

Isolation and Morphological Identification of Apple Anthracnose Fungus of *Colletotrichum* sp. KV-21

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Abstract: This study was undertaken to isolate and to identify a fungal pathogen *Colletotrichum* sp. KV-21 associated with apple anthracnose. Rotted Gala apples were used for the isolation of the fungus. The infected tissues were sterilized with 70% ethanol, washed with sterilized distilled water and were transferred to 50 ml containing potato broth (PDB) flasks. The peripheral hyphae of the fungal colony which developed from the infected tissues were isolated on to potato dextrose agar (PDA). On PDA plates the fungus grew well at 25°C and occupied more than half of a 9 cm petri dish within 5 days. The fungal cultures on PDA were used for morphological observation and identification of the fungus. Conidiophores were produced on the gray to whitish sporodochial structures scattered on PDA plates which gave rise to conidiogenous cells. The structures of the conidia produced on PDA plates were subcylindrical to obovoid, fusoid, tapered and 4 to 6 µm in size.

Key Words: *Colletotrichum* sp. KV-21, anthracnose, Gala apple, morphological identification

INTRODUCTION

The genus *Colletotrichum* comprises some of the most economically important fungi and destructive plant-pathogens, such as *Colletotrichum acutatum* (Simmonds), *Colletotrichum fragariae* (Brooks), *Colletotrichum gloeosporoides* (Penz) and *Glomerella cingulata* (Stoneman), responsible of anthracnose on apple fruits and plants. Apple anthracnose is an important disease hampering the quality and texture of apple fruits worldwide. *Colletotrichum* spp. can not be distinguished solely by symptoms and signs on infected tissues. Their identification is based on morphological characters. They are comprised of living filaments known as hyphae, or collectively as mycelium. They reproduce by spores which may be sexual or asexual. *Colletotrichum* spp.

are major causes of plant disease, accounting for perhaps 70% of all the major crop diseases and also known as post-harvest pathogens¹⁾. Some of these fungal plant pathogens are termed biotrophic because they establish an intricate feeding relationship with living host cells. Others are termed necrotrophic, because they invade the plant tissues aggressively, killing the host cells to obtain nutrients²⁾.

The apple is the pomaceous fruit of the apple tree, species *Malus domestica* in the rose family Rosaceae which has potential nutritive and therapeutic values. Research suggests that apples may reduce the risk of colon cancer, prostate cancer and lung cancer³⁾. Compared to many other fruits and vegetables, apples contain relatively low amounts of Vitamin C as well as several other antioxidant compounds⁴⁾. The fiber content, while less than in most other fruits, helps regulate bowel movements and may thus reduce the risk of colon cancer. They may also help with heart

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disease, weight loss, and controlling cholesterol, as they do not have any cholesterol, have fiber, which reduces cholesterol by preventing re-absorption, and are bulky for their caloric content like most fruits and vegetables^{5,6}. There is evidence that *in vitro* apples possess phenolic compounds which may be cancer-protective and demonstrate antioxidant activity⁷. The predominant phenolic phytochemicals in apples are quercetin, epicatechin, and procyanidin B2⁸.

Colletotrichum establishes infections in apple orchards, typically through wounds caused by insects or mechanically⁹. *Colletotrichum* spp. are distributed and propagated in Asia, Europe, USA, Africa and South America. The most destructive symptom caused is apple anthracnose. Anthracnose first manifests as a small superficial, brown, circular spot on the fruit, which gradually expands. As the lesion expands, conidiophores rupture the fruit epidermis, forming small tufts. Infected transplants are capable of spreading the disease from the nursery to the field, where typical anthracnose symptoms are later manifested¹⁰. Considerable yield loss can be inflicted by anthracnose under the appropriate environmental and cultural conditions¹¹. The conidiophores often form concentric rings, which radiate outwards from the initial point of infection. Soft ripe fruit, under moist conditions, with become entirely covered by conidial tufts or vegetative mycelium. Under dryer conditions, or on unripe fruits, mycelium is not present and conidial tufts are rare. Eventually affected fruits become mummified, after the whole fruit has discoloured and dehydrated. The spread of the disease to adjacent fruit occurs often, resulting in adhered clusters of mummified fruit which can persist between seasons¹².

