

Postharvest fruit rot caused by *Pestalotiopsis* sp. on grape in Korea

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Abstract In 2010, fruit rot disease was observed on the ‘Cheongsoo’ grape cultivar in a store house in Daejeon, Korea. The causal agent was determined to be a species of *Pestalotiopsis* not previously described in the literature. Pathogenicity tests revealed that the fungus is pathogenic to the fruit of the ‘Cheongsoo’ grape. This is the first report of fruit rot diseases on ‘Cheongsoo’ grape caused by *Pestalotiopsis* sp. in Korea.

Keywords ‘Cheongsoo’ grape · *Pestalotiopsis* · Morphology · Sequence analysis · Pathogenicity

Eight grape cultivars have been released in Korea since a grape breeding program started in the early 1960s. One grape cultivar, ‘Cheongsoo’ (1993), is an early season table grape that produces white seedless fruit with good quality and high productivity (Park et al. 2009a, b). In October 2010, severe fruit rot was observed on the ‘Cheongsoo’ grape during storage in Yuseong, Daejeon, Korea. Initial symptoms of fruit rot disease mostly occurred at the ruptured connection between the pedicel

and berry, and at wounds on the fruit (Fig. 1a). Following infection, the fruit skin turned reddish brown, after which the area of discoloured skin increased and whitish mycelium developed on the lesions. The severely infected fruits then became rotten and were separated completely from the pedicels.

Diseased fruits of the ‘Cheongsoo’ grape were collected from a store house in Yuseong, Daejeon, Korea. Infected grape fruits were surface sterilized with sodium hypochlorite (2 % available chlorine) for 2 min and then incubated in a moist chamber at 25 °C to isolate causal pathogens. Cultures of fungi belonging to the genus *Pestalotiopsis* were obtained from the areas of the fruit showing symptoms.

Colonies of the fungus grown on PDA were white and floccose cottony, forming a marked concentric ring with dense and thick mycelia 7.5–8.5 cm diam. in size (Fig. 2a, b). Hyphal tips of the fungus were inoculated on PDA medium. Autoclaved segments of carnation leaves (*Dianthus caryophyllus*) were placed on the developing colonies to promote sporulation (Fisher et al. 1982; Strobel et al. 1996). Acervuli that developed on carnation leaves were black, erumpent, pustule-like, unilocular, oval or irregular in outline and 100–200 (–250) μm (Fig. 2c, d). Conidiogenous cells were lageniform to ampulliform or subcylindrical, colourless, and smooth-walled (Fig. 2e). Conidia were fusiform, straight or slightly curved, five-celled, and 19.5–29.2 \times 4.6–7.2 μm (mean 25.2–6.2 μm , $n=50$) in size (Fig. 2f, g). The darker median cells were three celled (rarely two), subcylindrical to dolliiform, umber to fuliginous. Normally, the upper two cells were brown to umber with a darker band at the septa between them, while the lowest cell was lighter coloured. The apical and basal cells were conical in shape and colorless. Appendages appeared at the apex and base. Apical appendages were unknobbed. There were two to four appendages, mostly three, and they were 18–40 (–43) μm long. Basal appendages were single, centric, with unknobbed tips, and 3.8–12.2 μm long. The conidial morphology of the fungus was quite different from that of *Pestalotiopsis menezesiana* and *P. uvicola*, which

