GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Phenotypic and molecular characterization of *Colletotrichum* species associated with anthracnose of banana (*Musa* spp) in Malaysia

M.A. Intan Sakinah, I.V. Suzianti and Z. Latiffah

School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

Corresponding author: Z. Latiffah
E-mail: Lfah@usm.my / latiffahz@yahoo.com

Genet. Mol. Res. 13 (2): 3627-3637 (2014)
Received March 13, 2013
Accepted September 26, 2013
Published May 9, 2014
DOI http://dx.doi.org/10.4238/2014.May.9.5

ABSTRACT. Anthracnose caused by *Colletotrichum* species is a common postharvest disease of banana fruit. We investigated and identified *Colletotrichum* species associated with anthracnose in several local banana cultivars based on morphological characteristics and sequencing of ITS regions and of the β-tubulin gene. Thirty-eight *Colletotrichum* isolates were encountered in anthracnose lesions of five local banana cultivars, 'berangan', 'mas', 'awak', 'rastali', and 'nangka'. Based on morphological characteristics, 32 isolates were identified as *Colletotrichum gloeosporioides* and 6 isolates as *C. musae. C. gloeosporioides* isolates were divided into two morphotypes, with differences in colony color, shape of the conidia and growth rate. Based on ITS regions and β-tubulin sequences, 35 of the isolates were identified as *C. gloeosporioides* and only 3 isolates as *C. musae*; the percentage of similarity from BLAST ranged from 95-100% for ITS regions and 97-100% for β-tubulin. *C. gloeosporioides* isolates were more prevalent compared to *C. musae*. This is the first record of *C. gloeosporioides* associated with banana anthracnose in Malaysia. In a phylogenetic analysis of the combined dataset of ITS regions and β-tubulin using a maximum likelihood method, *C. gloeosporioides* and *C. musae* isolates were clearly separated into two groups. We concluded

that *C. gloeosporioides* and *C. musae* isolates are associated with anthracnose in the local banana cultivars and that *C. gloeosporioides* is more prevalent than *C. musae*.

Key words: Banana; Anthracnose; *Colletotrichum gloeosporioides*; *Colletotrichum musae*; ITS regions; β-tubulin

INTRODUCTION

Banana belongs to the family *Musaceae*, which is considered one of the most popular tropical fruits in Malaysia (Abdullah et al., 1990). The production of bananas is ranked second after durian, which is traditionally planted temporarily intercropped with oil palm, rubber and other perennial crops. The most popular varieties of banana for dessert cultivars are 'mas', 'berangan', 'rastali', 'cavendish', and 'embun'.

Anthracnose is one of the most important postharvest diseases of bananas, commonly caused by *Colletotrichum musae*, which infects wounded green fruits and also ripe fruits (Meredith, 1960; Stover and Simmonds, 1987). *C. musae* has also been reported causing blossom end rot, crown rot and tip rot of banana (Nazriya et al., 2007). This disease usually occurs during long transportation and storage period with relatively low temperature and high humidity (Thompson and Burden, 1995). Anthracnose of banana is characterized as brown spots, which become sunken lesions with orange or salmon-colored acervuli (Sun, 1988). When the fruits ripen, the disease will be induced by the accumulation of phytoaloxins, which can facilitate the penetration of the fungus (Jeger et al., 1995; Turner, 1995).

Although *C. musae* is the most common species associated with anthracnose of banana, *C. gloeosporioides* has also been reported to be associated with banana anthracnose (Wijesundera, 1994; Duduk et al., 2009). Most studies related to banana anthracnose in Malaysia have used morphological characteristics to identify *Colletotrichum* species, which may not be sufficient to confidently use in species delimitation, especially to differentiate closely related species. Moreover, *C. musae* is a member of the *C. gloeosporioides* species complex, which indicates that *C. musae* and *C. gloeosporiodes* are closely related.

Internal transcribed spacer (ITS) regions are useful to define the relationships of *Colletotrichum* spp within a species complex in phylogenetic analysis (Du et al., 2005; Phoulivong et al., 2010). The β-tubulin gene can provide a robust tree to support the relationships for species clusters (Shen et al., 2002; Phoulivong et al., 2010). Since *Colletotrichum* species associated with anthracnose of banana in Malaysia are not well characterized and since most studies have used morphological characteristics for identification, the objective of this study was to isolate and identify *Colletotrichum* species from anthracnose of banana by using morphological and molecular characteristics and to determine the phylogenetic relationship of *Colletotrichum* isolates associated with anthracnose of banana by using ITS regions and β-tubulin gene.

