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First Report of Post-Harvest Fruit Rot of *Aronia melanocarpa* Caused by *Fusarium tricinctum* in Korea

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ABSTRACT : Black chokeberry, *Aronia melanocarpa* (Michx.) Elliott, is commonly used as a source of jam and jelly in Korea and worldwide. A fungal isolate EML-CCB6 was isolated from the decaying fruit of black chokeberry. Based on the morphological characteristics and rDNA internal transcribed spacer sequence analysis, the isolate was identified as *Fusarium tricinctum* (Corda) Sacc. This is the first report of post-harvest fruit rot of black chokeberry caused by *F. tricinctum* in Korea.

KEYWORDS : ***Aronia melanocarpa*, *Fusarium tricinctum* species complex, Post-harvest fruit rot**

Black chokeberry, *Aronia melanocarpa* (Michx.) Elliott, native to North America, is a berry packed with essential phyto-nutrients, vitamins, and antioxidants [1, 2]. Some polyphenol compounds of *Aronia* have been tested for immunomodulatory activity and against influenza viruses [3, 4]. Despite their high antioxidant levels, chokeberry fruits can be contaminated with mycotoxin-producing fungi (Fig. 1A). Several fungi have been found to infect *Aronia melanocarpa*, including *Penicillium*, *Alternaria*, and *Botrytis* [5, 6].

Fungal mycelia were initially observed on the chokeberry fruit (Fig. 1B, 1C). The infected fruit samples were examined under a stereo-microscope; individual hypha tips of the fungi were moved to potato dextrose agar (PDA) and incubated at 25°C. A pure isolate was transferred into a slant tube and deposited at the Environmental Microbiology Lab Herbarium (Chonnam National University, Gwangju, Korea) with the accession number of EML-CCB6 and also deposited at Culture Collection of National Institute of Biological Resources (NIBR, Incheon, Korea) as ex-type (KOSPFGC000002016). Morphological characteristics of the isolate were observed after 7 days of incubation at 25°C. Colonies on the PDA showed 3 pigments, including brown, red, and white colours in the medium, reaching 25 mm in diameter at 25°C after 7 days (Fig. 1D). Conidiophores were simple or branched. Phialides were subcylindrical or cylindrical. Microconidia were abundant, oval, or pyriform, measuring 3.0~4.5 (av. 3.5) µm wide × 9.5~18.0 (av. 13.5) µm long and commonly having one septate. Macroconidia were oval-shaped and reniform, measuring 3.5~4.0 (av. 3.5) µm wide × 27.5~42 (av. 34.5) µm long and having 3~5 septate (Fig. 1). Chlamydospores were found in chains or clumps, mostly globose or subglobose. Based on the morphological characteristics and rDNA sequence analysis, EML-CCB6 was identified as *Fusarium tricinctum* (Corda) Saccardo [7] belonging to the *Fusarium tricinctum* species complex (FTSC) group. For the phylogenetic analysis of the genus *Fusarium*, several studies based on internal transcribed spacer (ITS) and large subunit ribosomal DNA, translation elongation factor-1α, β-tubulin gene sequences, and RNA polymerase II have been conducted [8-11].

To confirm the morphological identification, genomic DNA was directly extracted from mycelia and conidia using the Higene™ Genomic DNA prep Kit for fungi (Biopact Corp., Daejeon, Korea). The internal transcribed spacers (ITS1 and ITS2) and 5.8S gene were amplified using the primers ITS1 (5- CTTGGTCATTTAGAGGAA

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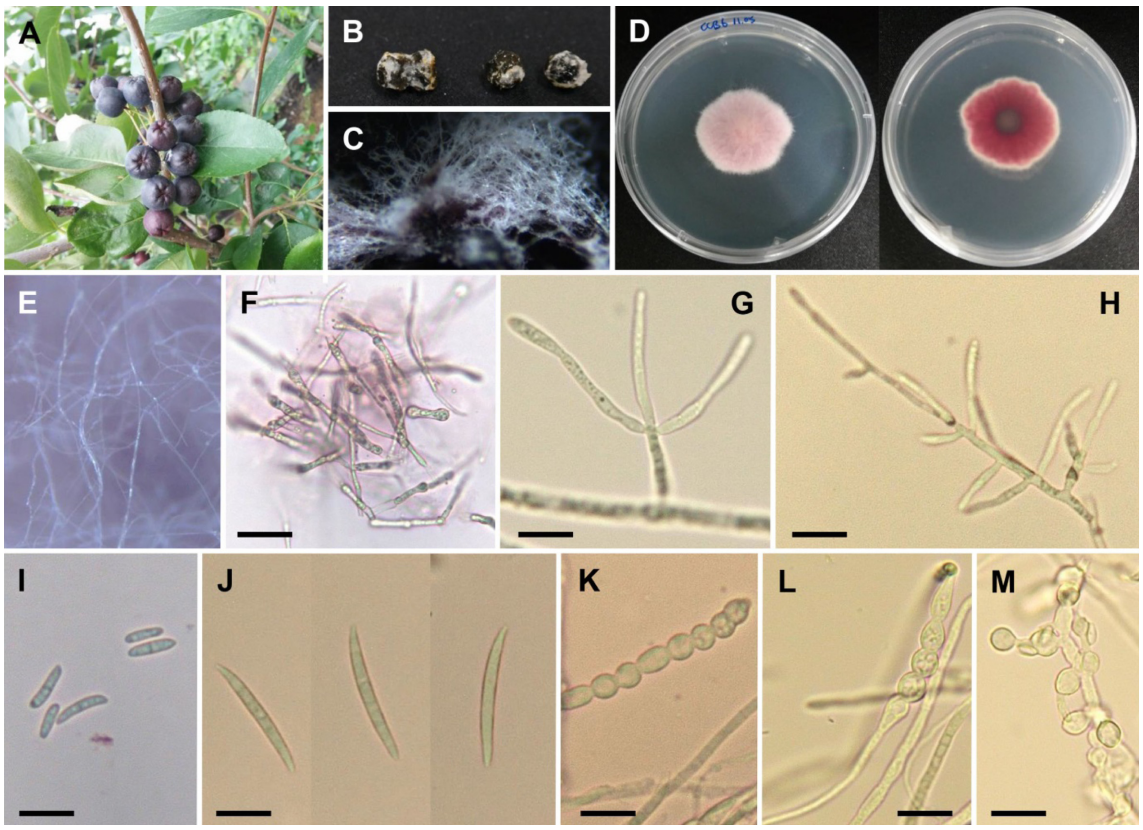

