Mycol Progress (2017) 16:677–686
DOI 10.1007/s11557-017-1304-2

CrossMark

ORIGINAL ARTICLE

DGfM

Diversity of *Colletotrichum* spp. isolated from chili pepper fruit exhibiting symptoms of anthracnose in Thailand

Sawita Suwannarat[1] · Siegrid Steinkellner[2] · Pattavipha Songkumarn[1] · Somsiri Sangchote[1]

Received: 24 November 2016 / Revised: 17 April 2017 / Accepted: 21 April 2017 / Published online: 10 May 2017
© German Mycological Society and Springer-Verlag Berlin Heidelberg 2017

Abstract *Colletotrichum* spp. are causal agents of anthracnose disease in chili fruits and other tropical crops. The disease is increasing in chili fruits in Thailand and significantly reduces fruit quality and fruit production. Forty-eight isolates of *Colletotrichum* spp. associated with chili anthracnose were collected from different areas of Thailand during 2010–2015. Based on morphological characteristic identification, 10 isolates were shown to belong to the *C. gloeosporioides* species complex, 24 isolates belong to the *C. acutatum* species complex and 14 isolates to *C. capsici*. For molecular identification, two primer sets, ITS1/ITS4 and ACT528/ACT738, were used for amplification of the internal transcribed spacer of rRNA gene (ITS1–5.8S–ITS2) and partial region actin gene (*ACT*), respectively. The phylogenetic analysis of individual and combined ITS region and actin nucleotide sequences identified the collected isolates into 4 species: *C. gloeosporioides*, *C. siamense*, *C. acutatum* and *C. capsici*. The pathogenicity test demonstrated that all four species were pathogenic on intact unwounded and healthy fruits. These results indicated that *C. capsici*, *C. acutatum*, *C. gloeosporioides* and *C. siamense* were the causal agents of chili anthracnose disease.

Section editor: Gerhard Rambold

✉ Somsiri Sangchote
agrsrs@ku.ac.th

[1] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, 50 Ngamwongwan Road, Chatuchak, Bangkok 10900, Thailand

[2] Division of Plant Protection, Department of Crop Science, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Strasse 24, 3430 Tulln, Austria

Keywords Anthracnose · Capsicum · *Colletotrichum* · Identification · rDNA-ITS · Actin

Introduction

The species of *Colletotrichum* comprise important plant pathogens that cause anthracnose disease on many crops including fruits, vegetables, cereals and ornamental plants. In chili production (*Capsicum annuum* L.), anthracnose is a serious problem especially in tropical and sub-tropical areas including India, Korea and Thailand (Sharma et al. 2005; Kim et al. 2008; Than et al. 2008). Chili anthracnose is caused by several *Colletotrichum* species: *C. capsici*, *C. acutatum*, *C. gloeosporioides*, *C. coccodes* and *C. dermatium* (Manandhar et al. 1995; Hong and Hwang 1998; Ivery et al. 2004; Sharma et al. 2005). In Thailand *C. capsici*, *C. gloeosporioides*, and *C. acutatum* have been reported to affect this crop (Than et al. 2008). To date, the species identification of *Colletotrichum* spp. has relied mainly on morphological characteristics such as colony characteristics, conidial shape and size, shape of appressoria, conidial mass color, and the presence and absence of setae (Sutton 1992). However, the effects of growth conditions, such as light and temperature, and re-subculturing can cause morphological changes. Nowadays, DNA-based molecular techniques have been developed and are widely used for taxonomic purposes using DNA sequencing from internal transcribed spacer region (ITS) and coding sequences for fungal identification. Phylogenetic analysis using a single DNA dataset or multilocus analysis which was generated from loci such as actin, calmodulin and chitin synthesis genes has been used to support the morphology characteristics.

Recently, Weir et al. (2012) has divided the *Colletotrichum gloeosporioides* complex into 22 species by using a

multilocus phylogenetic analysis and has reported that some isolates of *Colletotrichum* spp. from *Capsicum annuum* in Thailand belong to *C. siamense*. In this study, we report the characterization of isolates of *Colletotrichum* spp. from chili pepper exhibiting symptoms of anthracnose, using morphological characteristics, pathogenicity and DNA markers.