MATERIAL AND METHODS

Isolation of *Colletotrichum* isolates

Thirty-eight *Colletotrichum* isolates from banana anthracnose were isolated from anthracnose lesions of several local banana cultivars, namely 'mas,' 'berangan,' 'awak,' 'nang-

ka', and 'rastali' (Table 1). The banana fruits were obtained from several markets and farms in the States of Pulau Pinang and Perak, Peninsular Malaysia.

Table 1. *Colletotrichum* isolates used in this study.

Isolate	Location	Banana cultivar
BB1	Perak	Berangan
BB2	Perak	Berangan
BB3	Pulau Pinang	Berangan
BB4	Pulau Pinang	Berangan
BB5	Pulau Pinang	Berangan
BB6	Pulau Pinang	Berangan
BB7	Pulau Pinang	Berangan
BE8	Pulau Pinang	Mas
BB9	Pulau Pinang	Berangan
BR10	Pulau Pinang	Rastali
BR11	Pulau Pinang	Rastali
BB12	Pulau Pinang	Berangan
BB13	Pulau Pinang	Berangan
BA14	Pulau Pinang	Awak
BA15	Pulau Pinang	Awak
BA16	Pulau Pinang	Awak
BA17	Pulau Pinang	Awak
BA18	Pulau Pinang	Awak
BE19	Perak	Mas
BE20	Perak	Mas
BE21	Perak	Mas
BE22	Perak	Mas
BE23	Perak	Mas
BB24	Pulau Pinang	Berangan
BB25	Pulau Pinang	Berangan
BB26	Pulau Pinang	Berangan
BN27	Pulau Pinang	Nangka
BB28	Pulau Pinang	Berangan
BN29	Pulau Pinang	Nangka
BB30	Pulau Pinang	Berangan
BB31	Pulau Pinang	Berangan
BB32	Pulau Pinang	Berangan
BB33	Pulau Pinang	Berangan
BB34	Pulau Pinang	Berangan
BB35	Pulau Pinang	Berangan
BN36	Pulau Pinang	Nangka
BN37	Pulau Pinang	Nangka
BN38	Pulau Pinang	Nangka

Colletotrichum isolates were obtained by using direct isolation and surface sterilization techniques. For direct isolation, conidia from conidial masses from the anthracnose lesions were transferred to potato dextrose agar (PDA) and incubated at 27° ± 1°C until visible growth of mycelium was observed. For surface sterilization technique, tissues between infected and healthy areas were cut approximately 5 x 5 mm by using a sterile scalpel. The tissue pieces were surface sterilized by dipping in 1% sodium hypochlorite (10% Clorox) for about 3-5 min and rinsed with distilled water. Four pieces of sterilized tissue were plated on PDA and incubated at 27 ± 1°C until there was visible growth of mycelium from the tissues. For each isolate, single spores were isolated to obtain pure cultures.

Morphological identification of *Colletotrichum* isolates was performed using the species descriptions by Sutton and Waterston (1970), Mordue (1971), Cannon et al. (2008), Prihastuti et al. (2009), and Su et al. (2011). The shapes and sizes of conidia were determined by measuring 40 random conidia under a light microscope (Olympus BX41 with Soft Imaging System). Culture

characteristics such as colony color and formation of conidial masses were observed and recorded after 7 days of incubation. To induce appresoria formation, a small amount of mycelium was transferred to a cavity slide, mounted with distilled water and incubated at 27° ± 1°C for 24-48 h.

Phylogenetic analysis

All 38 *Colletotrichum* isolates were grown on PDA plates with membrane dialysis for 5-7 days. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germany) following the manufacturer protocol. The ITS regions were amplified using the ITS 4 and 5 primers (White et al., 1990), while the β-tubulin gene was amplified using the Bt2a and Bt2b primers (Glass and Donaldson, 1995). PCR amplification was performed in a 25-µL reaction mixture comprising deionized distilled water, 4 mM $MgCl_2$, 5X PCR buffer, 0.2 mM dNTP mix (Promega, USA), 0.5 mM of each primer, 0.625 U *Taq* polymerase (Promega) and 1.6 mM DNA template. PCR amplification for both ITS regions and β-tubulin gene was performed in a TM Peltier Thermal Cycler Model PTC-100 (MJ-Research, USA). PCR cycles started with an initial denaturation at 95°C, followed by 34 cycles of denaturation at 95°C for 1 min, 30 s annealing at 52°C and a 1-min extension at 72°C. A final extension for 10 min at 72°C was performed after the cycles ended. Agarose gel electrophoresis (1%) was used to detect the PCR product, and the electrophoresis was run for 80 min at 70 V and 400 mA. PCR products were purified using the QIAquick PCR Purification kit (Qiagen) according to the manufacturer protocol. The purified PCR products were sent to a service provider for DNA sequencing.

