



Biological characterization of emerging fungal pathogen *Colletotrichum* associated with mango (*Mangifera indica* L.) post-harvest anthracnose from Vietnam

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Abstract

Background Post-harvest anthracnose (PHA) of mango is a devastating disease, which results in huge loss to mango producers and importers. Various species of PHA, diverse pathogenicity, and different resistance towards fungicides make it essential to evaluate the pathogen taxonomic status and biological characterization.

Methods and results Two strains DM-1 and DM-2 isolated from the fruit of DaQing mango from Vietnam were identified as *Colletotrichum fructicola* and *C. asianum* respectively, based on the morphological features, along with the phylogenetic tree of ITS and *ApMat* combined sequences. The growth status of different *Colletotrichum* strains under different conditions was analyzed to reveal the biological characteristics. The optimum growth temperature of DM-1 and DM-2 was 28 °C and mycelia grew rapidly in the dark. Both strains could grow in media with pH 4–11, while the optimum pH value was 6. Maltose and soluble starch were the most suitable carbon source for DM-1 and DM-2 respectively, and the peptone was the most suitable nitrogen source for both strains. The lethal temperatures were recorded as 55 °C 5 min for DM-1, and 50 °C 10 min for DM-2.

Conclusions To the best of our knowledge, it is the first study reporting the identification of the pathogens: *C. fructicola* and *C. asianum* responsible for postharvest fruit anthracnose of mango in Vietnam.

Keywords Biological characteristics · *Colletotrichum fructicola* · *C. asianum* · Mango anthracnose

Abbreviations

PHA Post-harvest anthracnose

Introduction

Mango (*Mangifera indica* L.), belongs to the Anacardiaceae family is one of the most important fruit crops with high commercial value around the tropical and sub-tropical

areas of the world. The cultivation area of mango is the second largest after banana, with the production of 42 million tons per year [1]. Mango fruit rich in vitamins, protein, fat, carbohydrates, fiber, minerals, and carotenoids, is not only nutritious, but also unique flavor, sweet and delicious. Additionally, the phytochemicals from particular parts of the mango plant, such as leaf, bark, heartwood, fruit peel, pulp, and seed are known for their potential therapeutic effects on cancer [2]. Mango is worthy of “the king of fruits”.

China is the second largest mango producer around the world. Nowadays, various cultivars of mango have been widely cultivated in southern China. Among of them, an early-maturing variety, Daqing mango, originated from Vietnam has been cultivated in Yunnan, Guangxi, Sichuan, Hainan, and other mango growing areas of China and generally consumed due to its juiciness, delicious taste, small pit and less fiber. However, the fruits of Daqing are more susceptible to anthracnose disease which could cause the devastating fruit rot during the storage and transportation.

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Initially, it appears dark brown round spots on the fruit surface, then forms coalesced sunken lesions, eventually covering the entire surface of the fruit and rot which seriously affects the appearance and quality of mango fruit and causes huge economic losses [3]. The yield loss of mango during storage and transportation caused by anthracnose ranged between 30 and 50%, even up to 100% [4]. The common pathogen of mango anthracnose is fungus belonging to *Colletotrichum* with characteristics of latent infection. These species of *Colletotrichum* usually hide in the outer layer and cuticle of the fruit peel and after penetrate into mango fruits, which cannot further infect and present any symptoms in the orchard due to the presence of diolefins antifungal compounds. The pathogenicity of pathogens restores when the antimicrobial substances gradually decline as the fruit ripens and available nutrients increase during storage, transportation and on shelf [5]. Moreover, preharvest damage of *Colletotrichum* to young shoots, flowers and fruits under favorable conditions (high humidity, and moderate temperature from 24 to 32 °C) also significantly contributes to enormous losses in mango orchard [5]. Mango in major growing areas worldwide could be infected by various of *Colletotrichum* species [6]. At least 18 species of mango anthracnose pathogens have been reported worldwide, which belong to *C. gloeosporioides* species complex, *C. acutatum* species complex, *C. boninense* species complex and *C. gigasporum* species complex, and a single species, *C. cliviicola* [7, 8]. Fourteen *Colletotrichum* species associated with mango anthracnose have been reported in China, namely *C. alienum*, *C. cliviicola*, *C. cordylinicola*, *C. endophytica*, *C. gigasporum*, *C. karstii*, *C. liaoningense*, *C. musae*, *C. asianum*, *C. fructicola*, *C. siamense*, *C. scovillei*, *C. tropicale* and *C. gloeosporioides* [7, 9]. It is reported that *C. asianum* is the main causal agent of mango leaf anthracnose in Vietnam [10]. However, there is less reports on post-harvest mango anthracnose caused by *Colletotrichum* on the Daqing mango neither in Vietnam nor in China. Different *Colletotrichum* species possess different susceptibility, invasiveness and reproductive capability to fungicides. So, it is necessary to accurately identify the pathogens of mango PHA, understand its biological properties to develop effective management strategies, and explore effective and safe agents to avoid chemical residues in fruits.

