RESEARCH NOTE

First report of anthracnose crown rot caused by *Colletotrichum siamense* on strawberry in Korea

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ABSTRACT

Anthracnose crown rot (ACR) has been observed in greenhouses during the nursery and harvest seasons in Gangwon Province, Korea. Infected plants showed black leaf spot, dark sunken pink conidial masses on petioles, wilting, and eventually death. Five isolates were obtained from the lesions of strawberry plants and were identified as a *Colletotrichum gloeosporioides* species complex based on their cultural and morphological characteristics. Multilocus sequence analysis of actin, calmodulin, chitin synthase, glyceraldehyde-3-phophate dehydrogenase genes, and internal transcribed spacer rDNA regions showed that the isolates formed a monophyletic group with the type strain of *C. siamense*. Pathogenicity tests were performed on the isolate, and Koch's postulates were performed to verify the relationship between *Colletotrichum* sp. and the strawberry plant variety Seolhyang. The isolate was pathogenic to strawberry plants, which exhibited typical ACR symptoms. Based on morphological characteristics, pathogenicity, and DNA sequence analyses, the fungus isolated in Korea was identified as *C. siamense*. This is first time *C. siamense* has been confirmed in ever-bearing strawberry varieties in Korea.

Keywords: Anthracnose crown rot, *Colletotrichum siamense*, Strawberry

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Strawberry (*Fragaria* x *ananassa* Duch.) in Korea is a high-income crop, with a production value of $ 1.029 billion from 5,683 ha in 2020 (KOSIS, 2021). Most of strawberries grown in Gyeongnam and Chungnam provinces are June-bearing varieties such as Seolhyang [1], and Kuemsil [2]. However, some ever-bearing strawberry varieties, such as Goha [3] and Miha [4], are cultivated in highland regions in Gangwon province. Anthracnose crown rot (ACR) caused by *Colletotrichum fructicola* is one of the most damaging diseases in June-bearing strawberries [5]. Recently, ACR has occurred in ever-bearing varieties in Miha and Goha, during the summer and autumn seasons in highland region. The incidence of diseased plants in the field ranged from 10 to 30%. The symptoms of ACR presented more severe leaf blackening than in previous instances of ACR, and their occurrence was higher in summer season. The symptoms of

ACR are as follows: the petiole of the strawberry plants had a pink conidial mass on dark, sunken spots on the acervuli, the leaves form circular black spots resembling ink spots (Fig. 1A), the whole plant showed symptoms of wilting (Fig. 1B), and the crown changed entirely into a reddish-brown color (Fig. 1C).