Multiple sequence alignments were performed using MEGA5 (Tamura et al., 2011), and optimized manually. Consensus sequences were compared with sequences in the GenBank database by using Basic Local Alignment Search Tool (BLAST). For phylogenetic analysis, ITS regions and β-tubulin were analyzed as a combined dataset, which was performed using MEGA5. Gaps were treated as missing data. Maximum likelihood (ML) trees were constructed by using substitution model Tamura-Nei (Tamura and Nei, 1993), inferred from ML heuristic search option with the nearest neighbor interchange (NNI) algorithm. The trees were generated after 1000 bootstraps to estimate the reliability of the tree.

The epitype sequences of ITS regions and β-tubulin for *C. gleosporioides* and *C. musae* used as comparison are presented in Table 2. Other sequences of ITS regions and β-tubulin from GenBank were also included in the analysis (Table 2). Two *C. trichellum* isolates (GU227817 and GU228111), which are members of *C. acutatum* group were used as outgroup.

Table 2. Sequences from GenBank used in the phylogenetic analysis.

Species	Host	GenBank accession No.	
		ITS	β-tubulin
C. gloesporioides (epitype)	Citrus	EU371022	FJ907445
C. gloeosporioides	Mango	DQ454004	DQ454041
C. gloeosporioides	Mango	DQ454005	DQ454044
C. gloeosporioides	Citrus	AY376534	AY376582
C. gloeosporioides	Citrus	AY376532	AY376580
C. musae (epitype)	Banana	HQ596292	HQ596280
C. musae	Banana	HQ596293	HQ596281
C. musae	Banana	HQ596294	HQ596282
C. trichellum (outgroup)	*Hedera helix*	GU227817	GU228111

RESULTS

Morphological characterization of *Colletotrichum* species

On the basis of morphological characteristics, 32 isolates were identified as *C. gloeosporioides* and 6 isolates as *C. musae*. *C. gloeosporioides* isolates could be divided into two morphotypes according to variation in colony color, conidial morphology and growth rate (Figure 1, Table 3). Differentiation between *C. gloeosporioides* and *C. musae* isolates was based on the shapes and sizes of the conidia and the growth rate.