Previously, morphological and molecular techniques along with pathogenicity tests were generally used to confirm the dominant pathogenic *Colletotrichum* species on specific mango cultivars in certain growing areas. Previously, actin (ACT), the internal transcribed spacer region (ITS) of ribosomal DNA, chitin synthase (CHS-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β -tubulin (TUB2) genomic regions were selected for sequencing for phylogenetic multi-locus analyses to establish the taxonomy

of *Colletotrichum* species [11]. While recent studies demonstrated that *ApMat* gene provided better phylogenetic information than other loci, and can identify species within the *C. gloeosporioides* complex [8, 12]. The species can be discriminated in this complex through ITS + *ApMat* rapidly and efficiently.

Herein, the causal agents of post-harvest Daqing Mango anthracnose was confirmed based on morphology, ITS and *ApMat* sequence data after pathogenicity test. In addition, the aims of this study were to detect the biological characteristics of the pathogen and analyze their differences to improve the disease prediction, diagnosis, prevention and control of post-harvest mango anthracnose in Vietnam.

Materials and methods

Sample collection, fungal isolation

15 intact mangoes (cultivar Daqing) were collected from five distinct mango wholesale markets of Kunming city, Yunnan province, China which were imported from Vietnam. When the dark brown lesions were formed on the fruit surface after storage 5–7 d in room temperature (20–26 °C), the diseased mangoes with typical anthracnose symptoms were used for pathogenicity test. Tissue isolation and single spore isolation method were used to isolate the causative agents [13]. Mango disease samples were surface disinfected with 3% sodium hypochlorite for 30 s after soaking in 75% alcohol for 3 min, and then rinsed with sterile water for 3 times. Tissues of 0.5 cm×0.5 cm size were cut from the margin between healthy and diseased parts of mango fruit using sterile scalpel, dried with sterile filter paper, and transferred to PDA medium and incubated upside down at 28 °C. Picked the mycelia from the edge of the colony that has been cultured on for 5 d, and transferred to a new PDA medium for cultivation. After sporulation, pure cultures of the pathogenic fungi were obtained with the single spore isolation method. Cultures were maintained as spore suspensions and mycelial plugs in 40% glycerol at -80 °C.

Pathogenicity and virulence on detached fruits

Healthy mangoes (cultivar Daqing) at the first color break stage of ripening with smooth surface were used for pathogenicity test according to the Tovar-Pedraza et al. [8]. After surface disinfection with 75% alcohol and 3% sodium hypochlorite, Daqing mangoes were soaked and rinsed 3 times in sterile water, then dried with sterilized paper. Each fruit was pricked at 6 points at same depth (5 mm). A mycelial plug (6 mm in diameter) removed from the margin of a 7-day-old PDA culture was placed onto the fruit surface on each

wound. Three fruits were inoculated with sterile plugs as control. Every inoculated fruit placed in a petri dish (9 mm in diameter) was placed in a transparent plastic box containing sterile distilled water, and incubated in an artificial climate chamber (14 h of light, 1053 lx, 28 °C; 10 h of darkness, 26 °C, 95% ± 3% relative humidity) with three replicates for each treatment. The virulence of pathogenic fungi was characterized into strong virulence (> 2 cm), moderate virulence (> 1.0 to < 2 cm) and weak virulence (< 1.0 cm) based on the size of the lesion diameter according to assessment criteria of Li et al. [7]. The pathogenic agents were reisolated and purified again after 7d according to Koch's postulates, and confirmed whether they were consistent with the original inoculum by morphological identification. The experiment was repeated three times.

Morphological observations

Mycelial plugs (6 mm in diameter) were taken from pure colonies cultured for 7 d and placed on a new PDA (25 mL) petri dish (9 mm) for incubation at 25 °C in darkness. The colony appearance, including surface and reverse on the agar, were observed and recorded every day. To determine the growth rate of each isolate, crossover method was used to measure the diameter of the colony every day, and the average daily growth rate of the colony was calculated after the hyphae over spread the petri dish. The morphology of conidia was observed after 7 d incubation on PDA medium, under the microscope following the protocol reported by Jiang [14]. The spores were picked on a sterile concave slide containing a drop of water and a drop of 1% glucose solution, and then the concave slide was placed into a Petri dish containing sterile filter paper soaked with sterile water and incubated for 15–24 h to observe the germination of conidia and the morphological characteristics of appressorium. The experiment was conducted thrice with three replicates per trial.

DNA extraction, PCR amplification, and sequencing

The purified mycelium after cultivated for 7d at 28 °C were scraped and finely ground. Fungal DNA was extracted using a fungal DNA extraction kit (Omega Bio-tek). The ITS [15]: 5'-TCCGTAGGTGAACCTGCGG-3'; 5'-TCCTCCGCTTATTGATATGC-3', *ApMat* [16] 5'-TCATTCTACGTATGTG CCG-3' 5'-CCAGAAATACACCGAAGTTGC-3' primer pairs were used for PCR reaction (25 µL) consisted of 12.5 µL of 2×PCR Master mix (Beijing Biomed Genetic Technology Company Limited), 0.5 µL of upstream and downstream primers (10 µmol/L), 0.5 µL of DNA template, and dd H₂O. PCR reaction conditions were set for ITS as: 94°C pre-denaturation for 2 min; 94°C denaturation for 1 min;

55°C annealing for 30s; 72°C extension for 1 min; 72°C extension for 10 min; 34 cycles. And for *ApMat*: 94 °C pre-denaturation for 5 min; 94 °C denaturation for 45s; 64 °C annealing for 45s; 72 °C extension for 1 min; 72 °C extension for 7 min; 30 cycles. The PCR products were sequenced by Tsingke Biotechnology Company Limited after detection through 1% agarose gel electrophoresis.

