shown above the branches. The bar indicates the number of substitutions per position. The sections were referenced from previously published reports [14,19].

Table 2. Comparison of the conidial morphology of *Alternaria brassicifolii* sp. nov. with *Pseudoalternaria* species on the PCA medium.

	Conidia			
Species	Max. size (μm)	Septa	Sporulation pattern (Conidia in chains)	References
A. arrhenatheri	17.5–32.5 × 7.5–10	3–4	Simple or branched	[19]
A. brassicifolii	40–80 (∼87) × 9–17	5–10	Solitary or simple chain with 2 conidia	Present study
A. kordkuyana	30–50 (∼60) × 7–11	3–6 (∼7)	Mostly simple (5–8 (∼10) conidia per chain) or branched with 1–2 conidia	[20]
A. parvicaespitosa	10–25 × 7–12	1–3 (∼4)	Simple or sometimes produce 2 branches (≥3–4 conidia between branching points)	[24]
A. rosae	10–22 × 5–8	4–5	Simple or branched in short chains	[4]

were used to isolate and identify the fungus involved. The test was repeated thrice and showed similar results. No symptoms were observed in control samples. These results indicate that *A. brassicifolii* exhibits weak pathogenicity in the host, which may reduce its market value.

Disclosure statement

No potential conflict of interest was reported by the authors.

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