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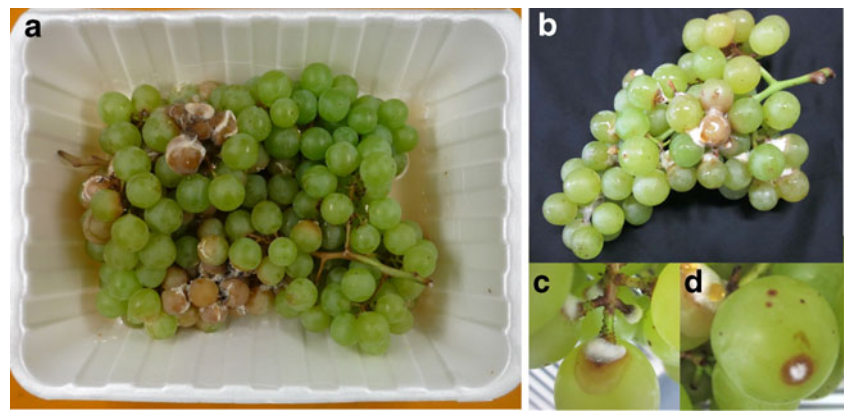
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Fig. 1 Symptoms on ‘Cheongsoo’ grape fruit caused by *Pestalotiopsis* sp. in a store house (a) and during pathogenicity tests (b–d)



are two causal agents of fruit rot disease on grape (Xu et al. 1999, Table 1). Moreover, the morphology was very similar to that of *P. longisetula* among related species, but it could be differentiated based on its relatively longer basal appendages (Table 1). Two cultures of the fungus (CNU 100093 and 100094) were deposited in the Culture Collection of Chungnam National University (CNU) Fungal Herbarium, Daejeon, Korea and the Korean Agricultural Culture Collection (KACC), Suwon, Korea.

Genomic DNA was extracted according to the method described by Park et al. (2005). The internal transcribed spacer (ITS) region of the ribosomal DNA gene was then amplified by primers ITS5 and ITS4 (White et al. 1990), while the β -tubulin (BT2) gene was amplified by primers Bt2a and Bt2b (Glass and Donaldson 1995). The sequences of the amplified products were then deposited in the GenBank database and assigned accession numbers JQ288418 (ITS) and JQ288419 (BT2). BLAST searches revealed that the present fungus was

identical to *Pestalotiopsis* sp. (UCD2559AR) in the BT2 gene sequence (HQ288311) and its ITS gene sequence differed by one nucleotide (HQ288234), while it differed from *Pestalotiopsis longisetula* by four and one nucleotides of the BT2 and ITS gene sequences, respectively. Their phylogenetic relationship (Fig. 3) was determined by RAXML analysis (Stamatakis et al. 2008). Apparently, the present fungus was closely related to *Pestalotiopsis* sp. 2 (UCD2559AR) isolated from *Vitis vinifera* associated with grapevine trunk diseases (Urbez-Torres et al. 2012). Although sequence data of the isolate (UCD2559AR) was deposited in the GenBank database (HQ288234 and HQ288311), the morphological description of the fungus was not reported. In order to give a new epithet to our fungus, morphological comparison between our fungus and *Pestalotiopsis* sp. 2 (UCD2559AR) is needed.

Pathogenicity tests of the fungus were conducted on two grape cultivars, ‘Cheongsoo’ and ‘Campbell Early’. To accomplish this, conidia of the fungus identified here produced on

Fig. 2 *Pestalotiopsis* sp. (CNU 100093) on ‘Cheongsoo’ grape. a–b Colonies on PDA at 25 °C for 7 days. c–d Acervuli on autoclaved segments of carnation leaf (scale bar=50 μ m). e Conidiogenous cells (scale bar=20 μ m). f–g Conidia (scale bar=20 μ m)