In this study, we isolated a fungal pathogen, associated with apple anthracnose in Gala apples in Korea and on the basis of its morphological features we identified this fungal pathogen as a *Colletotrichum* sp. KV-21.

MATERIALS AND METHODS

Fungal material and isolation of the fungal isolate

In this study, the rotted Gala apples were collected from the local area of Yeongcheon, Kyoungbook, Republic of Korea. Isolates of anthracnose fungi were originally obtained either by direct transfer of conidia

with a sterile needle from infected host tissues onto fresh potato dextrose agar (PDA) plates or by transferring infected host tissue samples into fresh 50 ml containing potato broth (PDB) flasks after surface sterilization with 70% ethanol for 1 min and by washing with sterilized distilled water. The plates with conidia or infected host tissue segments were incubated at 25°C in darkness for 7-9 days, and hyphal tips from developing colonies, which were in most cases producing conidia of *Colletotrichum* type, were transferred to fresh PDA slants for storage and subsequent study. The culture was deposited with an accession number (DU-KV-21) in the culture collection of the Laboratory of Genetic Engineering, Department of Biotechnology, Daegu University, Republic of Korea. Prior to use, fungus was grown on fresh PDA plates at 25°C in the dark, and small agar pieces (1-2 mm diameter) with mycelia were cut from the edge of the advancing colonies for inocula.

Culture media, myelial growth and colony appearance

The germination and the growth of fungus was maintained on potato dextrose agar (PDA) medium containing per liter 17 g enzymatic digest of casein, 3 g enzymatic digest of soybean meal, 5 g NaCl, 2.5 g dipotassium phosphate and 2.5 g dextrose in sterile distilled water.

Colony colors of the fungal isolates were described from isolates incubated at 25°C in dark. Three plates were used to determine the radial mycelial growth of fungal isolate.

Morphological observation

Isolates of the fungus were incubated at 25°C in dark. Cultures were transferred to fresh potato dextrose agar (PDA) to stimulate sporulation of conidia and facilitate identification. Morphological observations were made from structures mounted in lacto-phenol. The conidial measurements were derived from observations at 400x magnification (Nikon, Alphaphot-2/YS2, Shanghai, China).

RESULTS AND DISCUSSION

Abundant anthracnose infestations have been evident in several host plants and many countries during the summer seasons including apple fruits and plants.

This has resulted in massive collapse of economically important host plants. *Colletotrichum* spp. are known to have a wide host range and geographic distribution. Exact identification of apple anthracnose pathogens (*Colletotrichum* spp.) of fruit trees should be based on laboratory studies as well as field observations of disease epidemiology and symptomatology. These studies are necessary because the fungal organs we usually find on diseased plants tissues in the field are almost always the anamorphs or conidia of the *Colletotrichum* type, which are morphologically simple and similar to each other for different species¹³⁻¹⁵.

Colletotrichum spp. causing apple anthracnose, are predominantly the pathogens of fruit, stolons and crowns. We made an attempt to morphologically characterize a *Colletotrichum* sp. The fungus isolated here in this study from the rotted Gala apples of Yeongcheon area, Kyoungbook, Republic of Korea, was identified to be *Colletotrichum* sp. KV-21 (Fig. 1) through com-

paring its morphology with the documented description based on its microscopic features¹⁶. As shown in Fig. 2, the colony of *Colletotrichum* sp. KV-21 grew well on fresh PDA medium and maximum mycelial growth was observed at 25°C with entire colony margin, filling the whole plate surface in 7 days. The colony was fluffy, grayish white, sporulating well and grayish yellow in reverse. The results of this study strongly support the identity of the fungal isolate as *Colletotrichum* sp. KV-21 and were identical to the findings of others as evident by mycelial growth and colony appearance¹⁶. Lubbe et al. (2004) also characterized the *Colletotrichum* spp., and based on their morphological features these fungi were confirmed to be *C. acutatum* f. sp. hakea, *C. boninense* and these results were also in strong agreement with our findings¹⁶.