Phylogenetic analysis

The nucleotide sequences were blasted on NCBI (<http://www.ncbi.nlm.nih.gov>). The sequences of type strains and non-type strains which had high similarity and their closely related species were downloaded. Multiple sequence alignment was performed online MAFFT (<https://www.ebi.ac.uk/Tools/msa/mafft/>) [17]. The sequences of ITS and *Apmat* gene were manually adjusted to allow maximum sequence similarity with Gblocks of Phylo Suite v1.1.16. Then these sequences were connected end to end. The phylogenetic tree was constructed by maximum likelihood method and nucleotide replacement model of GTR + G (general time reversible) using MEGA 7.0.21 [18]. The bootstrap equals to 1000 with the self-expanding method.

Temperature, pH, and light

The effect of different temperature, pH value and light intensity on the growth rate of pathogenic fungi, as well as the lethal temperature and suitable carbon and nitrogen sources were examined. In the assays of the effect of different temperature, pH, and light on the growth of mycelium, same isolates, volume of medium, inoculation method, and standard of measurement were uniformed. Fungal mycelial plugs (6 mm in diameter) were taken from the edge of the six-day-old PDA culture and placed on the center of PDA plates and incubated at distinct temperature range (12, 16, 20, 24, 28, 30, 32, 34, 36, 38, and 40 °C), pH values (4–11), and light conditions (24 h light (1053 lx), 12 h light/dark and 24 h dark at 28 °C), respectively. The pH values were adjusted with 1 M HCl and 1 M NaOH solutions. The assays were conducted thrice with three replicates per trial.

Carbon and nitrogen sources

Czapek medium agar (CDA, NaNO₃ 3 g, K₂HPO₄ 1 g, MgSO₄ 0.5 g, KCl 0.5 g, FeSO₄ 0.01 g, sucrose 30 g, Agar 15 g, H₂O 1000mL) was used as the basal medium and sucrose was replaced with equal mass (30 g) of glucose, L-arabinose, D-xylose, lactose, maltose and soluble starch, respectively, CDA without carbon source served as control. The fungal mycelial plugs were placed in the center of a medium containing different carbon sources and incubated

upside down at 28 °C. CDA was used as the base medium and NaNO₃ was replaced with equal mass (3 g) of yeast extract, peptone, (NH₄)₂SO₄, (NH₄)₃PO₄, beef extract, and gelatin, respectively, with nitrogen-free source of CDA medium as control. Fungal mycelial plugs (6 mm in diameter) were put in the center of a medium containing different nitrogen sources and incubated upside down at 28 °C. The test was conducted thrice with three replicates per trial.

Lethal temperature

To assess the mycelial lethal temperature, mycelial plugs (6 mm in diameter) were placed in the center of a PDA plate after water bath (5, 10 and 15 min at different temperatures (40, 45, 50, 55 and 60 °C respectively) in the 2.0 mL centrifuge tube with 1 mL of sterile water, and incubated upside down at 28 °C to observe the growth of the colony. This experiment was conducted thrice with three replicates per trial.

Statistical analysis

Excel and SPSS 19.0.0 software (Duncan's method) were used to statistical data and the significance of differences analysis, and data were subjected to analysis of variance (ANOVA) followed by Fisher's Least Significant Difference" (LSD) tests for means separation. and Origin Pro 2021 9.8.0.200 software was used for mapping.

Results

Fungal isolation

In total, 23 isolates were isolated and purified from 15 mango fruit with typical symptoms of anthracnose. Five types of fungi with different colony morphology were used for further study.

Pathogenicity and virulence of fungal isolates on mango fruits

Pathogenicity and virulence test was performed on intact Daqing fruits. In total, 2 of all 5 isolates were pathogenic to mango fruits with different symptoms. Water-soaked lesions were observed on pericarp after inoculated with DM-1 5 d. Then the center of lesions became dark brown on the 6th day. Same round or near-round sunken necrotic lesions with black, appeared on pericarp inoculated with DM-2 after 4 d. The edges of the lesion were water-soaked and expanded outwards on the 7th day with a clear junction between disease and health. Fruits inoculated with both isolates showed typical anthracnose symptoms after 7d (Fig. 1- a, c). The sarcocarp had large necrotic lesions (Fig. 1- b, d), whereas control fruits remained disease free. Fungal colonies, mycelium and conidia reisolated from all symptomatic fruits were morphologically identical to the original inoculants according to the Koch's postulates.

The diameters of disease spots on mangoes, inoculated with DM-1 and DM-2 ranged from 0.47 to 3.76 cm and 1.34 to 3.80 cm, were various at different parts of mango after 7d inoculation. The results showed that the diameter of lesions on the fruit inoculated with DM-1 and DM-2 were 0.50 ± 0.03 and 1.86 ± 0.25 cm, respectively (Table 1) near



Fig. 1 Symptoms of fruit surface and sarcocarp after 7 d inoculated with *C. fructicola* DM-1 and *C. asianum* DM-2. **a, b**: inoculated with DM-1; **c, d**: inoculated with DM-2 **e**: inoculated with sterile plugs (control)