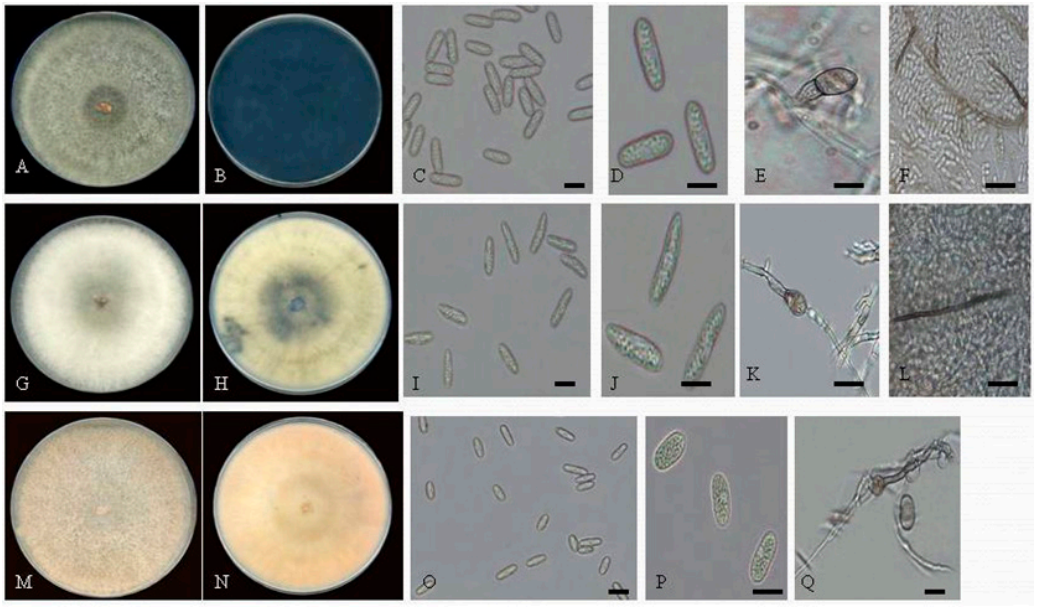

Figure 1. Morphological characteristics of *Colletotrichum gloesporioides* and *C. musae* isolated from anthracnose of banana. *C. gloeosporioides* morphotype 1 (A-F): (**A**) upper surface, (**B**) lower surface, (**C-D**) conidia, (**E**) appresoria, (**F**) seta. *C. gloeosporioides* morphotype 2 (G-I): (**G**) upper surface, (**H**) lower surface, (**I-J**) conidia, (**K**) appresoria (**L**) seta *C. musae* (M-Q): (**M**) upper surface, (**N**) lower surface, (**O-P**) conidia, (**Q**) appresorium (Bars = 10 µm).

Table 3. Morphological characteristics of *Colletotrichum gloesporioides* and *C. musae* isolates from anthracnose of banana.

Species	Colony color	Conidia (mean)			Appresoria	Setae	Growth rate (cm/day)
		Length (µm)	Width (µm)	Shape			
C. gloeosporioides (morphotype 1)	Gray to moss dark green	15-16	5-6	Cylindrical	Ovate to obovate formed	Present in brown color and slightly swollen at the base with tapered apex	±6.48
C. gloeosporiodes (morphotype 2)	Grayish white to grayish green	16-19	5-6	Fusiform to slightly rounded ends	Ovate to obovate formed	Present in brown color and slightly swollen at the base with tapered apex	±5.69 ±7.13
C. musae	White to orangish, white to gray	13-15	5-7	Cylindrical	Irregularly lobed	Absent	

C. gloeosporioides morphotype 1 consisted of 17 isolates in which colony appearance was gray to a moss dark-green color. The conidium shape was cylindrical with obtuse ends, and the average size of conidia was 15.0 x 5.39 µm. Appresoria were ovate to obovate, and setae were present with brown color and slightly swollen at the base with tapered apex but were absent in some isolates (Figure 1). The growth rate was ±6.48 cm/day.

C. gloesporioides morphotype 2 comprised 15 isolates, which had colonies appearing grayish white to grayish green. The shape of conidia varied from fusiform to having slightly rounded ends, and the average size of conidia was 16.84 x 5.68 µm. Appresoria were ovate to obovate. Setae were present with brown color and slightly swollen at the base with tapered apex but were absent in some isolates (Figure 1). The growth rate was ±5.69 cm/day.

Six isolates were identified as *C. musae* with white to orangish and white to gray colony colors. Conidia were elliptical or cylindrical and the average size was 13.14 x 5.86 µm. Appresoria were irregularly lobed and lacked setae (Figure 1). The growth rate of *C. musae* isolates was ±7.13 cm/day, which was faster than for *C. gloeosporioides* isolates.

Phylogenetic analysis

From BLAST, 35 isolates were identified as *C. gloeosporioides* and only 3 isolates as *C. musae*. The size of ITS regions was 600 bp and the size of β-tubulin, 500 bp. The percentage similarity from BLAST and accession number of the sequences are listed in Table 4. The percentage similarity obtained from BLAST ranged from 98 to 100% for ITS regions and from 97 to 100% for β-tubulin.

Maximum likelihood trees of combined datasets of ITS regions and β-tubulin are shown in Figure 2. Both *C. gleosporioides* and *C. musae* isolates were grouped in separate clades (clades I and II). *C. gloeosporioides* isolates from anthracnose of banana were grouped in seven subclades (1-7), and none of the isolates was grouped with the epitype strains (EU371022, FJ907445) or grouped with *C. gloeosporioides* from mango and citrus. Only *C. gloeosporioides* from citrus was grouped with *C. gloeosporioides* epitype strains. *C. musae* isolates formed a separate clade (clade II) with 82% bootstrap value and grouped with *C. musae* epitype strains (HQ596292, HQ596280).

DISCUSSION

The findings from the present study showed that *C. gloeosporioides* and *C. musae* were associated with anthracnose of banana, and *C. gloeosporioides* was more prevalent than *C. musae*. To our knowledge, this is the first documented report of *C. gloeosporioides* associated with banana anthracnose in Malaysia. The results showed that identification based solely on morphological characteristics can lead to misidentification, since morphological characters are easily influenced by environmental factors such as humidity, temperature and rainfall (Freeman et al., 1998). Therefore, for precise species identification, DNA sequencing data are needed to provide information for species delimitation.