Aerial mycelium of *Colletotrichum* spp. at first sparse, later developing concentric zones of dense mycelium

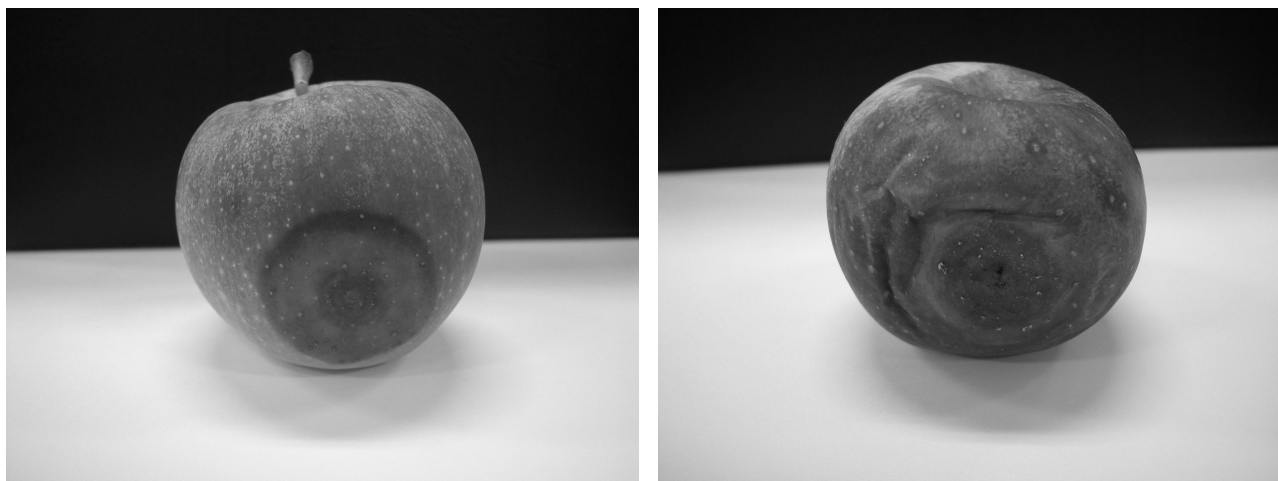
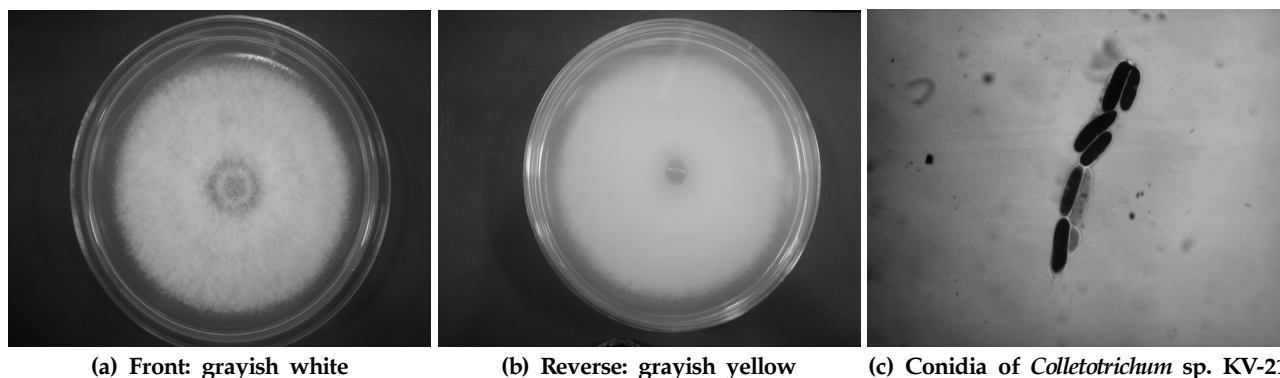


Fig. 1. Apple anthracnose of Gala apples collected from the local area of Yeongcheon City, Kyoungbook, Republic of Korea.