Table 1 Aggressiveness descriptions of isolates DM-1 and DM-2

Isolates	Lesion diameter on fruit (Mean \pm SEM*, cm)			Overall average(cm)
	pedicle	Middle	end	
DM-1	0.50 \pm 0.03c	2.94 \pm 0.07b	3.20 \pm 0.11a	2.22
DM-2	1.86 \pm 0.25 γ	2.69 \pm 0.36 β	3.03 \pm 0.08 α	2.53

*Note: Each factor in the table was analyzed separately, and different lowercase letters indicate significant differences ($p < 0.05$) between treatments

the pedicel, which were significantly smaller than that at the middle and top of the fruit. Mangoes inoculated with DM-1 had smallest necrotic spots near the pedicle with 0.50 \pm 0.03 cm in average diameter. In general, the total mean diameter of mango fruit lesions reached 2.22 cm (DM-1) and 2.53 cm (DM-2) respectively. The virulence of DM-1 and DM-2 were strong virulence according to the evaluation criterion.

Morphology and cultural characteristics of post-harvest pathogens

DM-1 and DM-2 were incubated in petri dish (9 mm) containing 15 mL PDA solid media at 28 °C in the dark, and the mycelium grew throughout the dishes at 6 and 7 d, with average growth rates of 14.00 mm/d and 12.00 mm/d, respectively. DM-1 showed sub-circular colonies on PDA media, initially white with flocculent and downy aerial mycelium, and turn to greenish grey on 3 d. It also exhibited variation in color from white to grey-brown on the back of the media, and pigmentation expanding with the development of colonies on 6 d (Fig. 2a1). Mycelium collapsed on 10 d. DM-2 colonies showed white with flocculent mycelium. The color

of the back of the media varied from white to yellow gradually on 4–6 d (Fig. 2a2), and black dots of pigmentation appeared on 8 d. Clusters of tangerine conidia formed on the colony surface on 15 d.

Conidia of DM-1 were hyaline, unicellular, and cylindrical with rounded extremes or one slightly pointed end under optical microscope (eyepiece \times objective = 10 \times 40), and approximately (12.08 ~ 16.27) μm \times (4.81 ~ 6.25) μm (Fig. 2-b). The conidia of DM-2 were also hyaline unicellular with the size of (14.29 ~ 25.41) μm \times (4.31 ~ 6.16) μm , but fusiform and more elongated than those of DM-1 (Fig. 2-g), and green plastids were also observed in the conidia. The conidia germinated after 7–15 h in 1% glucose solution (Fig. 2-c and -h). The brown reniform appressorium ((6.32 ~ 7.51) μm \times (3.69 ~ 4.52) μm) of DM-1, and nearly round or cylindrical brown appressoria ((8.37 ~ 9.18) μm \times (5.72 ~ 6.56) μm) of DM-2 were observed respectively at 15–24 h (Fig. 2-d and -i). Conidiophores and sporulation were observed 36 h later (Fig. 2-e and -j).

Phylogenetic analysis based on ITS and *ApMat* genes

The sequencing results of ITS and *ApMat* gene showed that the length of ITS and *ApMat* sequence of DM-1 and DM-2 were 550, 1083 bp and 550, 1185 bp respectively, which were consistent with the theoretical values. Initially, DM-1 and DM-2 were recognized as *Colletotrichum gloeosporioides* species complex through the ITS-only evolutionary tree containing all *Colletotrichum* taxa. Further, the *ApMat*-only tree was also constructed to resolve the clades containing the isolates obtained in this study (data not shown).

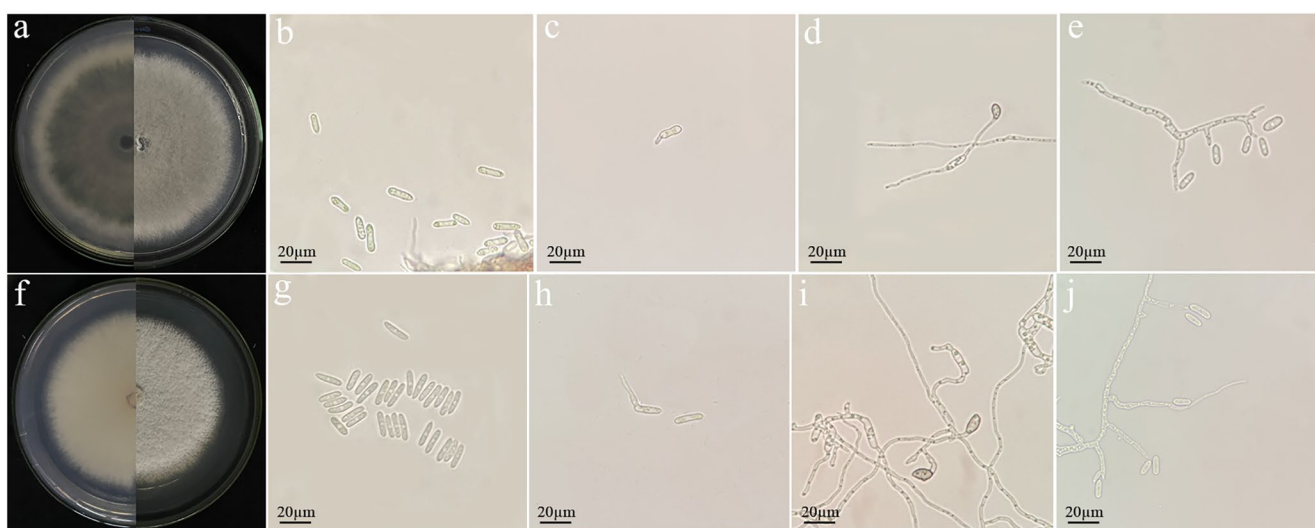


Fig. 2 Colony morphology and conidia of DM-1 and DM-2 after incubated at 28 °C under continuous dark. **a–e**: DM-1. Reverse and upper view of colony (**a**), Conidia (**b**), Spore germination (**c**), Appressorium