On the basis of morphological characteristics, *C. gloeosporioides* isolates can be divided into two morphotypes and the descriptions of the isolates in both morphotypes fall within the range of descriptions of *C. gloeosporioides* by Mordue (1971), Cannon et al. (2008)

and Prihastuti et al. (2009). Generally, the six *C. musae* isolates fall within the description of morphological characteristics by Su et al. (2011), except the colony color of several isolates in the present study was white to orangish with floccose aerial mycelium. However, according to the sequences of ITS regions and β-tubulin, only three isolates (BB9, BN27 and BN29) were identified as *C. musae* with 99-100% similarity.

Table 4. Identity of *Colletotrichum* isolates from anthranose of banana based on sequence similarity of ITS region and β-tubulin gene.

Isolates	Species name	Sequence similarity (%)		GenBank accession No.	
		ITS	β-tubulin	ITS	β-tubulin
BB1	*C. gloeosporioides*	99	99	JX163228	JX431527
BB2	*C. gloeosporioides*	100	99	JX163231	JX431541
BB3	*C. gloeosporioides*	100	99	JX163201	JX431542
BB4	*C. gloeosporioides*	100	100	JX163217	JX431543
BB5	*C. gloeosporioides*	98	96	JX163229	JX431528
BB6	*C. gloeosporioides*	99	99	JX163230	JX431529
BB7	*C. gloeosporioides*	100	99	JX163215	JX431544
BE8	*C. gloeosporioides*	100	98	JX163223	JX431533
BR10	*C. gloeosporioides*	99	97	JX163221	JX431538
BR11	*C. gloeosporioides*	99	96	JX163219	JX431539
BB12	*C. gloeosporioides*	99	99	JX163202	JX431545
BB13	*C. gloeosporioides*	100	99	JX163203	JX431546
BB14	*C. gloeosporioides*	100	99	JX163226	JX431526
BA15	*C. gloeosporioides*	100	92	JX163204	JX566758
BA16	*C. gloeosporioides*	95	99	JX163225	JX431547
BA17	*C. gloeosporioides*	99	99	JX163224	JX431548
BA18	*C. gloeosporioides*	99	100	JX163222	JX431549
BE19	*C. gloeosporioides*	100	99	JX163205	JX431550
BE20	*C. gloeosporioides*	99	99	JX163216	JX431551
BE21	*C. gloeosporioides*	100	98	JX163206	JX431534
BE22	*C. gloeosporioides*	100	100	JX163218	JX431552
BE23	*C. gloeosporioides*	100	95	JX163207	JX431535
BB24	*C. gloeosporioides*	99	99	JX163227	JX431553
BB25	*C. gloeosporioides*	99	98	JX163208	JX431554
BB26	*C. gloeosporioides*	99	98	JX163209	JX431555
BB28	*C. gloeosporioides*	99	99	JX163210	JX431531
BB30	*C. gloeosporioides*	99	98	JX163211	JX431532
BB31	*C. gloeosporioides*	99	96	JX431560	JX566751
BB32	*C. gloeosporioides*	99	99	JX163220	JX431556
BB33	*C. gloeosporioides*	99	99	JX163212	JX431557
BB34	*C. gloeosporioides*	99	100	JX163213	JX431558
BB35	*C. gloeosporioides*	99	99	JX163214	JX431559
BN36	*C. gloeosporioides*	99	96	JX431561	JX566763
BN37	*C. gloeosporioides*	99	99	JX431540	JX566764
BN38	*C. gloeosporioides*	100	99	JX431562	JX566758
BB9	*C. musae*	99	99	JX163232	JX431530
BN27	*C. musae*	99	99	JX163233	JX431536
BN29	*C. musae*	100	97	JX163234	JX431537

Although identification using morphological characteristics could lead to misidentification, it is useful for grouping the isolates to their morphotypes as mentioned by Prihastuti et al. (2009). On the basis of phylogenetic analysis of combined datasets as well as an individual dataset (data not shown), *C. gloeosporioides* and *C. musae* were clearly separated into different clades. The analysis also showed that location, banana cultivar and morphotype did not contribute to phylogenetic relatedness. The results were similar to those of a study by Rampersad (2011) on papaya anthracnose in which geographical distribution, host cultivar or morphotype was not an indication of the phylogenetic relatedness of the papaya isolates.