Materials and methods

Isolation of fungi

Chili fruit showing anthracnose symptoms were collected from chili producing fields in twelve provinces of five regions of Thailand (Fig. 1). Tissue from the margins of anthracnose lesions was cut into small pieces, sterilized with 10% sodium hypochlorite for 5 min and dried on sterile paper. Tissue samples were placed on Petri dishes containing potato dextrose agar (PDA) and incubated at 25 °C under 12-h light:12-h dark for 5–7 days. Single spores were isolated and maintained on the same medium for further studies.

Fig. 1 Samples of chili anthracnose were collected from different provinces in Thailand, indicated by the orange dots

Morphological identification

Intact fruits were inoculated with each isolate by spraying with a conidial suspension at approximately equal to 800 conidia/cm^2 of fruit surface area. After symptoms developed and a conidial mass was produced on the lesion, conidia were transferred to PDA plates. Approximately 100 conidia were mounted on glass slides to determine the conidial shape and size and then isolates were identified using Sutton's key (Sutton 1980, 1992). To investigate the colony morphology, mycelium agar plugs were cut with a cork borer (0.5 cm in diameter) from 5-day-old cultures of *Colletotrichum* and transferred to the center of a Petri dish which contained 20 ml potato dextrose agar (Difco, USA). Three plates per isolate were used. All plates were incubated at 25 °C under 12-h light:12-h dark for 9 days. Then, the colony characteristics were observed. Appressoria were produced by using a slide culture technique according to Johnston and Jones (1997) and were observed under a microscope.

DNA extraction

Fungal mycelium was cultured in malt broth (MB; malt extract 20 g/L) for 4 days. The mycelium was harvested with forceps and then suspended in extraction buffer (Alexander et al. 2007). After that chloroform:isoamyl alcohol (24:1) was added and the suspension was incubated on ice for 5 min and centrifuged at 14,000*g* for 10 min. The clear supernatant was transferred to a new microtube and 400 μl of isopropyl alcohol was added for nucleic acid precipitation. That tube was centrifuged at 14,000*g* for 5 min and the isopropanol discarded. The pellet was washed 2 times with 70% of ethyl alcohol. After washing, the DNA was suspended with 25 μl RNase water and stored at −20 °C.

PCR amplification

The internal transcribed spacer (ITS) region including ITS1, 5.8sRNA gene and ITS2 was amplified by using ITS1 primer (5'GCCGTAGGTGAACCTGCGG3') and ITS4 primer (5'TCCTCCGCTTATTGATATGC3') (White, 1990). PCR was performed in 25 μl reaction mixtures containing 0.25 mM dNTPs, 0.5 U Taq polymerase (Thermostatic dream taq; Thermostatic), 0.1 μM of each primer and 50 ng DNA template. The PCR cycle consisted of 1 cycle of 5 min at

