Mycobiology 39(1) : 52-53 (2011)
DOI:10.4489/MYCO.2011.39.1.052

First Report of *Botrytis cinerea* as a Postharvest Pathogen of Blueberry in Korea

Jin-Hyeuk Kwon[1]*, Mi-Geon Cheon[1], Okhee Choi[2] and Jinwoo Kim[2]

[1]*Gyeongsangnam-do Agricultural Research and Extension Services, Jinju 660-360, Korea*
[2]*Department of Applied Biology and Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660-701, Korea*

(Received January 25, 2011. Accepted February 18, 2011)

Gray mold of blueberry caused by *Botrytis* sp. is reported for the first time in Korea. A detailed description of the fungus is given, along with its rDNA internal transcribed spacer sequence. The fungus was identified as *Botrytis cinerea* based on mycological characteristics and molecular data.

KEYWORDS : *Botrytis cinerea*, Gray mold, Postharvest disease

Blueberry (*Vaccinium corymbosum*), a flowering plant with dark-purple berries, is sold fresh, processed as frozen fruit and juice, or as dried goods such as jellies, jams, and blueberry pies. A gray mold that was a morphologically distinct *Botrytis* sp. was observed on blueberries in a storage room at Gyeongsangnam-do Agricultural Research and Extension Services, Jinju, Korea. Infection rates reached 8% in August and September of 2010. *Botrytis* diseases appear primarily as blossom blights or fruit rots, but they also cause harvest diseases in various crops [1]. The pathogen favors cool and humid conditions, causing considerable losses during storage [2].

Symptoms. Symptoms of gray mold were observed on harvested fruits after 2~3 days of storage in a refrigerated room. The symptoms usually started with wrinkles, atrophy, crouch down, and depression on the fruit surfaces (Fig. 1A). Accordingly, the heavily infected fruits rotted. Because the symptoms appeared quickly in a refrigerated room, the fruits may have been infected while they were in the field.

Mycological characteristics. A causal fungal pathogen was isolated from freshly infected fruits. The optimum temperature for mycelia growth or sclerotia formation was 20°C. The sclerotia, which were flat or irregular in shape and black in color, formed abundantly on potato dextrose agar (Fig. 1B). Detailed microscopic examinations of a representative isolate were performed using a model 1420VP scanning electron microscope and an Axioplan 2 light microscope (Carl zeiss, Göttingen, Germany). The conidia were one-celled, ellipsoid or ovoid in shape, colorless or pale brown, and 8~16 × 5~10 μm in size. Conidiophores were brown in color and 16~31 μm in length (Table 1, Fig. 1C).

*Corresponding author <E-mail : kwon825@korea.krr>

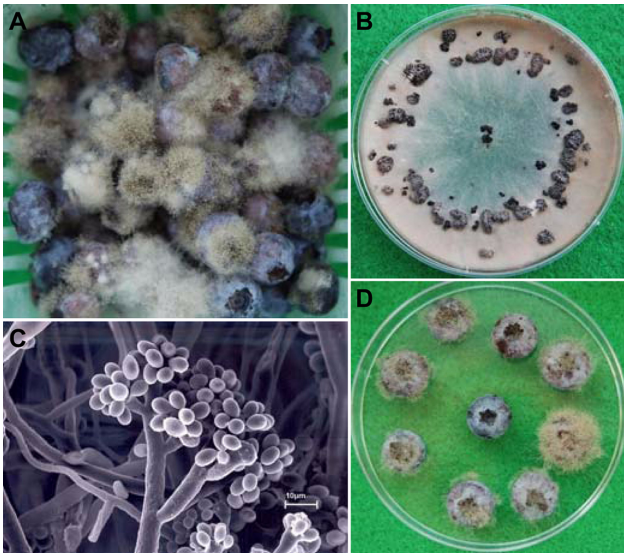

Fig. 1. Symptoms of gray mold of blueberry (*Vaccinium corymbosum*) and morphological characteristics of the causal organism, *Botrytis cinerea*. A, Symptoms of gray mold; B, Appearance of mycelial mat and sclerotia on potato dextrose agar; C, Scanning electron micrography image of conidia and conidiophores; D, Symptoms induced by artificial inoculation.