(**d**), The conidiophores and conidia (**e**). **f–j**: DM-2. Reverse and upper view of colony (**f**), Conidia (**g**), Spore germination (**h**), Appressorium (**i**), The conidiophores and conidia (**j**)

ITS + *ApMat* are enough to discriminate species in the *Colletotrichum gloeosporioides* species complexe [8, 12].

A phylogenetic tree of the combined ITS and *ApMat* sequences was constructed using MEGA 7.0.21 software. The gene sequences of DM-1 and DM-2 separately clustered into different clades with the sequences of type strains *C. fructicola* LC0033^T and *C. asianum* LC0037^T (Fig. 3). Combined with the morphological identification, DM-1 and DM-2 were identified as *C. fructicola* and *C. asianum*, respectively. The ITS and *ApMat* gene sequences of DM-1 and DM-2 were uploaded to the National Microbial Science Data Centre (NMDC) database under registration numbers NMDCN00016NS, NMDCN00016NT and NMDCN00011PH, NMDCN00011PI respectively.

It was reported that PHA of multiple mango cultivars were caused by *C. fructicola* and *C. asianum* in various mango growing areas in Yunnan, Sichuan, Guizhou, Fujian,

Guangdong, Guangxi, Hainan, and Taiwan of China [6, 7, 11]. Additionally, *C. fructicola* and *C. asianum* could also harm coffee, apple, citrus, pear, kiwi, cabbage, strawberry, pearl plum and other important commercial crops because of the characteristic of cross infection [19–21]. Therefore, crops that are also hosts of *C. asianum* and *C. fructicola* should be avoided in the vicinity of mango orchards, and weeds should be removed.

Temperature effects on colony growth

The growth and biological properties of fungi could be affected by environmental conditions. It is important to study the biological characteristics under certain circumstances of pathogen for proposing the disease control strategies. Fungal germination and growth rate depend on temperature, humidity, and nutrients.

