

First Report of Postharvest Gray Mold Rot on Carrot Caused by *Botrytis cinerea* in Korea

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In February 2014, gray mold rotting symptoms were observed in carrots in cold storage at Gangneung, Gangwon province, Korea. The typical symptom of gray mold rot showed abundant blackish gray mycelia and conidia was observed on the infected root. The pathogen was isolated from infected root and cultured on PDA for further fungal morphological observation and confirming its pathogenicity according to Koch's postulates. Results of morphological data, pathogenicity test and rDNA internal transcribed spacer (ITS 1 and 4) sequence showed that the postharvest gray mold rot of carrot was caused by *Botrytis cinerea*. This is the first report of postharvest gray mold rot on carrot in Korea.

Keywords : *Botrytis cinerea*, Carrot, Pathogenicity, Postharvest gray mold rot

Carrots (*Daucus carota* var. *sativa* DC.) are biannual crop under the *Apiaceae* (*Umbelliferae*) family. The edible portion is the storage taproot, which contains high levels of carbohydrates (sugars) and β -carotene (pre-vitamin A). In 2011, carrot was grown in Korea on 2,849 ha of land to yield a total of 93,694 Mt with productivity of 32.88 t ha⁻¹ (Anonymous, 2012).

Botrytis cinerea (teleomorph *Botryotinia fuckeliana*) also known as gray mold fungus is a ubiquitous filamentous fungal pathogen and may cause enormous damage both during plant growth and postharvest (Jarvis, 1977). The pathogen is a necrotroph, inducing host cell death resulting in serious damage to plant tissues culminating in rot of the plant or the harvested product (Govrin and Levine, 2000; Staats *et al.*, 2005). *Botrytis* spp. are often considered a cool weather pathogen with best growth, sporulation, spore release, germination, and establishment of infection occurring at an optimum of 18 to 23°C. However, it is also active at low temperatures and can cause considerable loss of plant material maintained at 0 to 10°C. This fungus has been reported on the leaves and petioles of carrots infected at field condition in Korea (Park *et al.*, 2011) and postharvest carrot disease in the USA (Davis and Raid, 2002) but not reported in Korea as a postharvest carrot disease.

Isolation of the fungi and pathogenicity test. In February 2014, rotting symptoms were observed in carrots in cold

storage at Gangneung, Gangwon province, Korea (Fig. 1A). The symptoms observed were abundant blackish gray mycelia and conidia on the infected root. For pathogen isolation, small pieces from infected root were sterilized by immersion in 0.1% sodium hypochlorite (NaOCl) for 1 min, rinsed with sterile distilled water three times and cultivated on potato dextrose agar (PDA, Difco, USA) and incubated at 20 ± 2°C for 7 days.

To determine the pathogenicity of the fungus, 10 ml conidial suspension (2 × 10⁴ spores per ml) was sprayed onto carrot sliced roots in plastic box lined with moist filter papers. The control was sprayed with sterile distilled water served as controls. The plastic box with sliced roots was incubated at 20 ± 2°C under laboratory condition. After 5 days, blackish gray mycelia were developed on sliced roots. The fungal pathogen was re-isolated from the disease lesions of the inoculated roots and the re-isolated pathogen exhibited the same morphological characteristics as those of the original isolates (Fig. 1B). Thus, the fungal pathogen fulfilled the criteria stipulated by the Koch's postulates and was identified as the causal agent of the postharvest gray mold rot of carrot.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequence analysis. For DNA extraction, the mycelia isolated from diseased lesion were grown in 250 ml flasks containing 100 ml potato dextrose broth, which were incubated for 4 days at 20°C on a rotary shaker at 125 rpm. Mycelia were harvested by vacuum filtration through Whatman No. 1 filter paper and then lyophilized for 24 h before grinding them to a fine