Fig. 1. Post-harvest fruit rot on black chokeberry and morphology of the causal fungus. A, Fresh black chokeberry fruit; B, Post-harvest fruit rot of black chokeberry; C, White mycelia on the rot fruit; D, Colonies on potato dextrose agar (PDA); E, F, Hyphae on PDA; G~H, Conidiophore and phialide on PDA; I, Microconidia; J, Macroconidia; K~M, Chlamydospores on PDA (scale bars: F~M = 20 μm).

GTAA-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) as described by White et al. [12]. The polymerase chain reaction (PCR) amplification parameters were as follows: initial denaturation at 95°C for 5 min, followed by 35 thermal cycles with denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. The PCR products were purified using the Accuprep PCR Purification Kit (Bioneer Corp., Daejeon, Korea). DNA sequencing was performed in an ABI 3700 Automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The sequence was deposited in the GenBank database with accession no. KU948155. BLASTN searching revealed that the rDNA ITS sequence of EML-CCB6 is identical to those of *F. tricinctum* BBA69828 and BBA64485 (GenBank accession no. AF111054 and AF405482, respectively) belonging to FTSC group. The sequences were initially aligned using CLUSTAL X [13] and the alignment was edited manually. Phylogenetic analyses were performed using the maximum likelihood method in MEGA 6 [14] with the default settings. A sequence of *Fusarium concolor* was used as the outgroup. In the phylogenetic tree (Fig. 2), the EML-CCB6 isolate was placed within the FTSC clade with strong bootstrap support.

Fusarium species are of major concerns in cultivation of wheat and other grains, causing *Fusarium* head blight, root rot, and seedling blight. Different species of *Fusarium* vary in their contributions to yield loss and grain contamination with mycotoxins, as particular species can produce mycotoxins [11]. Particularly, *F. tricinctum* is known to produce trichothecane family toxins [15-17]. Therefore, appropriate control strategies should be considered for preventing the fungal contamination during storage.

To determine the growth rates, EML-CCB6 was cultured on four different media; PDA, malt extract agar (33.6 g MEA in 1 L of deionized water; Becton Dickinson Korea, Seoul, Korea), oatmeal agar (OA; 1.5% oatmeal and 1.5% agar; Junsei Chemical, Tokyo, Japan, and 1 L deionized water), and Sabouraud dextrose agar (SDA; 1% peptone, 4% dextrose and 1.5% agar; Junsei Chemical, and 1 L de-