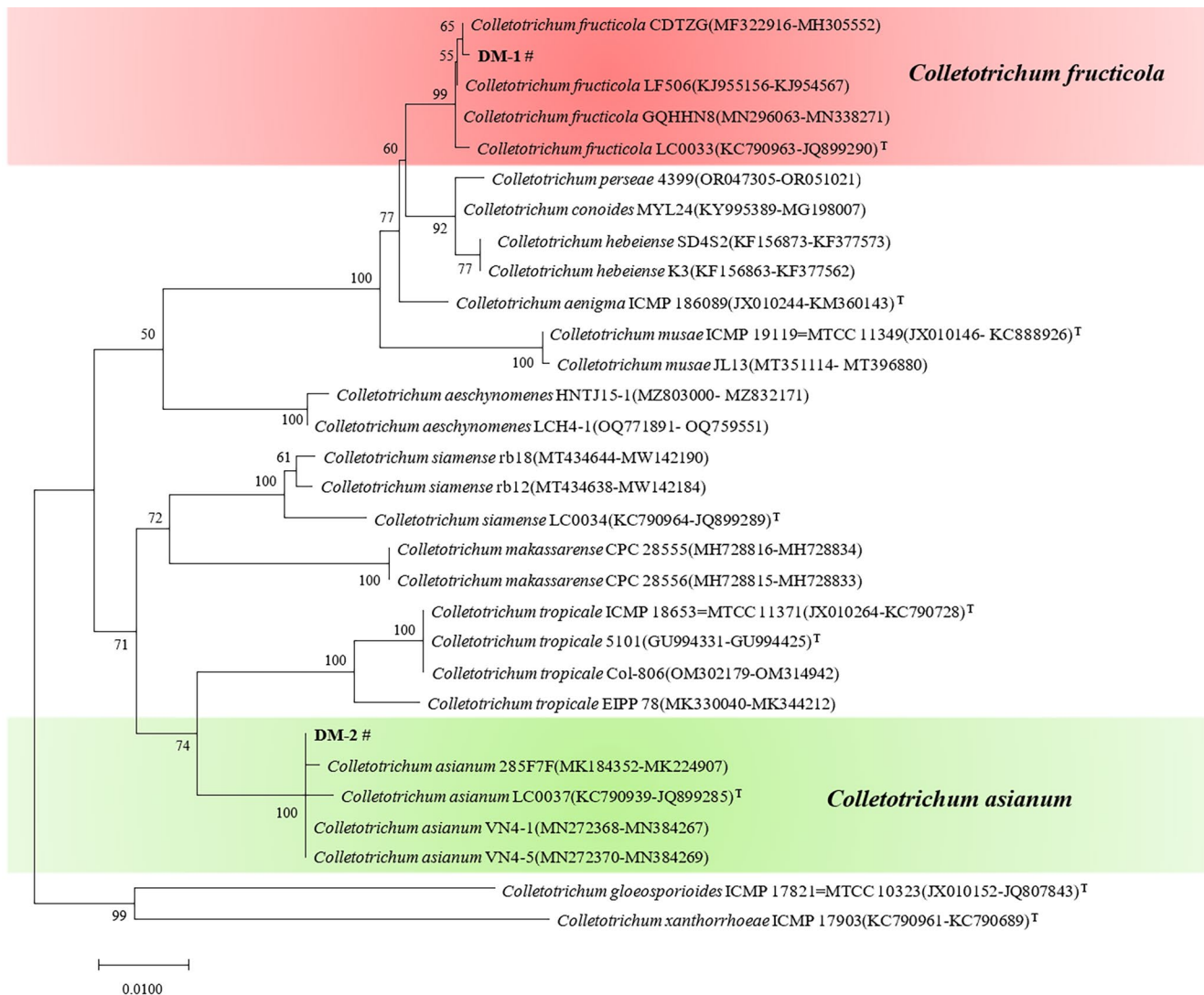


Fig. 3 Phylogenetic tree constructed based on the combined ITS and *ApMat* sequences to analyze the relationship between DM-1, DM-2 and its related species by the Maximum Likelihood method. BOOTSTRAP RUNS = 1000. T, indicates type strain; #, indicates strain isolated for this study

In this study, we revealed that the optimum growth temperature of *C. fructicola* DM-1 and *C. asianum* DM-2 was 28 °C, and the colony diameter reached 84.20 and 73.63 mm respectively, which was significantly higher than the other treatments ($p < 0.05$). The mycelium could not grow at 12 and 40 °C. but in the range from 16 °C to 36 °C, the expansion rate of colony increased first and then decreased with the increase of temperature. However, the range of optimum growth temperature of DM-1 is 24~30°C, and DM-2 is 24~28°C (Fig. 4a). The colony diameters of DM-1 were larger than those of DM-2 at different temperature gradients, indicating that DM-1 grew faster and had stronger expansion ability at the same temperature.

pH effects on colony growth

Mycelium of *C. fructicola* DM-1 and *C. asianum* DM-2 could grow in the pH ranging from 4 to 11 (Fig. 4b), and the optimal pH for growth was 6 for both. The growth rate of DM-1 did not change significantly in the range of pH (4–11), indicating that pH value had little effect on its growth, and it could grow in strong acidic and alkaline environment. There was no significant difference in mycelial expansion diameter when DM-2 developed in 4–6 pH value, but mycelial growth rate tended to decrease with the increase of pH value, indicating that neutral and alkaline environments were not suitable for DM-2 mycelial growth.

pH value is another key factor in fungal-host interactions. The optimum pH value of DM-1 and DM-2 was 6, when the mycelium grows the fastest. The mycelium can grow normally in the pH value of 4~11, indicating that these two