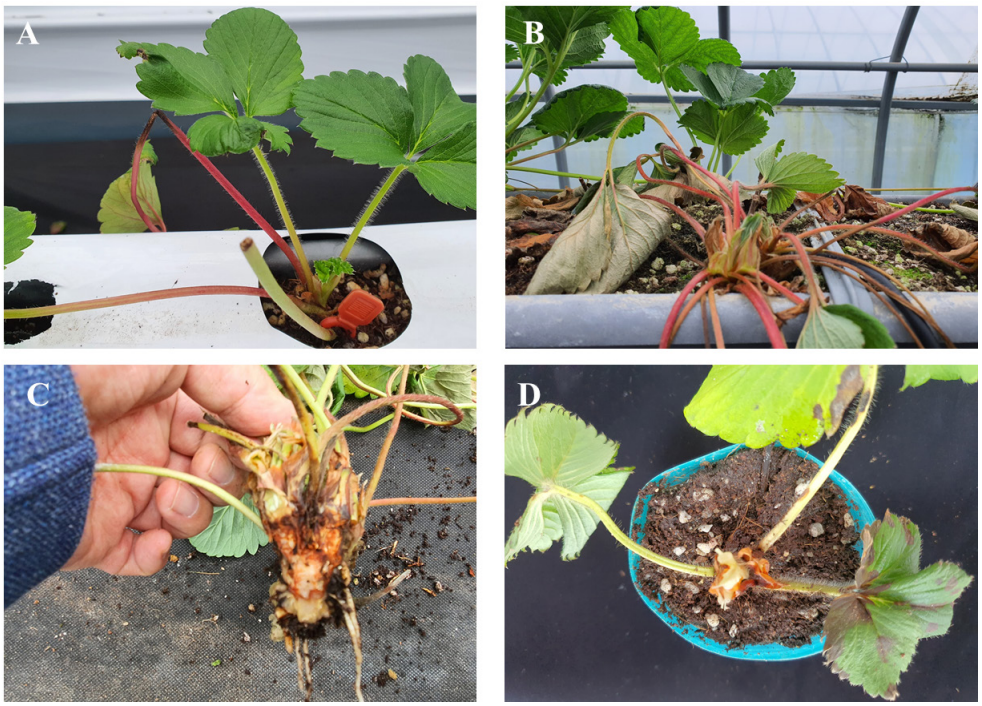

Fig. 1. Anthracnose symptoms on strawberry of *Colletototrichum siamense*. (A) Strawberry plant with anthracnose crown rot girdling on a petiole. (B) Wilting plant. (C) Longitudinal section of an infected crown showing marbled reddish-brown necrosis. (D) Symptoms of artificial inoculation test with an isolate.

Diseased strawberry plant tissues were surface-sterilized with 70% alcohol for 2min and rinsed thrice with sterile water. It was then dried on sterile tissue paper. The samples were incubated at 25°C after being placed on water agar. The edges of any fungal mycelia grown from the tissue ware aseptically transferred to potato dextrose agar (PDA; Difco, Becton Dickinson, Franklin Lake, NJ, USA). Single spore isolation was also carried out using the procedure described by [6] and pure cultures were stored at 4°C.

Morphological characteristics of the five isolates were investigated using a compound microscope (Olympus, Tokyo, Japan) to examine the shape and size of the conidia. All isolates were identified as *C. gloeosporioides* species complexes based on morphological characteristics as previously reported [7]. Colonies of all isolates initially formed greyish white mycelia on the PDA and became pale brown to pink on the front side and pink on the back side (Figs. 2A and B). Conidia were one-celled, smooth-walled, hyaline, cylindrical with both ends bluntly rounded (Fig. 2C), with size ranging 12.5-18.5×4.0-6.0 μm (Table 1). Appressoria were ovate, globose, or ellipsoidal with dark brown in color, and ranged from 11 to 20×5 to 12 μm in size (Fig. 2D).

Table 1. Morphological characteristics of the strain used in this study compared with a previous report on *Colletotrichum siamense*.

Characteristic	*C. siamense* CGS211102	*C. siamense*[a]
Colony morphology	Cottony, white to pale gray to dark gray	Cottony, aerial mycelium grayish white, orange conidial masses at the center
Conidia size (μm)	12.5-18.5×4.0-6.0	11.0-14.5×4.0-5.0
Conidia shape	Cylindrical, with round obtuse ends	Cylindrical, both ends bluntly rounded

[a]Described by [18].