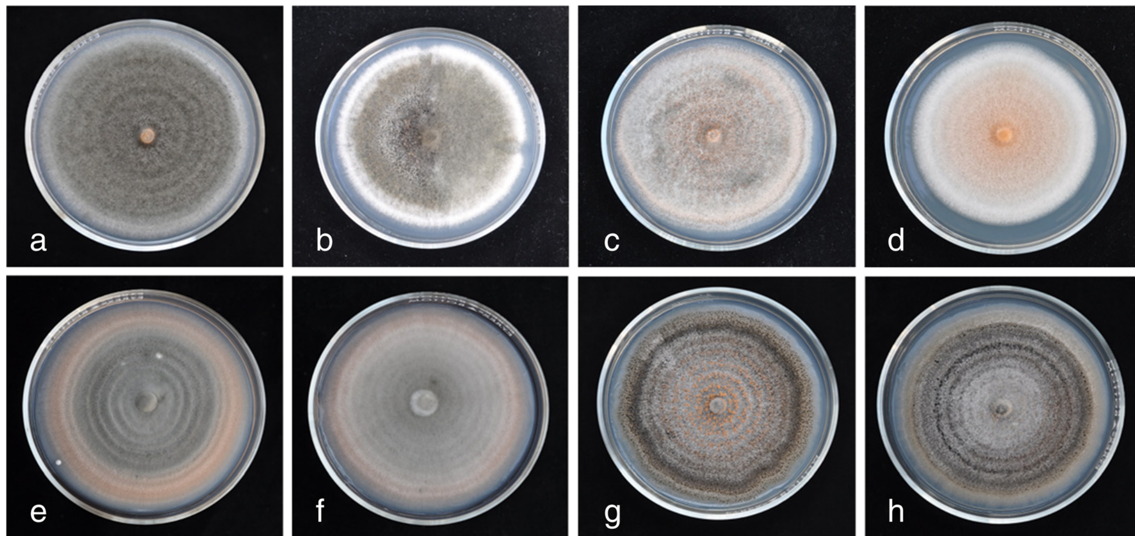

Fig. 2 Colonies of *Colletotrichum* isolates after 9 days on PDA: **a**, **b** *C. gloeosporioides* NAN2002 and NAN1603, **c**, **d** *C. siamense* KPS2 and CN4, **e**, **f** *C. acutatum* NAN1103 and PR264, **g**, **h** *C. capsici* CC9.4 and CN2

95 °C, 30 cycles of 45 s at 95 °C, 45 s at 60 °C, 45 s at 72 °C and a final cycle of 72 °C for 5 min. A partial actin fragment was amplified using ACT512F (5'AGTTGCAAGGCCGGTTTCG3') and ACT738R (5'TACGAGTCCTTCTGGCCCAT3') primer (Carbone and Kohn 1999). The PCR started with 95 °C 5 min for 1 cycle, followed by 30 cycles, with step of 30 s at 95 °C, 30 s at 52 °C, 30 s at 72 °C and a final cycle 72 °C for 5 min. All PCR products were electrophoresed in a 1.5% agarose gel, stained with a fluorescence dye (Novel juice; Genedirex) and visualized under UV light.

Phylogenetic and sequence analysis

The sequences which corresponded to each region were assembled and edited using BioEdit (Hall 1999), and consensus sequences of each isolate were created. The consensus sequences of each region were submitted to the Basic Local Alignment Search Tool (BLAST) from NCBI (http://blast.ncbi.nlm.nih.gov) for finding similar sequences. For phylogenetic analysis, data were generated from the combined dataset (ITS + *ACT*) and separate datasets (ITS, *ACT*).

