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Isolation and Identification of *Colletotrichum musae* from Imported Bananas

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Colletotrichum musae **was isolated from dark-brown anthracnose lesions on commercial banana (*Musa sapientum* L.) to establish the causal agent of the symptom. The fungus grew fast and produced white aerial mycelium on PDA. Acervuli developed abundantly on culture plates after incubation for 10 days at 25°C. Pinkish conidial masses were produced on the acervuli, which mostly coalesced together. Conidia were aseptate, hyaline, straight, ellipsoid to globose, and 14.5 × 6.9 μm in size. Black, clavate, round, or irregular-shaped appressoria measuring 8.8 × 6.8 μm were readily formed from germ tubes. Setae-like structures were not found either on the lesion or on the cultures. Sclerotia were also absent. Among the media, PDA medium was the best for mycelial growth. The optimum temperature for mycelial growth was 28°C, while the optimum pH ranged from pH 5.5 to 6.5. The isolates of *C. musae* caused black necrotic lesions on banana fruits by needle-wound inoculation, and orange-colored spore masses were produced on the lesions. The fungus also caused discoloration on apple fruits inoculated.**

Keywords : anthracnose, banana, *Colletotrichum musae*.

Banana (*Musa sapientum* L.) is one of the most popular fruits in Korea but it is entirely imported from tropical countries such as Taiwan and the Philippines. For international trading, banana fruits are usually harvested before ripening, and stored at relatively low temperature during transportation and market process. Long distance transport and extended storage period in the market may make banana sensitive to disease incidence (Thompson and Burden, 1995).

According to the report of the National Plant Quarantine Services of Korea, 2,647 MT of imported bananas were disinfected and 402 MT were discarded in 2000 and 2001 (www.npqs.go.kr). Insect control was the major reason for disinfecting and discarding. Even though various symptoms have been reported, some of which can develop during transport and storage, bananas can still be imported without any treatment because no abnormal lesions are observed on the fruits at the time of inspection.

Anthracnose caused by *Colletotrichum musae* (Berk. & Curt.) Arx. is one of the most important and widely distributed diseases of ripening and ripe bananas, and is particularly associated with wastage following injuries in the form of scratches and other wounds sustained by the fruits during handling and transport (Wardlaw, 1934). There are also losses in local markets because of disease infection of ripe fruits. The fungus can infect banana fruits at any time during the growing season in the field (Simmonds and Mitchell, 1940). Banana anthracnose usually starts as quiescent infections on green fruit in the field. However, successful penetration of the fungus is restricted by accumulation of phytoalexins as the fruits ripen (Jegger et al., 1995; Turner, 1995). Therefore, symptoms generally can be seen only in overripe fruits. Anthracnose becomes a serious problem when bananas are shipped as bunches for a long time and ripened under high temperature (Meredith, 1960a).

Colletotrichum musae is the most important pathogen on wounded green and ripe banana fruits (Meredith, 1960b; Stover and Simmonds, 1987b). Occasionally, the fungus invades necks of green fingers when damaged by flexing (Wardlaw, 1995). Lesions are sunken and covered with salmon-colored acervuli (Sutton and Waterston, 1970). Infections stimulate ripening of fruits and lesions elongate with ripening. On ripening fruits, sunken brown spots develop with orange acervuli (Stover and Simmonds, 1987b).

This study was conducted to identify the casual agent associated with anthracnose lesions on banana fruits collected from the grocery store and to examine mycological characteristics and pathogenicity of the fungal isolates.

Materials and Methods

Isolation of fungi. Fungi were isolated from anthracnose lesion on bananas sold in the market. Pieces of lesion tissue were surface-disinfected with 70% EtOH for 1 minute, rinsed with sterilized distilled water, and then air-dried on a clean bench. Dried samples were placed on potato dextrose agar (PDA) amended with streptomycin 100 μg/ml and lactic acid 25 μg/ml and incubated at 25°C. After 5 days of inoculation, growing

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mycelial tips were transcultured on new PDA medium. Single spore was isolated by dilution method on water agar and cultured on PDA for 5 days at 25°C to obtain monoconidial culture of the fungi. The isolates were transferred to PDA slants and kept at 4°C for preservation and further use.

Morphology and cultural characteristics. The shape, size, and color of conidia of the isolates were examined under the microscope after incubation at 25°C for 10 days on PDA. In addition, a drop of conidial suspension (1×10^5 conidia/ml) was inoculated on cellophane membrane on a slide glass and incubated for 24 h at 25°C to induce appressorial formation.