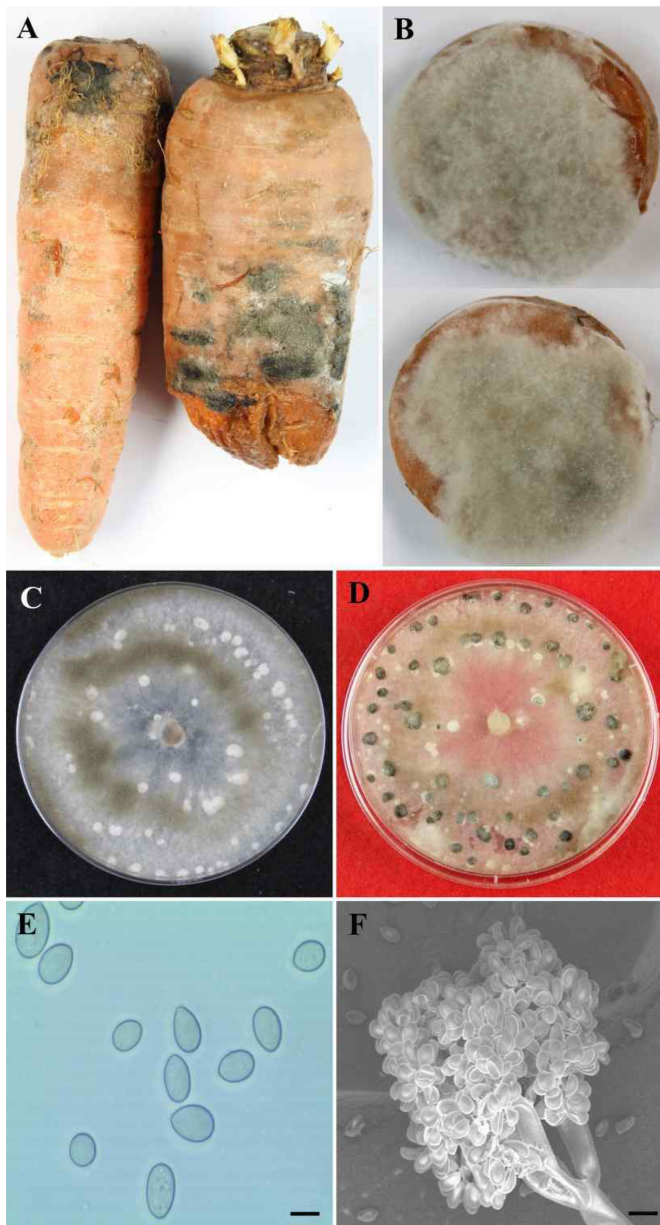


Fig. 1. Gray mold rot on carrot caused by *Botrytis cinerea*. **A:** Disease infected carrot root showing blackish gray mycelia and conidia, **B:** In pathogenicity test, produced similar symptoms on carrot sliced by artificial inoculation, **C and D:** One and three-wk-old colony showing black sclerotia growing on potato dextrose agar (PDA) medium, **E:** Conidia under light microscope, **F:** Conidiophore and conidia by SEM. Scale bar: E = 20 μm and F = 50 μm .

powder. Next, 100 mg of the ground powder was transferred to a 1.5 ml Eppendorf tube and DNA was extracted by using the CTAB extraction method (Moller *et al.*, 1992).

To confirm identity of the causal fungus, the complete ITS rDNA of the representative fungal pathogen was amplified and sequenced using by universal primers for internal transcribed spacer (ITS) 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990).

The amplification was performed in a 25 μl reaction mixture containing 0.5 μl of each primer, 0.5 μl of *Taq* DNA polymerase (Bioneer, Korea), 0.5 μl of each dNTP, 2.5 μl of 10 \times PCR reaction buffer, 18.5 μl of distilled water, and 2.0 μl of template DNA. The reaction was performed in Mastercycler Gradient (Eppendorf, Germany) under the following conditions: pre-denaturation at 94 $^{\circ}\text{C}$ for 5 min, followed by 35 cycles of denaturation at 94 $^{\circ}\text{C}$ for 35 s, annealing at 52 $^{\circ}\text{C}$ for 55 s, and elongation at 72 $^{\circ}\text{C}$ for 1 min and then post elongation at 72 $^{\circ}\text{C}$ for 10 min. The obtained nucleotide sequences were searched through BLASTn at the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>). Phylogenetic analysis of *B. cinerea* was performed by using the MEGA5 program with the neighbor-joining method (Tamura *et al.*, 2011). Sequence data were deposited in GenBank (accession number KJ685806).