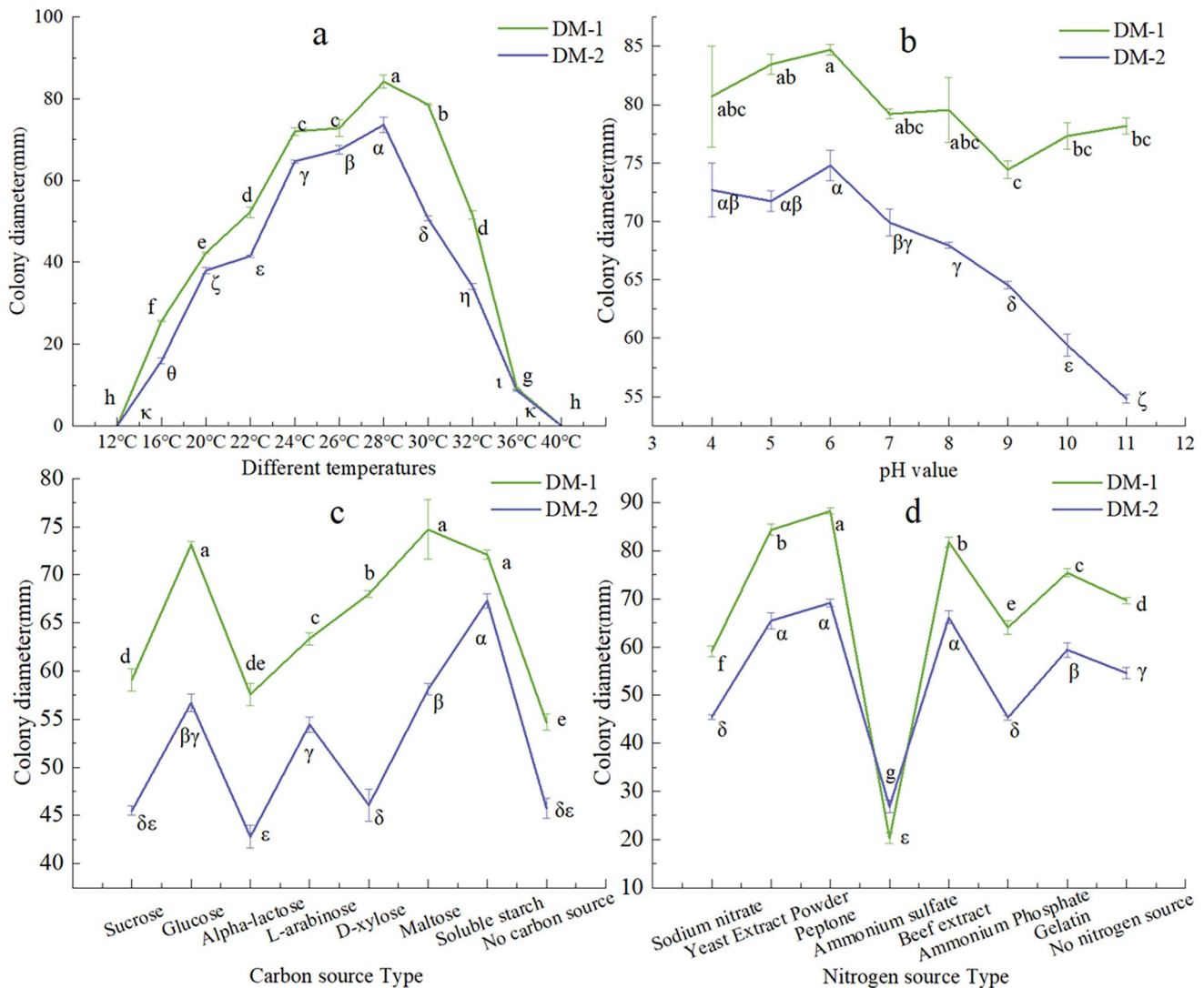


Fig. 4 Effects of different conditions on the mycelia growth of *C. fructicola* DM-1 and *C. asianum* DM-2. **a:** temperatures, **b:** pH values, **c:** carbon sources, **d:** nitrogen sources

isolates can adapt to the strong acidic and alkaline environment. A study reported that the optimum pH for growth of *C. fructicola* HN47-2, GZ15-1 and *C. asianum* YN55-1, FJ11-1 was 5, and the growth rate of *C. asianum* in neutral environment was slower than that in acidic and alkaline environment [22]. In contrast, the growth of *C. asianum* DM-2 strain was inhibited with increasing pH under neutral and alkaline conditions in this experiment, which was contrary to the results of aforementioned study. It is likely that the differences among physiological race are due to different environmental conditions in different geographical areas. In addition, pH value is an important regulator of pectin depolymerase secretion of *C. gloeosporioides*. Pectate Lyase (PL) and polygalacturonase (PG) are important pathogenic factors of *C. gloeosporioides*, which can degrade the outer cuticle of the host, and facilitate the invasion of pathogens [23, 24]. pH in the host environment can modulate the activity of PL and PG, thereby altering the virulence of the pathogen [25]. The secretion of PL increases with elevated pH value (5.8 to 6.5), while no PL was produced on pH below 5.8 [26]. While the optimal pH value of PG is 6.0. The pH value of mango is about 3.8 when it is picked, and it is between 5.5 and 6 when it reaches the best edible state after ripening. This means that the metabolism of the host (mango fruit) changes as its ripens. The level of antifungal compounds in host decreases, and the available substances for pathogen increases, so that the latent pathogen restore growth and further expands. The secretion of PL is regulated by the increase of pH in pericarp which initiates the activity of pathogenic enzymes [26]. Therefore, delaying fruit ripening is an effective measure to avoid PHA. For example, lower temperature during the transportation and storage, so as to prolong the shelf life of fruits and reduce the losses.

Light effects on colony growth

C. fructicola DM-1 and *C. asianum* DM-2 developed rapidly under dark conditions, with colony diameters could reach up to 84.20 and 73.63 mm (Table 2), respectively, which were significantly different from those under continuous light and alternating light and dark conditions. The results are similar

Table 2 Effects of different light on the mycelia growth of *C. fructicola* DM-1 and *C. asianum* DM-2

Factor	Treatments	Colony diameter (mm)	
		<i>C. fructicola</i> DM-1	<i>C. asianum</i> DM-2
Light	Lighting	24.64 ± 2.39 c	20.23 ± 0.63 γ
	Lighting/darknes	68.40 ± 1.37 b	39.81 ± 1.41 β
	darkness	84.20 ± 1.61 a	73.63 ± 1.93 α

*Note: Each factor in the table was analyzed separately, and different lowercase letters indicate significant differences ($p < 0.05$) between treatments

to Shu's study [22]. The colony diameters showed a trend of dark > 12 h alternating light and dark > light, indicating that the mycelial growth of DM-1 and DM-2 were both sensitive to light. While the growth of mycelium of DM-1 and DM-2 is more sensitive to light, and the growth of DM-2 is more affected by light. But on the contrary, Sun et al. found that *C. asianum* T0408 had the fastest growth rate under light conditions [27]. There are differences in the light requirements of different species and different isolates of same species in the nutritional growth [22].

Carbon sources effects on colony growth

C. fructicola DM-1 and *C. asianum* DM-2 could grow on seven carbon sources, while the mycelial growth was minimal on the medium without carbon source. The most suitable carbon source for DM-1 was maltose, followed by glucose and soluble starch, and the colony diameters reached 74.7, 73.12 and 72.1 mm after incubation 6 d respectively, without significant differences. The optimal carbon source of DM-2 was soluble starch, and the colony diameter was recorded as 67.3 mm after 6 days, which was significantly different from other carbon sources. Maltose and glucose followed, with colony diameters of 58.1 mm and 56.69 mm, respectively. Both strains had poor utilization of α -lactose (Fig. 4c).