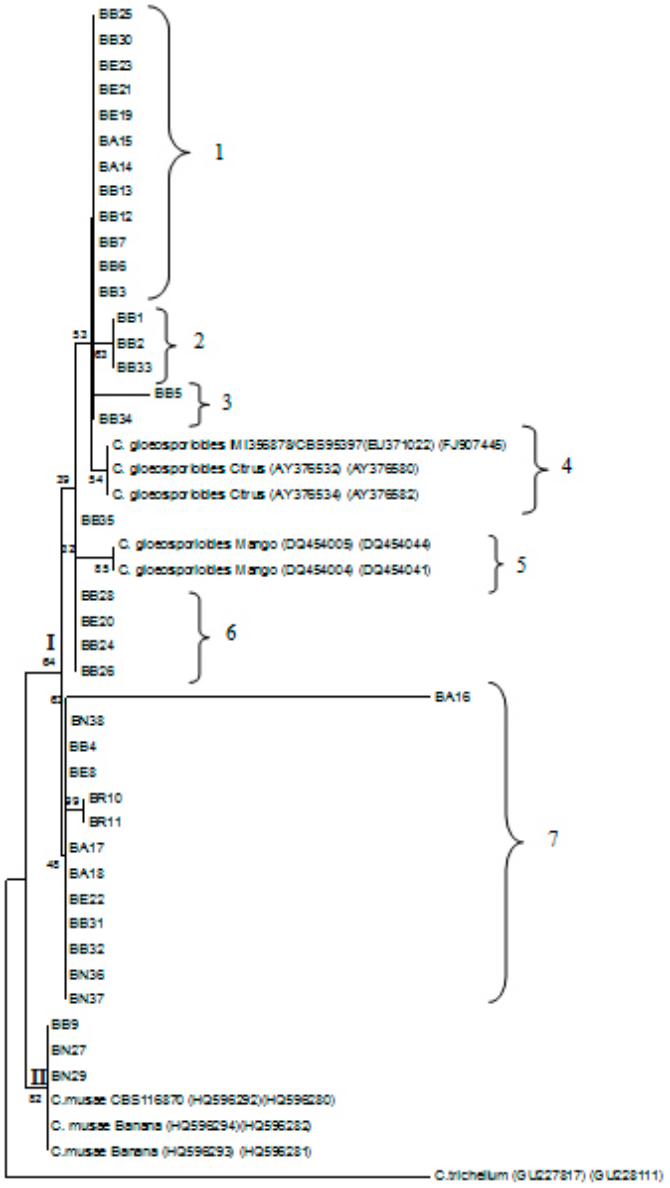

Figure 2. Maximum likelihood showing phylogenetic relationships among *Colletotrichum* based on ITS regions and β-tubulin gene using Tamura-Nei substitution model and nearest-neighbor-interchange search options with 1000 bootstrap replicates. *Colletotrichum trichellum* isolate was the outgroup.

C. gloeosporioides and *C. musae* are part of the taxa in *C. gloeosporioides* species complex in which the species in this complex has broad genetic and biological diversity (Damm et al., 2010). *C. gloeosporioides* is found on a wide variety of fruit crops such as almond, avocado, apple, coffee, banana, guava, mango, and strawberry (Freeman et al., 2000;

Sanders and Korsten, 2003; Xiao et al., 2004; Photita et al., 2005; Prihastuti et al., 2009). On the other hand, *C. musae* appears to be specific to *Musa* spp (Su et al.., 2011). Although *C. musae* was not prevalent in the present study, several studies have shown that *C. musae* is the most common species associated with anthracnose of banana. In Korea and Saudi Arabia, *C. musae* was associated with anthracnose of imported banana (Lim et al., 2002; Abd-Elsalam et al., 2010). Thangamani et al. (2011) reported that according to morphological and physiological characterization, *C. musae* was pathogenic to banana in India.

The grouping of *C. gleosporioides* isolates into several subclades on the basis of individual dataset (data not shown) and combined datasets, indicated variability, which further confirmed the complexity of the species. Grouping of *C. gloeosporioides* into several subclades suggest that the isolates may represent a sub-population of *C. gloeosporioides* with distinct genetic characters. Similar results were reported by Prihastuti et al. (2009) and Waller et al. (1993) in which *C. gloeosporioides* on coffee berries showed several distinct genetic and phenotypic species. It was also noted that none of the *C. gloeosporioides* isolates from banana was grouped with the ex-epitype of *C. gloeosporioides*, which could also suggest that the isolates belong to *C. gloeosporioides*-like species, possibly indicating the presence of more than one distinct species. The results of the present study were similar to those of a study by Phoulivong et al. (2010) in which 22 strains of *C. gloeosporioides* from tropical fruits did not cluster with an ex-epitype of *C. gleosporioides*, indicating the presence of more than one distinct species.