Cultural characteristics of the isolate were observed from the colony on culture plates incubated for 7 days at 25°C. Mycelial growth on the media was observed after incubation for 7 days at 25°C. The media used included PDA (potato dextrose agar), MEA (malt extract agar), CMA (corn meal agar), CDA (Czapek-Dox agar), OA (oatmeal agar), and WA (water agar). The optimum pH for mycelial growth was determined on PDA, where pH was

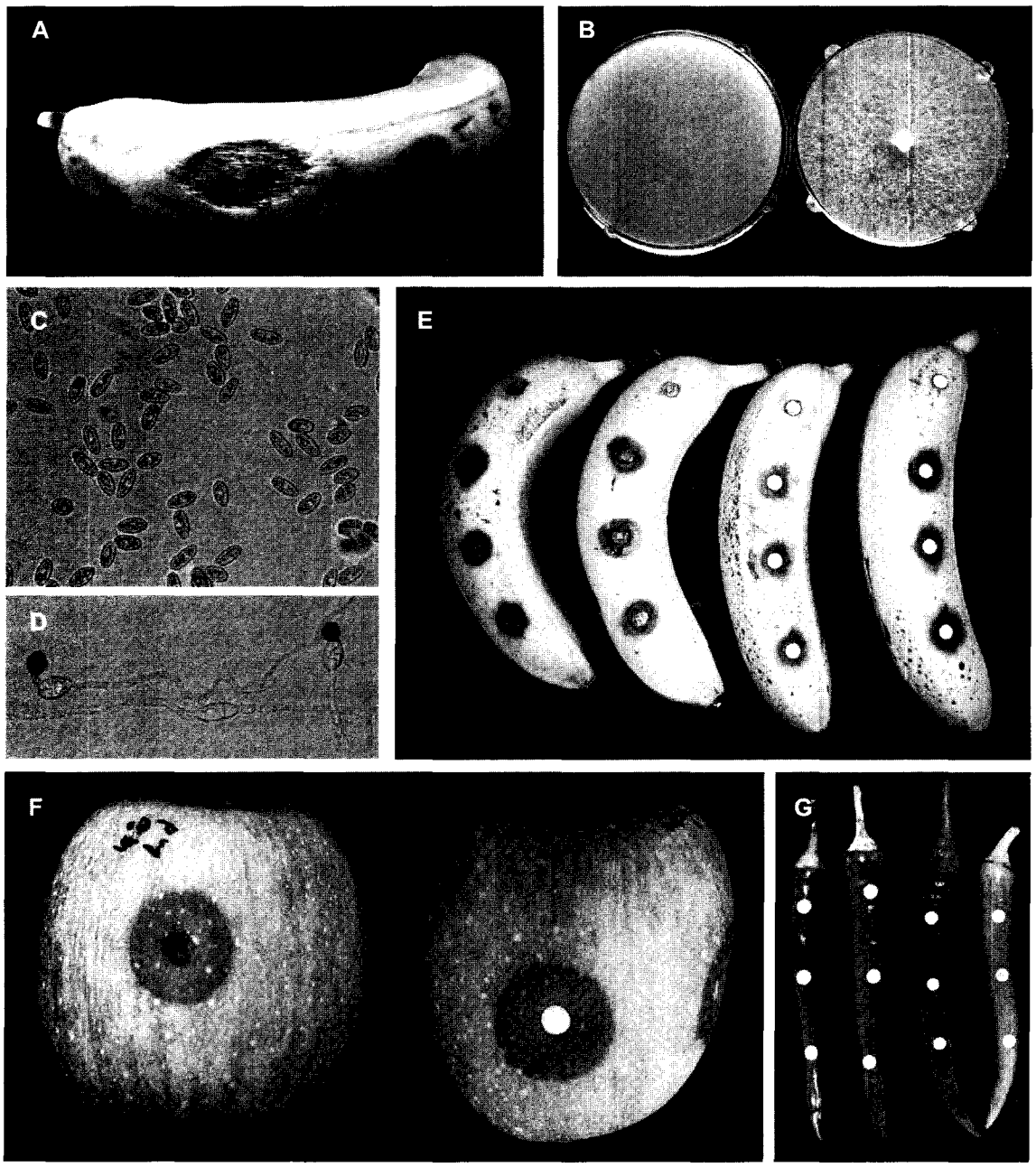

Fig. 1. Anthracnose lesion (A), morphology (B-D), and pathogenicity test (E-G) of *Colletotrichum musae* ST-01 isolated from the anthracnose lesion on commercial banana fruit. **A**) the original lesion, **B**) colony on PDA (left: lower, right: upper), **C**) conidia, **D**) appressoria, **E**) pathogenicity on bananas (top on each fruit: uninoculated check, left two fruits: inoculated with mycelial agar plugs, right two fruits: inoculated with conidial paper discs), **F**) on apples (left: mycelial plug, right: conidial paper disc), **G**) on peppers (no lesion).

adjusted from 4.0 to 7.0 at 0.5 intervals. Effect of temperature on mycelial growth was examined on PDA incubated for 7 days at different temperatures from 0 to 30°C, at 5°C intervals. Mycelial growth of *C. musae* was compared with that of *C. gloeosporioides* isolated from anthracnose lesion on pepper fruits.