(a) Front: grayish white

(b) Reverse: grayish yellow

(c) Conidia of *Colletotrichum* sp. KV-21

Fig. 2. Colonies and morphology of the isolate of *Colletotrichum* sp. KV-21 isolated from the rotted Gala apples.

and light buff sporogenous tissue, but in many isolates only darkening of the mycelium with age occurs^{12,17}. Primary hyphae at advancing edge of colony are thin-walled with one or more branches initiated before the first septum with dense and granular contents. Secondary and subsequent branch hyphae are often much narrower than primary hyphae^{17,18}.

As shown in Fig. 2, on fresh PDA, the conidia of *Colletotrichum* sp. KV-21 were subcylindrical, to abovoid, fusoid, hyaline, smooth with 4 to 6 μm in size and generally tapering from the lower part towards a truncate apex, which the unique feature of genus *Colletotrichum*. Several researchers have been reported these conidial characteristics of *Colletotrichum* spp. as also evident by the findings of others¹⁶. On the host, conidial sporodochia occur on all infected organs; discrete sclerotia are not usually formed, but infected fruits develop dry substratal stromata ('mummies') in which a stromatic layer replaces most of the pericarp¹⁹. Although, acervular conidiomata were not present, all strains produced conidiogenous cells directly on the agar surface and/or throughout the aerial mycelium of the colony¹⁵.

The presence of pathogen on apple fruits probably occurred when fungus enzymatically penetrates the cuticle and then remains as sub-cuticular hyphae until the post climacteric stage of fruit growth is attained. Grover and Bansal (1970) reported the isolation of *C. capsici* from the rotten stems, leaves and seeds of *C. frutescens*²⁰. Wahid (2001) had also reported that the pathogen apple anthracnose has a wide host range, which includes mango, pear and guava fruits²¹. The attack of fruits by *Colletotrichum* spp. inducing anthracnose diseases especially in the rainy season has been reported²². Nevertheless, despite the fact that *Colletotrichum* affect a wide spread number of cultures, its pathogenic range increases caused by a rising number of species identified under this Genus that were classified as anthracnose's agent²³.

Colletotrichum spp. an ubiquitous pathogen infecting several crops causing anthracnose diseases. It is possible that insect vectors are involved in dissemination of the pathogens propagule into the plant during pollination or during feeding on the fruits. Adelaja (1997) reported that fruit fly stings enhance the entry of *Colletotrichum* spp. into African star apple fruits by their oviposition on the fruits²⁴. The prevalence and

the rapid spread of these diseases during the peak of the rainy season could be due to the humid condition prevailing at that time of the year, which supports the rapid production of conidia. *Colletotrichum* spp. have been reported to cause rapid infection during heavy dew or rain fall^{25,26}. Reasons for the above observation might be related to the fact that rainfall or rain-splash probably played an important role in the dispersal of the pathogen's propagules in the field.

On the other hand, market survey has revealed that the anthracnose infected fruits attracted low prices²⁷. The industrial use of apple fruits in jam, cheese, ice cream, apple chutney, apple crisp and pies will be unattainable if the apple fruits produced are of low quality due to fungal infection. Changes in nutrient composition caused by fungal infection of the apple fruits will also adversely affect their uses for several apple food products. While its commercial or market value as a means of livelihood to peasant farmers the women and the children will equally be affected. Therefore, identification of apple anthracnose fungi which cause severe losses to pome and stone fruits including Gala apple fruits would be an addition to plant pathology to understand the disease range of such pathogens in pre- and post-harvest technology. Thus, based on the aforementioned morphological as well as phytopathological characteristics, the isolated fungus was characterized as *Colletotrichum* sp. KV-21. However, further study is warranted on the identification *Colletotrichum* sp. KV-21 at molecular levels to further authenticate the outcomes of this study.

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