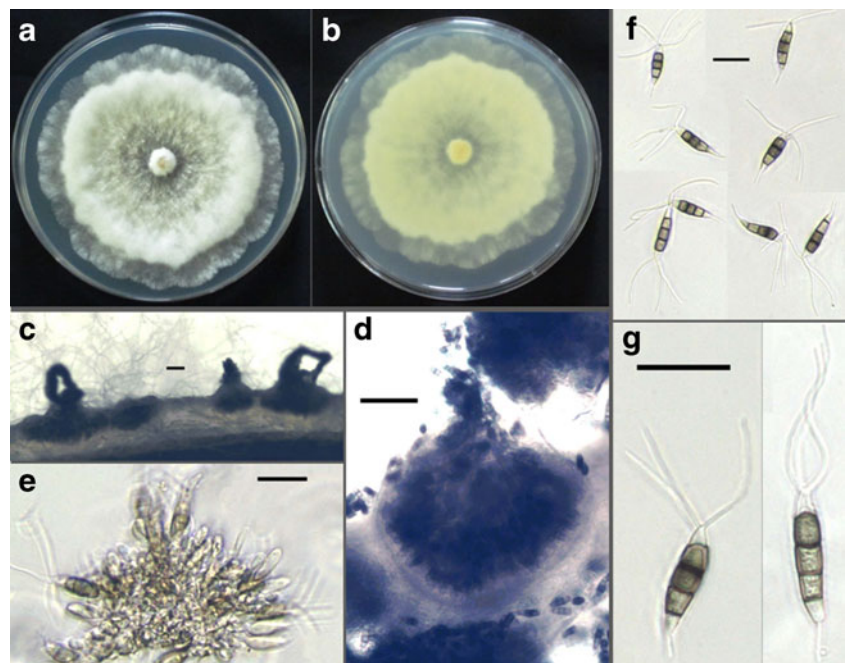


Table 1 Morphological comparison of the present fungus (*Pestalotiopsis* sp. CNU 100093) with related species

Species	Conidial size (µm)	Number of apical appendages	Length (µm) of appendages		Reference
			Apical	Basal	
Present fungus	19.5–29.2×4.6–7.2	2–4 (mostly 3)	18–40 (–43)	3.8–12.2	In this study
<i>P. crassiuscula</i>	20–28×7.5–9	3	10–23	2.5–5	Guba 1961
<i>P. lambertiae</i>	18.9–24.7×5.9–7.3	3	12.4–16.9	1.3–5.9	Liu et al. 2006
<i>P. longisetula</i>	21–29×6–7	3	20–32	3–6	Kasra et al. 2008
	25–33×6.6–8	3–4	22–44	3.2–8 (–9.5)	Maas 1998
<i>P. photiniae</i>	20–24×7–8	3	16–18	up to 7	Guba 1961
<i>P. menezesiana</i>	19–26×6.5–9	3	9–31	6	Guba 1961
	19–26×6–8	3	9–32	6	Xu et al. 1999
<i>P. uvicola</i>	18–25×5.5–7.5	2–3	13–22	7	Guba 1961
	20–28×6–8	2–5(3–4)			Ryu et al. 1993
	17–27×5–6.5	2–3	9–26	7	Xu et al. 1999

PDA media were flushed with distilled water and adjusted to 1×10^6 spores/ml. Grape clusters were then surface sterilized with sodium hypochlorite (2 % available chlorine) for 5 min and rinsed three times with distilled water. The spore suspension was subsequently sprayed on sterile grape clusters, placed in moistened clean boxes and incubated for 2 weeks at 25 °C. A control was treated with distilled water. Disease symptom development was then observed every 2 days and the number

of infected grapes was counted. The severity of disease was determined by the percentage of the infected grapes. Severe infection of the ‘Cheongsoo’ grape was observed 14 days after artificial inoculation with the present fungus and the symptoms were similar to those of grapes with the fruit rot disease observed in store houses (Fig. 1b); however, the ‘Campbell Early’ grape cultivar did not show any disease symptoms. Normally, white hyphae initially appeared at the point of rupture (the area connecting pedicel and fruit berry) after 2 days. Later, pale reddish brown fruit skin was observed near the point of rupture, which enlarged with time. In addition, the grape fruits became rotten (Fig. 1c). The fungus could infect grape berries from scars of fruit skin (Fig. 1d). After 2 weeks, 30–40 % of grapes in each cluster were infected, while little infection (0–2 %) was found in the control group. Disease severity is shown in Fig. 4.