Pathogenicity test. Pathogenicity was tested on green banana, apple, and pepper. The fruits with or without wound were inoculated with conidial suspension (1×10^5 conidia/ml) and mycelial plugs (ϕ 5 mm) prepared from 7-day-old culture of each isolate. The inoculated fruits were wrapped with plastic film, and incubated at 10°C or 28°C for 5 days with or without moist condition.

Results and Discussion

Morphology and identification of fungal isolates. Fungus was isolated from anthracnose lesions on bananas. After single spore isolation, the isolate ST-01 was selected and details of the characteristics of the isolates were investigated. ST-01 grew well on PDA medium. The colony was loose with white aerial mycelium, which later became orange in color (Fig. 1A). Several black, acervulus-like masses developed on the culture plates after incubation for 10 days at 25°C with dark-orange drops of conidia. Conidial masses mostly coalesced together. Conidia were aseptate, hyaline, mostly ellipsoid, ranging from 10-18×5-9 μm (average of 14.5×6.9 μm) in size (Table 1 and Fig. 1B). Dark brown and clavate, round, or irregular shape of appressoria developed from germ tubes, ranging from 6-11×5-10 μm (average of 8.8×6.8 μm) in size (Table 1 and Fig. 1C). No setae were found on either the lesion or the cultures. Sclerotium was absent.

In the comparison of the morphological characteristics with the reports of Mordue (1971) and Sutton and Waterson (1970), ST-01 was identified as *Colletotrichum musae*. Conidia of ST-01 were as long as that of *C. musae*, and both fungi shared the same morphological characteristics (Table 1). In addition, the morphological characteristics of appressoria of ST-01 were almost the same as that of *C. musae*. Except for the width of ST-01 conidia (slightly wider than conidia of *C. gloeosporioides*), most characteristics including host species, color of conidium mass, and colony shape on PDA proved that ST-01 is an isolate of *C. musae*.

Table 1. Comparison of morphological characteristics of ST-01 isolated from anthracnose lesion on banana fruit with those of *Colletotrichum musae* and *C. gloeosporioides*

	ST-01	*C. musae*[a]	*C. gloeosporioides*[b]
Conidia			
Shape	Ellipsoidal	Ellipsoidal, Cylindrical	Cylindrical
Color	Hyaline	Brown	Hyaline
Size (μm)	10-18×5-9	11-17×3-6	9-24×3-6
Appressoria			
Shape	Clavate, rounded, irregularly lobed	Irregularly lobed	Obovate
Color	Dark brown	Dark brown	Brown
Size (μm)	6-11×5-10	6-12×5-10	6-20×4-12

[a] Sutton and Waterson (1970)
[b] Mordue (1971)

Cultural characteristics of ST-01. Among the six culture media tested, PDA was the best for mycelial growth of the isolate. Colony diameter of *C. musae* ST-01 reached to 82.4 mm on PDA after 7 days at 25°C (Fig. 2). OA was the next with colony diameter of 73.5 mm. *C. musae* ST-01 had the slowest growth on CDA.

The optimum temperature for mycelial growth of *C. musae* ST-01 was 25-30°C (Fig. 3A). However, mycelial growth was not much inhibited even at 35°C. Mycelia grew very slowly below 10°C. The good mycelial growth of *C. musae* ST-01 at high temperatures of over 30°C seemed to be attributed to its host, banana, which is a tropical plant. It has been reported that mycelial growth of *C. musae* is good at high temperatures (Chakravarty, 1957; Stover and Simmonds, 1987a).

The optimum pH for mycelial growth was pH 5.5-7.0. At this pH level, mycelial growth did not show much difference (Fig. 3B), suggesting an insignificant relationship.