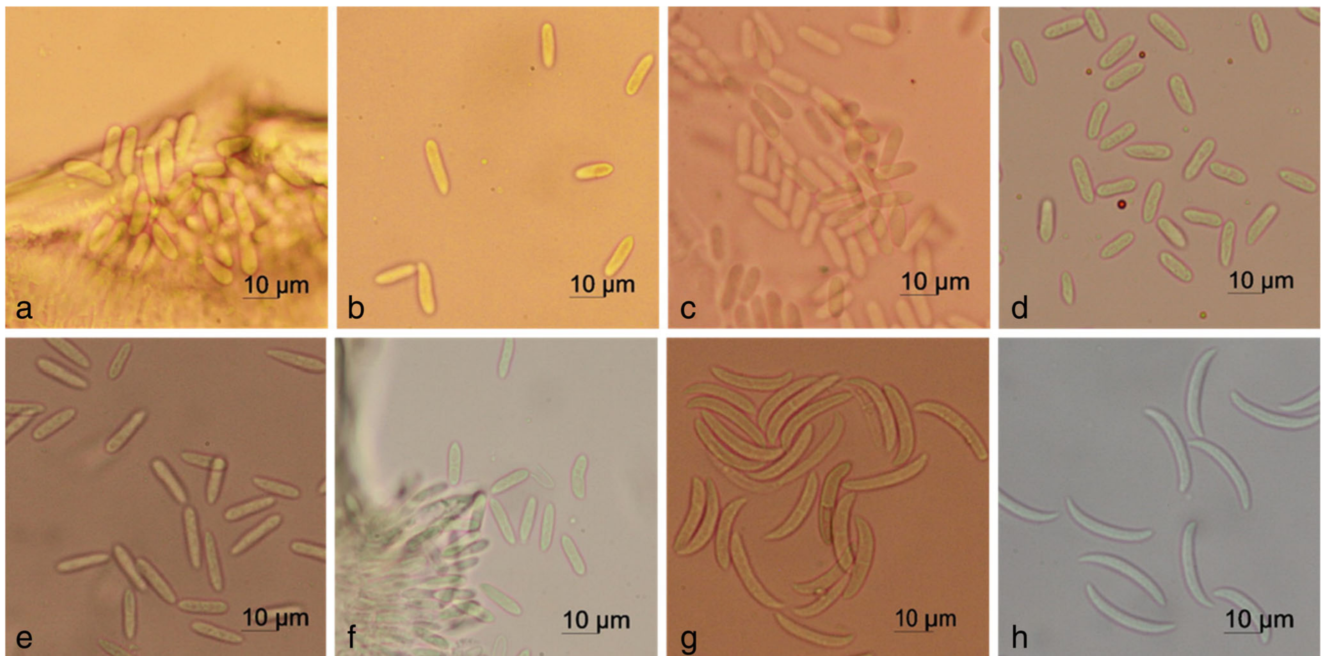

Fig. 3 Conidia of selected *Colletotrichum* isolates: **a**, **b** *C. gloeosporioides* NAN2002 and NAN1603, **c**, **d** *C. siamense* KPS2 and CN4, **e**, **f** *C. acutatum* NAN1103 and PR264, **g**, **h** *C. capsici* CC9.4 and CN2

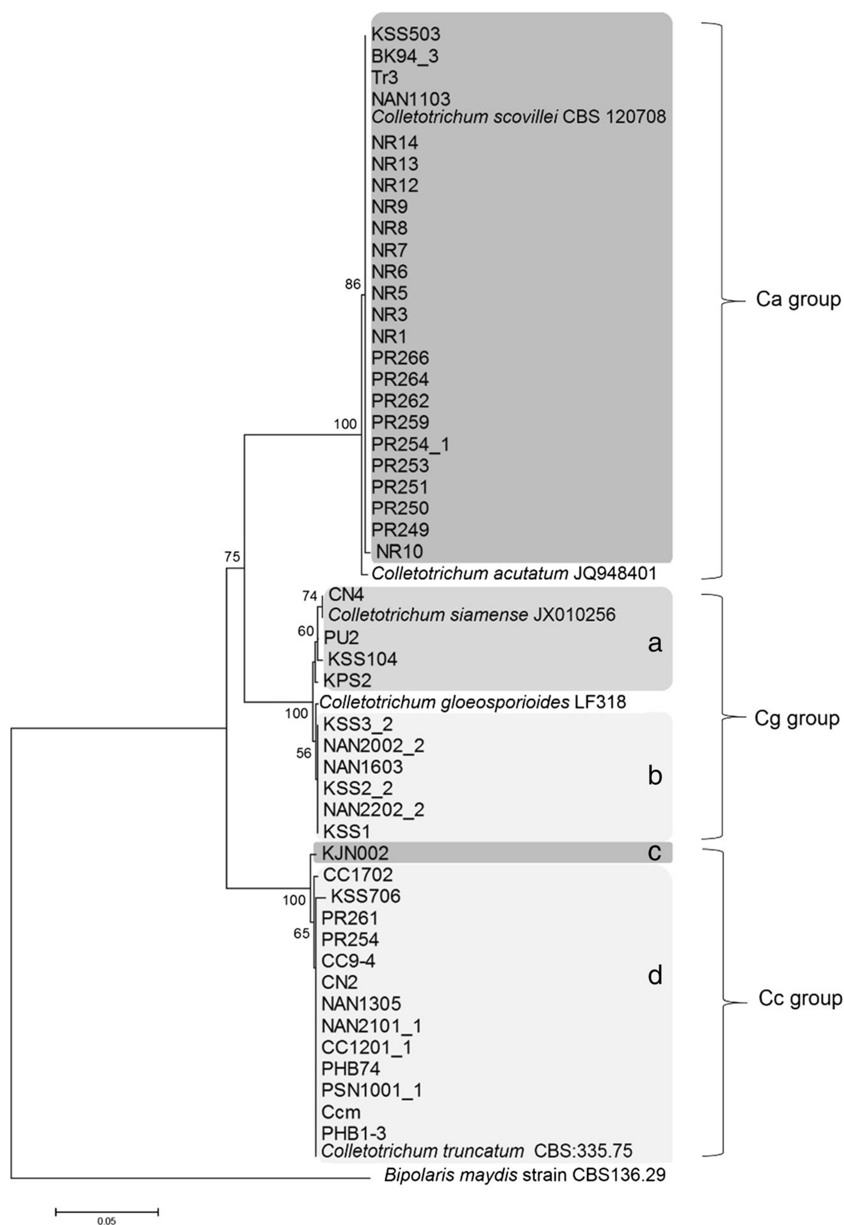

Fig. 4 The phylogenetic tree constructed by using the neighbor-joining method of the ITS region from *Colletotrichum* species associated with chili fruits in Thailand and reference species. The *number above each branch* indicates the bootstrap percentage value with 1000 replications. Branch lengths are drawn proportionally to genetic distances and the *bar* at the bottom of the tree indicates a length corresponding to 0.05 nucleotide substitutions per site

The phylogenetic trees were constructed from multiple alignment from ClustalW (Thomson 1994) included in the MEGA5 software (Tamura 2011). The analysis was conducted using the neighbor-joining (NJ) likelihood method in MEGA 5.0. All positions containing gaps and missing data were eliminated. The bootstrap analysis was assessed with 1000 replicates to determine the reliability of branches. The new sequences in this study were deposited in DDBJ (DNA Data Base of Japan, Japan).