Pathogenicity test. Thirty berries of the 'Northland' cultivar (*V. corymbosum*), were surface-sterilized with 1% NaOCl, rinsed in sterile distilled water three times, and allowed to dry. The surface-sterilized blueberries were placed on moist filter papers in a plastic box (29 × 22 × 15 cm). The conidial suspension (3×10^4 conidia/mL) of the causal pathogen was added dropwise to the surface of the blueberries. The inoculated blueberries were kept in a humid chamber with 100% relative humidity at 20°C for 24 hr then placed on a laboratory table at room temperature for observation. The first symptom appeared on the blueberries with mycelia and conidia after 4 days of inoc-

Table 1. Comparison of morphological characteristics of gray mold fungus isolated from blueberry (*Vaccinium corymbosum*), with the previous descriptions of *Botrytis cinerea*

Characteristics		Present isolate	*B. cinerea* [3]
Colony	Color	Grayish brown	Grayish brown
Conidia	Shape	Ellipsoidal or ovoid	Ellipsoidal or ovoid
	Size (μm)	8~16 × 5~10	6~18 × 4~11
	Color	Colorless or pale brown	Colorless or pale brown
Conidiophore	Size (μm)	16~31 × 2	16~30 × 2
Sclerotia	Shape	Flat or irregular	Flat or irregular
	Color	Black	Black

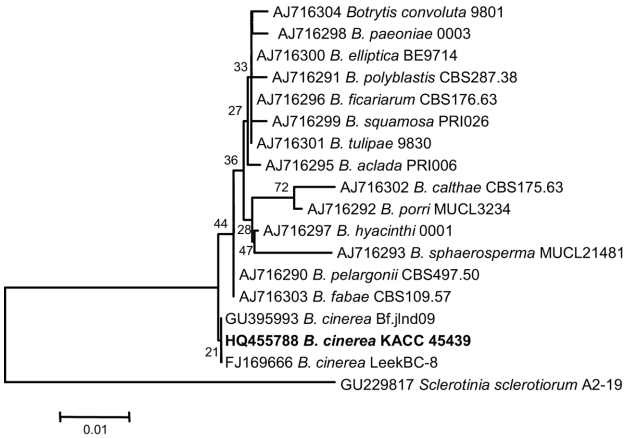

Fig. 2. Phylogenetic tree based on internal transcribed spacer sequences, showing closest known relatives of *Botrytis cinerea*, including the gray mold fungus infecting blueberry. DNA sequences from the National Center for Biotechnology Information nucleotide database were aligned using ClustalW, and a phylogenetic tree was constructed using the neighbor-joining method and visualized with TreeView. Numbers above the branches indicate bootstrap values. Bars indicate number of nucleotide substitutions per site. The isolate infecting blueberry is indicated in bold font.

ulation and rapidly spread to nearby fruits (Fig. 1D). The causal pathogen was re-isolated from the lesions to confirm Koch's postulates. The pathogenicity test was conducted twice.

Internal transcribed spacer (ITS) sequence analysis. To confirm identity of the causal fungus, the complete ITS rDNA of the representative fungal pathogen was amplified and sequenced using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White *et al*. [4]. The resulting 613-bp sequence was deposited in GenBank (accession No. HQ455788). Phylogenetic analysis was conduced using MEGA4 software, with the neighbor-joining method and the Tajima-Nei distance model. In the phylogenetic tree (Fig. 2), the isolate was placed within a clade comprising reference isolates of *Botrytis cinerea* [5].

On the basis of mycological characteristics, pathogenicity testing in host plants, and molecular data, the fungus was identified as *B. cinerea* Persoon: Fries [3]. To our knowledge, this is the first report of gray mold on blueberry caused by *B. cinerea* in Korea [6]. The representative culture of the fungus is stored at the Korean Agricultural Culture Collection (KACC 45439), National Academy of Agricultural Science, Rural Development Administration, Suwon, Korea.

Acknowledgements

This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ007345)" Rural Development Administration, Korea.

References

1. Agrios GN. Plant pathology. 5th ed. New York: Academic Press; 2005.
2. Caruso FL, Ramsdell DC. Compendium of blueberry and cranberry diseases. St. Paul: APS Press; 1995.
3. Ellis MB, Waller JM. *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*). CMI descriptions of pathogenic fungi and bacteria, No. 431. Kew: Commonwealth Mycological Institute; 1974.
4. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. New York: Academic Press, Inc.; 1990. p. 315-22.
5. Staats M, van Baarlen P, van Kan JA. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity. Mol Biol Evol 2005;22:333-46.
6. Korean Society of Plant Pathology. List of plant diseases in Korea. 5th ed. Seoul: Korean Society of Plant Pathology; 2009.