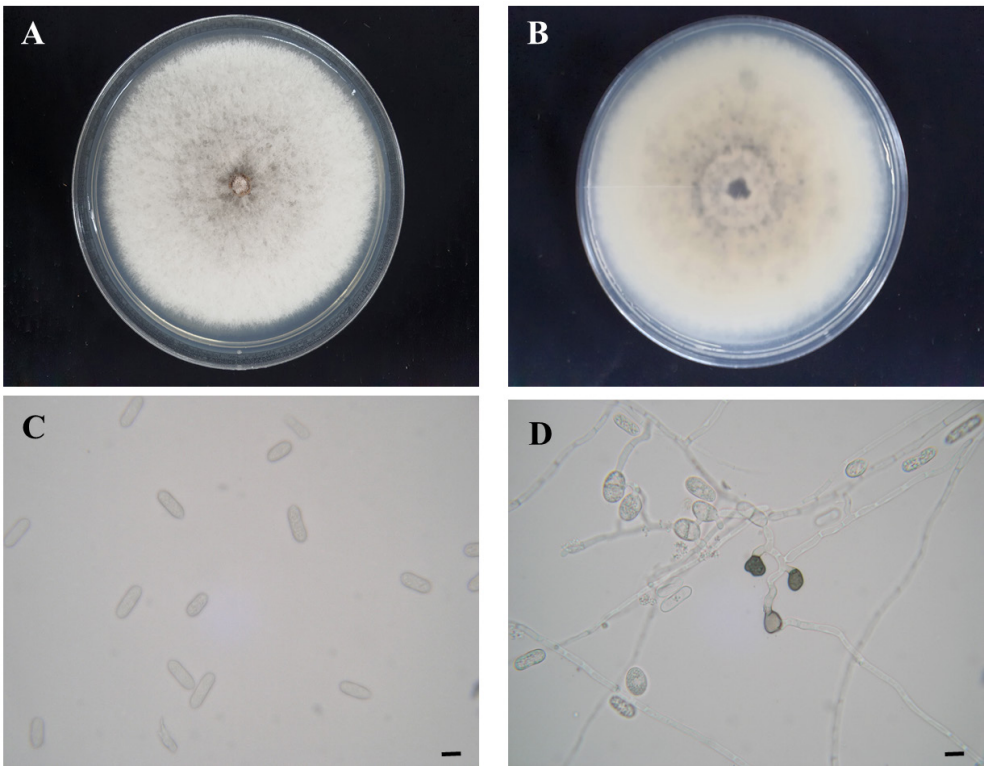

Fig. 2. *Colletotrichum siamense*. (A) and (B), Cultures of CGS211001 on potato dextrose agar; 7-d growth single conidia from above and below. (C), Conidia. (D), Appressoria. Scale bar=10 μm.

Genomic DNA was extracted from fungal strains grown on PDA using a modified cetytrimethylammonium bromide (CTAB) extraction protocol [8]. PCR amplifications of internal transcribed spacer (ITS) rDNA, actin (*ACT*), calmodulin (*CAL*), chitin synthase (*CHS*-1), and glyceraldehyde-3-phophate dehydrogenase (*GAPDH*) were performed using previously described methods [9]. DNA sequencing was performed at Macrogen (Seoul, Korea) on an ABI PRISM 3730XL analyzer (Life Technologies, Carlsbad, CA, USA). Each sequence was assembled and proofread using MEGA ver. 5.0 [10] deposited in GenBank. All sequences were aligned with *Collectrichum* reference sequences from GenBank using MAFFT v7 [11]. Maximum likelihood phylogenetic analyses for the concatenated data set (ITS, *ACT*, *CAL*, *CHS*-1, and *GAPDH*) were performed using RAxML [12] implemented on CIPRES web portal, using the GTR+G model with 1,000 bootstrap replicates. The sequences of ITS, *ACT, CAL, CHS*-1, and *GAPDH* from five isolates CGS211101, CGS211102, CGS211103, CGS211104, and CGS211105 were deposited in GenBank (Nos. ON926518~ON926537, and ON878198~ ON878202).

Phylogenetic analysis revealed that five isolates (CGS211101, CGS211102, CGS211103, CGS211104, and CGS211105) formed a monophyletic group with *C. siamense* CBS 130417 (ex-type) (82% bootstrap value) (Fig. 3).

The mycelial growth rate of *C. siamense* was the highest at 30°C, and a higher temperature was preferred compared to *C. fucticola,* which was highest at 25°C (Fig. 4). The mycelial growth rate of *C. siamense* based on temperature was similar to that of *C. fructicola* (30°C or lower), but showed a high growth rate >32°C [8]. As shown in the above results, *C. siamense* requires a higher temperature than *C. fructicola*; therefore, there is a high risk of occurrence in the nursery field and after transplanting strawberries.