Pathogenicity. To confirm the pathogenicity of *C. musae* ST-01, the mycelial disc and conidial suspension were inoculated on fruits of green banana, apple, and pepper either through wound or without wound. Wound-inoculation with conidial suspension was the most efficient among the inoculation methods. Dark-brown necrotic

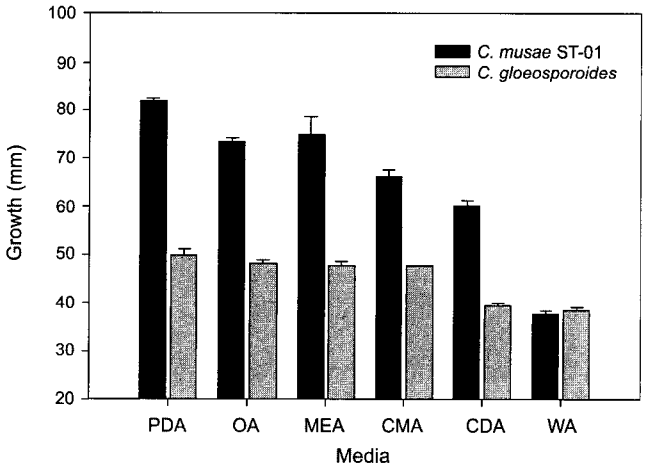

Fig. 2. Mycelial growth of *Colletotrichum musae* ST-01 of banana anthracnose and *C. gloeosporioides* of pepper anthracnose on various media after 7 days of incubation at 28°C (PDA: potato dextrose agar, OA: oatmeal agar, MEA: malt extract agar, CMA: corn meal agar, CDA: Czapek-Dox agar, WA: water agar).

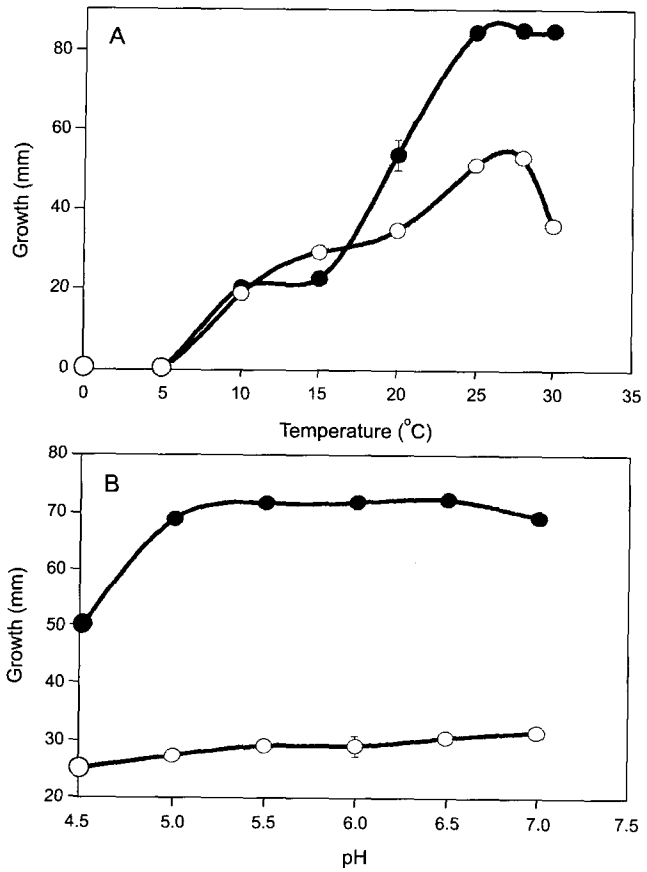

Fig. 3. Mycelial growth of *Colletotrichum musae* ST-01 (●) of banana anthracnose and *C. gloeosporioides* (○) of pepper anthracnose on PDA at different temperatures (A) and pH (B).

lesions appeared on inoculated green bananas at different temperatures, which played a very important role in symptom development. The higher the temperature was, the faster the symptom appeared. Dark-brown, necrotic, circular, and sunken lesions developed on green banana after 15 days at 10°C (Fig. 1D), but it took only 4 days at 28°C. The lesions progressed rapidly at high temperature. Whitish mycelia grew from the lesions and produced orange-colored conidial masses later. *C. musae* ST-01 also induced discolored and sunken lesions on apples (Fig. 1E). On the other hand, no symptoms were observed on green and red peppers inoculated (Fig. 1F).

Lesions seemed to develop easily on yellow bananas. However, it was not easy to induce typical lesions on yellow banana by inoculation because the bananas senesced rapidly and turned dark brown. Therefore, fresh and green bananas were used in this inoculation test. Green bananas were hard and kept their color for around 20 days at low temperature.

These results suggest that the pathogen, *C. musae*, may infect the banana fruits either in the field or during transport. As in anthracnose of grapevine caused by *C. gloeosporioides*, *C. musae* may not induce lesions on green bananas until the fruits become ripe (yellow). Conditions unfavorable to anthracnose during storage and transportation seem to suppress symptom development. These results also suggest that *C. musae*, which has not been reported yet in Korea, could be introduced into the country along with imported bananas and may cause diseases on other plant species. The isolate of *C. musae* ST-01 identified in this research was deposited to the Korean Agricultural Culture Collection (KACC, NIAST, RDA) with access code of KACC 40947.

Acknowledgment

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