Xu et al. (1999) isolated several fungi such as *Botrytis*, *Alternaria*, *Pestalotiopsis*, *Penicillium*, *Stemphylium* and

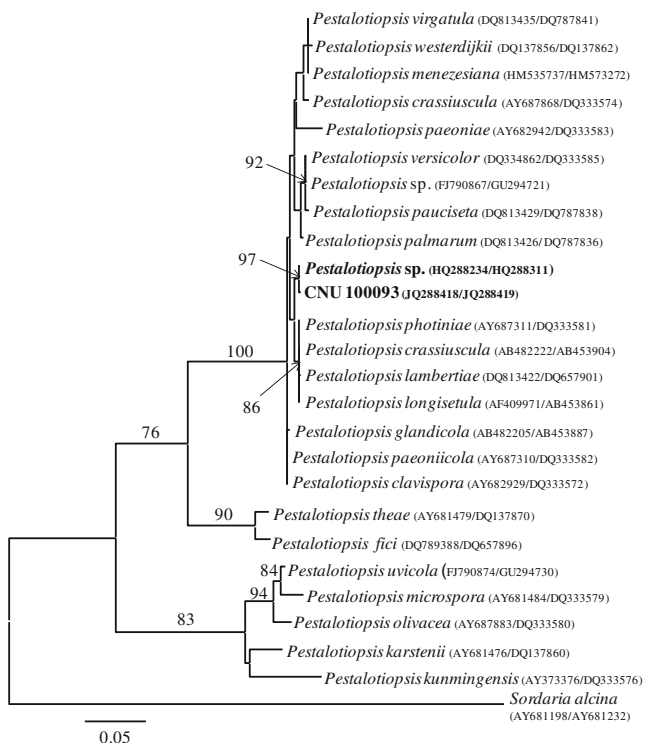


Fig. 3 Maximum likelihood tree generated from the combined data of ITS and β-tubulin gene sequences of *Pestalotiopsis* species. Bootstrap values (>70 %) from 1,000 replicates are shown at each node. The bar indicates the number of substitutions per position

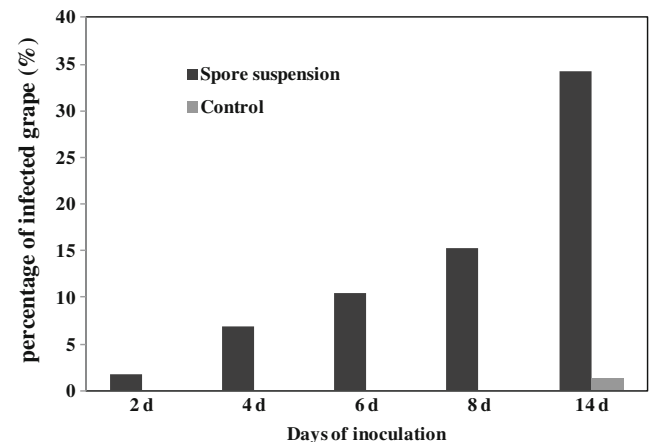


Fig. 4 Disease severity of *Pestalotiopsis* sp. inoculated on ‘Cheongsoo’ grape. The columns indicate the percentage of infected berries after 2, 4, 6, 8 and 14 days

Rhizopus from rotted berries of grapes in Japan. They also found that *Pestalotiopsis menezesiana* and *P. uvicola* initiate postharvest disease of grapes. The presence of *Pestalotiopsis* spp. on canes, woody tissue, berries, flowers, and leaves of grapevines has also been reported in Australia (Castillo-Pando et al. 2001; Sergeeva et al. 2005). *Pestalotiopsis* was one of the prevalent fungi isolated from the cankers of grapevines in Arkansas and Missouri (Urbez-Torres et al. 2009), and it was the second most common fungus isolated from grapevine cankers in Texas (Urbez-Torres et al. 2012). Urbez-Torres et al. (2012) reported that *Pestalotiopsis* sp. and *P. uvicola* occurred on cankers and bleached canes of grapevines, and could induce canker of grape canes. In Korea, *P. uvicola* has been reported to cause fruit rot disease of grape (Ryu et al. 1993), but limited information on conidial morphology of the species was described (Table 1). This is the first report of *Pestalotiopsis* sp. causing fruit rot disease on the grape cultivar ‘Cheongsoo’.

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