In the present study, three *C. musae* isolates were identified using ITS regions and β-tubulin sequences and these isolates were grouped with isolates with accession numbers HQ596292 and HQ596280, which confirms that the three isolates were *C. musae*. This species is a member of the *C. gloesporioides* species complex and has been recognized as a separate species limited to banana or *Musa* spp (Du et al., 2005). For sequence-based identification of *C. musae*, ITS sequence is recommended (Weir et al., 2012), which separates the species from other *Colletotrichum* species.

In the present study, important information was obtained. *C. musae* was not a common species associated with several types of local banana cultivars, although *C. musae* has been reported to be the most common causal anthracnose of many banana cultivars worldwide (Lim et al., 2002; Abd-Elsalam et al., 2010; Thangamani et al., 2011). *C. gloeosporioides* is the most prevalent species associated with anthracnose of banana, suggesting the potential of cross-infection to other tropical fruits. Moreover, pathogenic *C. gloeosporioides* is known to show a mutualistic and commensal association with various plant species. These associations can change under certain conditions (Redman et al., 2001; Photita et al., 2005). Phylogenetic analysis has shown that *C. gloeosporioides* from banana, mango and citrus form separate groups, which supports the hypothesis that *C. gloeosporioides* from tropical fruits may consist of more than one distinct species as reported by Phoulivong et al. (2010).

The findings of this study have an important bearing on the epidemiology of anthracnose disease since different species of *Colletotrichum* may have different conidial dispersal capacities (Ntahimpera et al., 1999; Rampersad, 2011). Accurate identification of the anthracnose-causing pathogen is also important, since different species of *Colletotrichum* may vary in their reaction to different types of fungicides (Rampersad, 2011). Sequence analysis using other protein-coding genes should be tested to confirm the existence of more than one distinct species of *C. gloeosporioides*, since this species has wide geographic distribution and host range.

ACKNOWLEDGMENTS

Research supported in part by ScienceFund grant from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia (#305/PBIOLOGY/613225).

REFERENCES

Abd-Elsalam KA, Roshdy S, Amin OE and Rabani M (2010). First morphogenetic identification of the fungal pathogen *Colletotrichum musae* (*Phyllachoraceae*) from imported bananas in Saudi Arabia. *Genet. Mol. Res.* 9: 2335-2342.

Abdullah H, Pantastico EB, Tirtosoekotja S, Nanthachai P, et al (1990). Status of the Banana Industry in ASEAN. In: Banana: Fruit Development, Postharvest Physiology (Hassan A and Pantastico EB, eds.). Handling & Marketing in ASEAN, Kuala Lumpur, 1-22.

Cannon PF, Buddie AG and Bridge PD (2008). The typification of *Colletotrichum gloeosporioides*. *Mycotaxon* 104: 189-204.

Damm U, Baroncelli R, Cai L, Kubo Y, et al. (2010). *Colletotrichum*: species, ecology and interactions. *IMA Fungus.* 1: 161-165.

Du M, Schardl CL, Nuckles EM and Vaillancourt LJ (2005). Using mating-type gene sequences for improved phylogenetic resolution of *Collectotrichum* species complexes. *Mycologia* 97: 641-658.

Duduk N, Ivanovic M and Duduk B (2009). Morphological, serological and molecular analyses of anthracnose-causing agent on banana fruit. *Pestic. Fitomedicina* 24: 281-286.

Freeman S, Katan T and Shabi E (1998). Characterization of *Colletotrichum* species responsible for anthracnose disease of various fruits. *Plant Dis.* 82: 596-605.

Freeman S, Minz D, Jurkevitch E, Maymon M, et al. (2000). Molecular analyses of *Colletotrichum* species from almond and other fruits. *Phytopathology* 90: 608-614.

Glass NL and Donaldson GC (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microbiol.* 61: 1323-1330.

Jeger M, Eden GS, Thresh J and Johanson A (1995). Banana Disease. In: Banana and Plantains (Gowen S, ed.). Chapman & Hall, London, 317-381.

Lim JY, Lim TH and Cha B (2002). Isolation and identification of *Colletotrichum musae* from imported bananas. *Plant Pathol. J.* 18: 161-164.

Meredith DS (1960). Studies on *Gloeosporium musarum* Cke. & Massee causing storage rots of Jamaican bananas. I. Anthracnose and its chemical control. *Ann. Appl. Biol.* 48: 279-290.