Hou and Shi revealed that the starch content of freshly harvested mango was 8.05% and soluble sugar was 4.11%, while the starch decreased and the soluble sugar increased as the fruit matured [28]. The soluble sugars in mango are mainly sucrose, fructose, and glucose. It is indicated that the preferred carbon source of DM-1 and DM-2 is available carbon source in mango fruit.

Nitrogen sources effects on colony growth

Similarly, *C. fructicola* DM-1 and *C. asianum* DM-2 could grow on the seven nitrogen sources respectively, as well as on the nitrogen-free control medium, but mycelial growth was minimal on the carbon-free medium. The optimal nitrogen source for DM-1 and DM-2 was peptone, and the colony diameters were 88.20 mm and 69.2 mm, respectively, after cultivation for 6 d. Yeast extract powder and beef extract were also suitable for the growth of the two isolates, and the difference between the two kinds of nitrogen sources on the growth of DM-1 and DM-2 was not significant. While both isolates grew most slowly on the medium with ammonium sulfate as the nitrogen source (Fig. 4d).

In the pathogenicity test, the Daqing fruit inoculated with DM-2 was infected earlier than that inoculated with DM-1 under the same conditions, indicating that the growth of the pathogen on nutrient-sufficient media could not reflect its growth in the host. In addition, the lesion size and the

severity of the disease also varied at different inoculation sites of the same mango. For example, the disease spots were slightly smaller at the sites inoculated with DM-2 near the stem end, while the sites inoculated with DM-1 near the stem end did not develop the disease, which was most likely related to the different nutrients, maturity, and pH values of the different parts of the mangoes.

Lethal temperature effects on colony growth

In the temperature range of 40~60 °C, *C. fruticola* DM-1 and *C. asianum* DM-2 could not grow on the medium after heating treatment at 55 °C for 5 min, and 50 °C for 10 min respectively. So, the lethal temperatures of DM-1 and DM-2 were 55°C for 5 min and 50°C for 10 min, respectively, indicating that DM-1 was more resistant to high temperature.

Heat treatment is a common method to inhibit pathogen after harvest. It is important to explore the lethal temperatures of different pathogenic fungi for the research of mango post-harvest heat treatment. Previously, studies have shown the control effect of mango post-harvest diseases through hot water soaking treatment at 52 °C for 20 min on three cultivars of Tainong, Guifei and Guiqi was up to 40% [29]. In this study, the lethal temperature of DM-1 and DM-2 was different. In the next step, temperature gradient could be set according to the higher lethal temperature of DM-1 (55°C), and find suitable temperature for post-harvest heat treatment available in production.

With the growing demand for mangoes, huge quantities of fresh mangoes are sold to Chinese markets from domestic and foreign mangoes growing areas. Mangoes from Vietnam are favored by consumers because of good quality, earlier maturation, and long supply period. It was reported that the total export of 96.72% Vietnamese mangoes were sold to Chinese fruit market in 2018 [30]. However, the loss of mango caused by PHA during storage and transportation is still a difficult problem to be solved urgently. Li et al. reported the causal agent of mango leaf anthracnose was *C. asianum* in Vietnam [10], but there is no record associated with post-harvest mango anthracnose. The sensitivity to fungicide, invasiveness, and reproductive potential of different *Colletotrichum* are different, so it is necessary to identify the pathogenic *Colletotrichum* accurately, and understand its biological properties, for further developing effective management strategies [31].

Conclusions

Emerging post-harvest pathogens on different fruits results in devastating losses to agriculture and fruit industry. We found that *C. fruticola* DM-1 and *C. asianum* DM-2 were the causal agents which induce PHA of DaQing mango from Vietnam. We found the biological characteristics of *C. fruticola* DM-1, *C. asianum* DM-2, and other isolates reported in literatures were distinguishing. This study has deepened our understanding on the biological characteristics of the pathogen. The results provide scientific basis for the prediction, diagnosis, prevention and control of post-harvest mango anthracnose in different regions. China is not only the largest importer of Vietnamese mangoes, but also the producer of Daqing mango originated from Vietnam. It is possible that the physiological races with different pathogenicity of *C. fruticola* and *C. asianum* may be introduced with mango fruit into different cultivation areas of China with the huge amount of imported mango fruit. This study reports the first assessment of *C. fruticola* and *C. asianum* related to mango PHA in Vietnam, which contributes to the developing prevention and control measures of mango PHA to producer and trader of Vietnam and China.

However, whether the different biological characteristics between DM-1 and DM-2 is related to pathogenicity, and whether these two strains can also infect the branches, leaves, and flowers of mango still need to be further studied. In addition, we will isolate and identify the anthracnose pathogens from Daqing mango cultivated in Yunnan China, and further compare their pathogenicity and biological characteristics with *C. fruticola* DM-1 and *C. asianum* DM-2. It is proposed that DaQing mango could be soaked in hot water (55 °C) 5–10 min after harvest to kill the latent pathogenic fungi in the peel, and turn down the temperature during the transportation and storage, which can prolong the shelf life of fruits and reduce the losses caused by PHA.

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Data availability Sequence data that support the findings of this

study have been deposited in the National Microbial Science Data Centre (NMDC) database of China with registration numbers of NMDCN00016NS, NMDCN00016NT and NMDCN00011PH, NMDCN00011PI, respectively.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no competing interests.

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