Pathogenicity test

The pathogenicity test for each species was performed on red intact chili fruits. Fruits were washed with sterilized distilled water and then dried at room temperature. The selected isolates were cultured on PDA medium at 25 °C under 12-h light:12-h dark for 9 days. Conidial suspension was prepared from 8-day-old cultures by adding sterilized distilled water to the plate. The suspension was filtered through cheesecloth. The conidia concentration was adjusted to 2×10^6 conidia/ml.

Chili fruits were inoculated by spraying the conidial suspension on one side of the fruit surface with an air brush apparatus at approximately 800 conidia/cm^2 of fruit surface area. For the negative control, the other side of inoculated fruits was sprayed with sterilized distilled water. Each inoculated fruit was covered with moist plastic bags for 48 h at 25 °C. Afterwards, the bag was removed and the fruit was observed for the symptom. Three fruits were analyzed for each isolate.

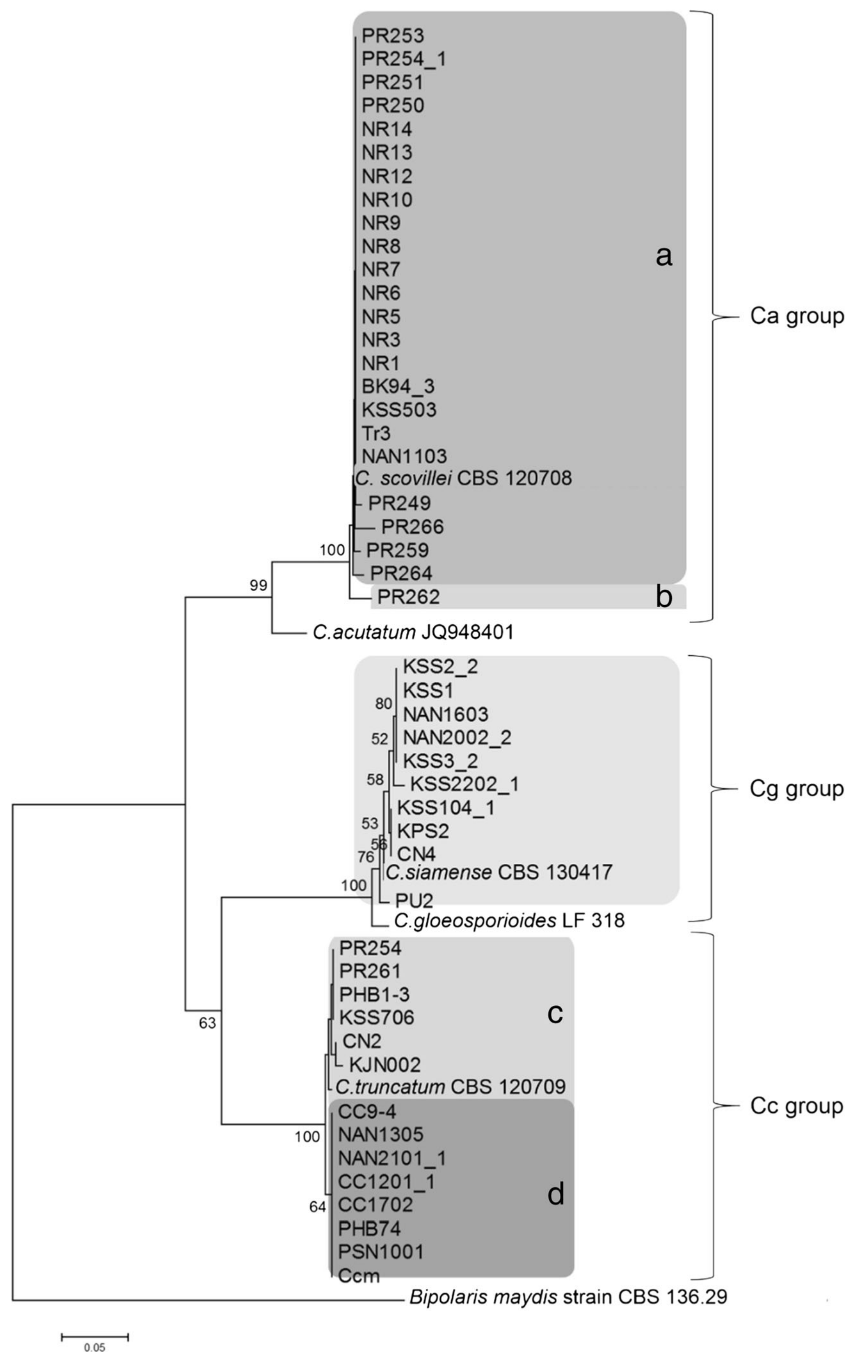

Fig. 5 The phylogenetic tree constructed by using the neighbor-joining method of the ACT gene from *Colletotrichum* species associated with chili fruits in Thailand and reference species. The *number above each branch* indicates the bootstrap percentage value with 1000 replications. Branch lengths are drawn proportionally to genetic distances and the *bar* at the bottom of the tree indicates a length corresponding to 0.05 nucleotide substitutions per site

Results

Morphological identification

The colonies of *C. gloeosporioides* were found in two types. One type showed a pale gray to dark gray mycelium covered with an orange conidial mass and the second type exhibited white to pale gray mycelium with a pinkish and orange conidial mass. The colony of *C. siamense* showed white to pale gray mycelium with a pinkish and orange conidial mass. The colony of *C. capsici* had greenish gray to light brown mycelium with a pale to a salmon orange conidial mass. The colony of *C. acutatum* showed gray to white puffy mycelium with a salmon to pinkish conidial mass (Fig. 2).

Conidia of the isolates were distinguished into three types (Fig. 3). The first type was hyaline, smooth, and cylindrical with a round end, 9.3–19.2 × 2.2–5.3 μm. The second type of conidia was hyaline and falcate 14.7–29 × 2–5 μm. The third type of conidia was hyaline, smooth, and fusiform 8.2–19.5 × 2–4.5 μm.

The appressoria of *C. gloeosporioides* groups were clavate and brown to dark brown. *C. capsici* appressoria were elliptical