Mordue JEM (1971). *Glomerella cingulata*. No. 315. In: CMI Descriptions of Pathogenic Fungi and Bacteria. CAB, Kew.

Nazriya MNF, De Costa DM and Azhaar AS (2007). Genomic variation of *Colletotrichum musae* morphotypes infecting banana varieties of Sri Lanka. *Proc. Peradeniya Univ. Res. Sessions* 12: 1-2.

Ntahimpera N, Wilson LL, Ellis MA and Madden LV (1999). Comparison of rain effects on splash dispersal of three *Colletotrichum* species infecting strawberry. *Phytopathology* 89: 555-563.

Photita W, Taylor PWJ, Ford R, Hyde DK, et al. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand. *Fungal Divers.* 18: 117-133.

Phoulivong S, Cai L, Chen H, McKenzie EHC, et al. (2010). *Colletotrichum gloeosporioides* is not a common pathogen on tropical fruits. *Fungal Divers.* 44: 33-43.

Prihastuti H, Cai L, Chen H, McKenzie EHC, et al. (2009). Characterization of *Colletotrichum* species associated with coffee berries in Northern Thailand. *Fungal Divers.* 39: 89-109.

Rampersad SN (2011). Molecular and phenotypic characterization of *Colletotrichum* species associated with anthracnose disease of papaya in Trinidad. *Plant Dis.* 95: 1244-1254.

Redman RS, Dunigan DD and Rodriguez RJ (2001). Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? *New Phytol.* 151: 705-716.

Sanders GM and Korsten L (2003). Comparison of cross inoculation potential of South African avocado and mango isolates of *Colletotrichum gloeosporioides*. *Microbiol. Res.* 158: 143-150.

Shen Q, Geiser DM and Royse DJ (2002). Molecular phylogenetic analysis of *Grifola frondosa* (maitake) reveals a species partition separating eastern North American and Asian isolates. *Mycologia* 94: 472-482.

Stover R and Simmonds N (1987). Bananas. 3rd edn. Longman, London.

Su YY, Noireung P, Liu F, Hyde KD, et al. (2011). Epitypification of *Colletotrichum musae*, the causative agent of banana anthracnose. *Mycoscience* 52: 376-382.

Sun SK (1988). Postharvest Disease of Subtropical and Tropical Fruits and Their Control. In: Postharvest Handling of Tropical and Subtropical Fruit Crops (Jan Bay-Petersen IO, ed.). Food and Fertilizer Technology Center for the Asian and Pacific Region, Taiwan, 113-122.

Sutton BC and Waterston NW (1970). *Colletotrichum musae*. CMI Description of Pathogenic Fungi and Bacteria, No. 222. Eastern Press, London.

Tamura K and Nei M (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10: 512-526.

Tamura K, Peterson D, Peterson N, Stecher G, et al. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28: 2731-2739.

Thangamani PR, Kuppusamy P, Peeran MF, Gandhi K, et al. (2011). Morphological and Physiological Characterization of *Colletotrichum musae* the causal organism of banana anthracnose. *World J. Agric. Sci.* 7: 743-754.

Thompson A and Burden O (1995). Harvesting and Fruit Care. In: Banana and Plantains & Hall (Gowen SR, ed.). Chapman, London, 403-433.

Turner D (1995). The Response of the Plant to the Environment. In: Bananas and Plantains (Gowen SR, ed.). Chapman & Hall, London, 206-229.

Waller JW, Bridge PD, Black R and Hakiza G (1993). Characterization of the coffee berry disease pathogens, *Colletotrichum kahawae* sp. nov. *Mycol. Res.* 97: 989-994.

Weir BS, Johnston PR and Damm U (2012). The *Colletotrichum gloeosporioides* species complex. *Stud. Mycol.* 73: 115-180.

White TJ, Bruns T, Lee S and Taylor JW (1990). Amplication and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols: A Guide to Methods and Application (Innis MA, Gelfand DH, Sninsky JJ and White YJ, eds.). Academic Press, San Diego, 315-322.

Wijesundera RLC (1994). Variation in *Colletotrichum gloeosporioides* isolates from banana. *J. Nat. Sci. Council Sri Lanka* 22: 145-150.

Xiao CL, Mackenzie SJ and Legard DE (2004). Genetic and pathogenic analyses of *Colletotrichum gloeosporioides* isolates from strawberry and noncultivated hosts. *Phytopathology* 94: 446-453.