Identification and characterization of *Botrytis cinerea*.

Total 6 fungal strains were obtained from the gray mold rot of storing carrots, and strain GM4 among 6 strains was examined for identification. Strain GM4 was identified as *B. cinerea* by analyzing the morphological characteristics of the isolated fungus and by performing rDNA sequencing analysis. The fungus produced gray to dark colonies with dark mycelium and produced abundant conidia on potato dextrose agar at 20 \pm 2 $^{\circ}\text{C}$ for 7 days (Fig. 1C). The conidia were one-celled, ellipsoid or ovoid in shape, dark brown, and 6.3~11.8 \times 5.2~8.0 μm in size (Fig. 1E). Conidiophores solitary, cylindrical, terminally branched, 15 to 28 μm wide, grayish to olivaceous gray, and smooth (Fig. 1F). After 3 weeks, the fungus formed several black sclerotia as survival structures near the PDA plate edge of petri dish (Fig. 1D). The morphological characteristics of the identified species are summarized in Table 1. The ITS sequence was compared to the GenBank database sequences by using the NCBI BLAST search tool (Fig. 2). The sequences identified based on ITS of rRNA gene alignment were 100% similar to those of several *B. cinerea* species (accession nos. HM989942.1, KC172064.1, EF207415.1, HQ171053.1, and KC683713.1). Thus, *B. cinerea* was identified as the causal agent of gray mold rot on carrot during storage in

Table 1. Comparison of morphological characteristics of gray mold fungus isolated from carrot

Characteristics		Present isolate	<i>Botrytis cinerea</i> ^a
Colony	color	greyish brown	greyish brown
Conidia	shape	ellipsoidal or ovoid	ellipsoidal or ovoid
	size (μm)	6.3–11.8 \times 5.2–8.0	6.0–18.0 \times 4.0–11.0
	color	dark brown	pale brown
Conidiophore	size (μm)	15–28	16–32
Sclerotia	shape	flat or irregular	flat or irregular
	color	black	black

^aDescribed by Ellis and Waller (1974).

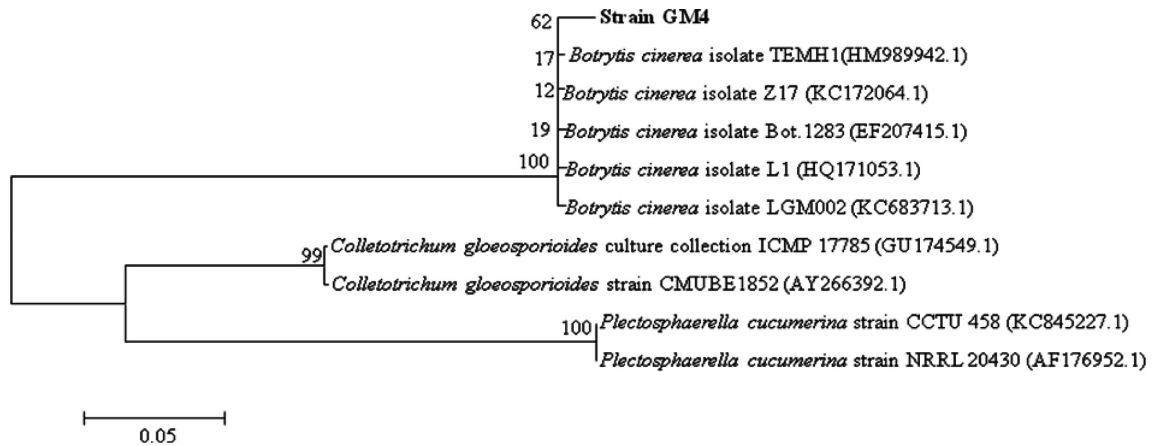


Fig. 2. Neighbor-joining phylogenetic tree of *Botrytis cinerea* strain GM4 and related species identified from GenBank based on internal transcribed spacer gene sequences. Numbers at the nodes indicate bootstrap values from a test of 1000 replications. The scale bar indicates the number of nucleotide substitutions.

Korea. To our knowledge, this is the first report of postharvest gray mold rot on carrot in Korea.

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