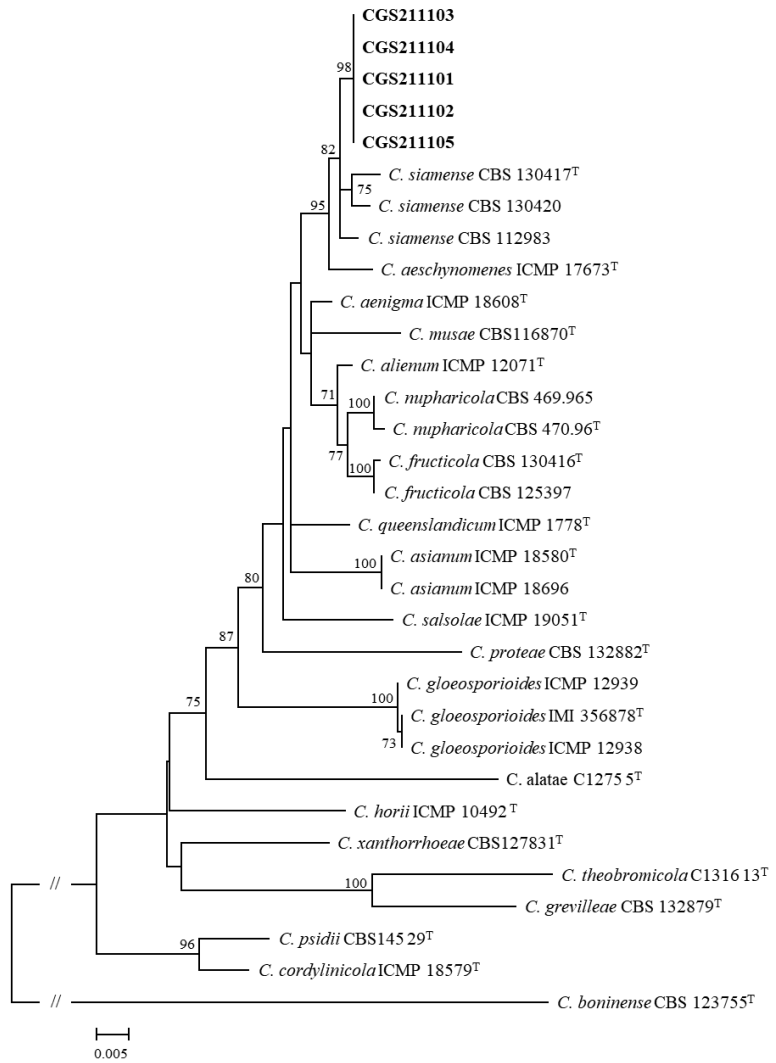

Fig. 3. Maximum likelihood phylogenetic tree based on the concatenated dataset (*ITS*, *ACT*, *CAL*, *CHS-1*, and *GAPDH*) used to identify *Colletotrichum* strains isolated from strawberry in Korea. Bootstrap scores greater than 70 are presented at the nodes. The scale bar indicates the number of nucleotide substitutions per site and the letter ^{T} indicates ex-type strains. The strains originating from strawberry are indicated in bold.

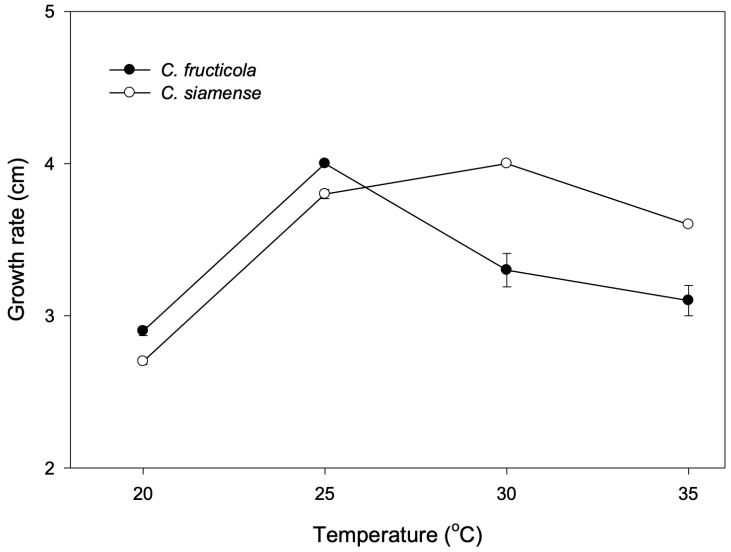

Fig. 4. Colony growth rate of the *Colletotrichum fructicola* and *C. siamense* from strawberry plants grown on potato dextrose agar under different temperatures for 5 days.

Based on the DNA sequence analysis and morphological features, five isolates were identified *as C. siamense*. Notably, *C. fructicola* has been previously reported to cause ACR in strawberries in Korea, and not *C. siamense* [5]. *C. siamense* in strawberry plant has been reported in the United States [7], Brazil [13], Japan [14], Taiwan [15], Bangladesh [16], and China [17]. Moreover, anthracnose by *C. siamense* in Korea was reported on apples [18], persimmons [19], Japanese plums [20], peaches [21], and chili peppers [22]. Therefore, it is necessary to investigate whether the *C. siamense* occurring in strawberry plants in Korea originated from another country or host plant.

Isolate CGS 211101 was used to confirm its pathogenicity in strawberry plants by artificial inoculation. Koch's postulates were performed by spraying 1mL of conidial suspensions (1×10^5 conidia/mL) per plant on the Seolhyang strawberry variety. Five plants were used for each test. Black spots were observed on all inoculated leaves and petioles after five days and necrosis eventually formed on the entire plants (Fig. 1D). The fungus was successfully re-isolated and identified as *C. siamense* according to the above description.

In this study, *C. siamense,* causing ACR in strawberries in Korea, was identified based on morphological features and molecular phylogenetic analysis. *C. siamense* causing ACR has been found in highland regions, but outbreaks in other strawberry-growing regions should be continuously monitored.

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