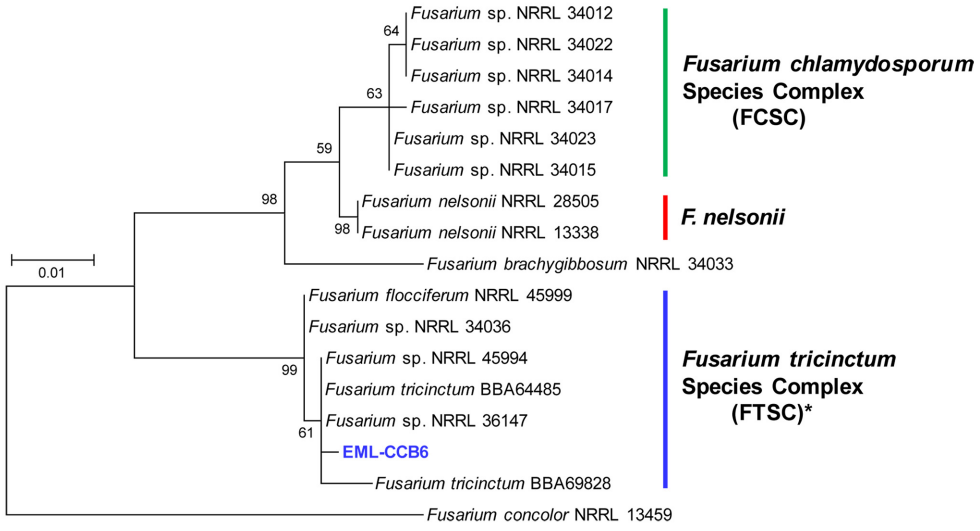

Fig. 2. Phylogenetic tree based on maximum likelihood analysis of internal transcribed spacer (ITS) rDNA sequence for EML-CCB6. *Fusarium concolor* was used as an outgroup. Bootstrap values are shown above/below the branches supported by more than 50% of 1,000 replications. *Fusarium complex system by O'Donnell et al. [11].

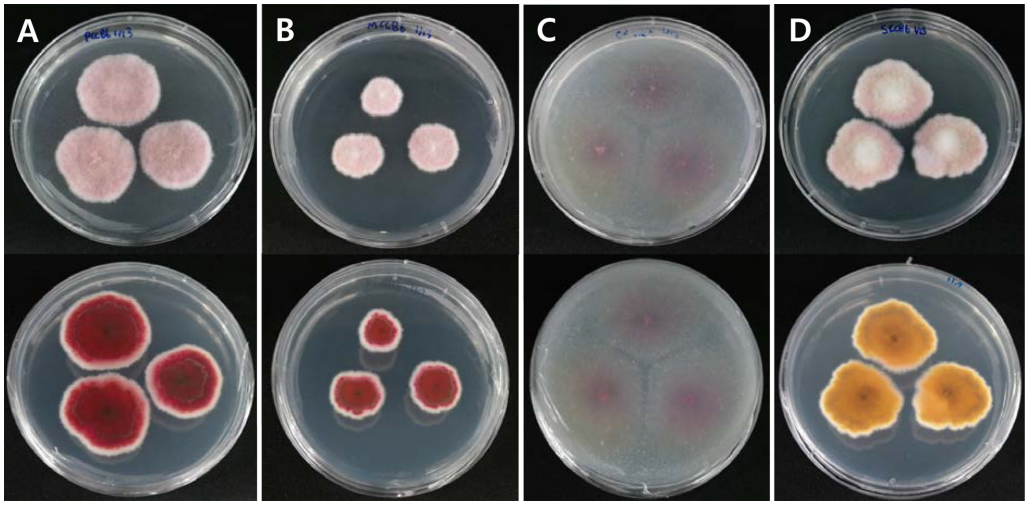

Fig. 3. Colony shapes of EML-CCB6 in potato dextrose agar (A), malt extract agar (B), oatmeal agar (C), and Sabouraud dextrose agar (D) after 7 days.

ionized water). The plates were incubated at 20°C, 23°C, 27°C, 30°C and 35°C in the dark for 7 days. Colony morphology of EML-CCB6 are diverse depending on different media (PDA, MEA, OA and SDA), respectively (Fig. 3). The average growth rates of EML-CCB6 on PDA, MEA and OA were 9.5 mm/day, 8.0 mm/day, and 17.5 mm/day, respectively. The optimal growth temperature ranged between 20°C and 27°C, but the slowest growth was observed at 35°C. Among the different temperatures and culture media, the best mycelial growth was found at a combination of 23°C and OA media (Figs. 3, 4).

The antimicrobial activity of *F. tricinctum* was reported in many studies [18-20]. Notably, the EML-CCB6 isolate showed moderate antifungal activity against several fungi, including *Botrytis cinerea*, *Fusarium oxysporum*, and *F. verticillioides* (data not shown). Thus, our results suggest that the strain can be used for biological control of the fungal diseases.

This is the first report of *F. tricinctum* on *Aronia melanocarpa* in Korea. Additional studies of pathogens associ-

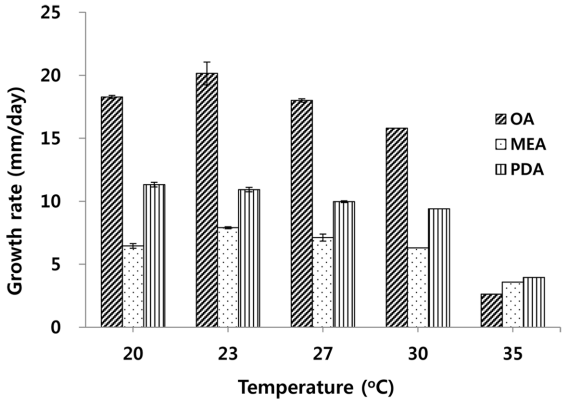

Fig. 4. Effect of temperature and culture media on mycelial growth of EML-CCB6 isolate. Mycelial growth was evaluated on three media (PDA, MEA, and OA) and the isolate was incubated at 20°C, 23°C, 27°C, 30°C, and 35°C. PDA, potato dextrose agar; MEA, malt extract agar; OA, oatmeal agar.

ated with *Aronia melanocarpa* in Korea are needed.

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