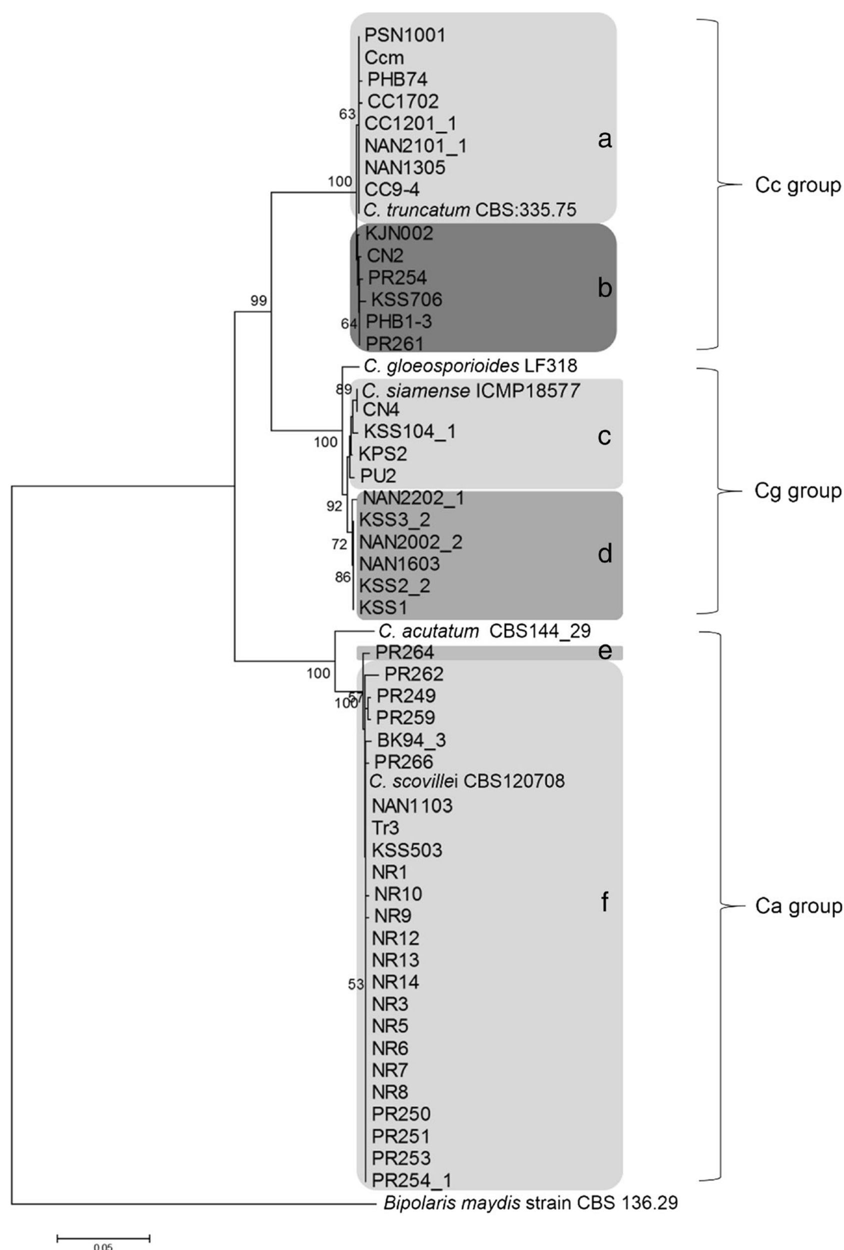

Fig. 6 The phylogenetic tree constructed by using the neighbor-joining method of the combined dataset of ACT and ITS sequences from forty-eight isolates of *Colletotrichum* species associated with chili fruits in Thailand and reference species. The *number above each branch* indicates the bootstrap percentage value with 1000 replications. Branch lengths are drawn proportionally to genetic distances and the *bar* at the bottom of the tree indicates a length corresponding to 0.05 nucleotide substitutions per site

to clavate, but some produced an irregular brown lobe. Appressoria of *C. acutatum* were elliptical to circular and dark brown.

These characteristics were described for *C. gloeosporioides*, *C. capsici* and *C. acutatum*, respectively, according to Sutton (1992).

PCR amplification and sequencing analysis

DNA fragments from ITS and Act locus were successfully amplified from total genomic DNA of 48 isolates. A PCR product size of approximately 550 bp was obtained by using ITS1/ITS4 primer and a product fragment size with ACT738/ACT582 primer was approximately 230 bp. Based on the BLAST search of the ITS region (ITS1–5.8 s rDNA-ITS2), the result showed that the species were consistent with *C. gloeosporioides*, *C. siamese*, *C. capsici*, *C. truncatum*, *C. dematium*, *C. acutatum*, *C. scoville* and *Colleotrichum* sp. from GenBank with 98–100% nucleotide sequence identity.

Then, ITS sequences of 48 isolates of *Colletotrichum* species including reference sequences and out-group species were used for phylogenetic tree construction. The phylogenetic tree was clearly separated into three main clades by using NJ. All clades were separated from the out-group, *Bipolaris maydis* (CBS 136.29). These clades were

C. capsici (Cc) clade, *C. gloeosporioides* (Cg) clade, and *C. acutatum* (Ca) clade (Fig. 4). Of the collected species 50% were in the Ca clade, followed by Cg and Cc with 20% and 30%, respectively. The Cg clade was separated into 2 sub-groups, A and B. In the Cc clade, there were 2 sub-groups: sub-groups C and D. A further phylogenetic tree was performed by using coding region sequences (partial actin gene). The tree was separated into 3 main clades as Cc, Cg and Ca (Fig. 5). In the Ca clade, there were 2 sub-clades (A and B). The Cc clade was divided into 2 groups as sub-groups C and D. A combined dataset analysis with NJ divided the isolates into 3 main clades representing the species: *C. capsici*, *C. gloeosporioides* and *C. acutatum* (Fig. 6). In the Cc clade, it showed 2 sub-groups with 100% bootstrap support. The Cg clade was divided into 2 groups as sub-groups C and D. In the Ca clade, there were 2 sub-groups, E and F.

Pathogenicity of *Colletotrichum* spp.

Colletotrichum isolates CC9.4, NAN1603, NAN1103, and CN4 were selected from *C. capsici*, *C. gloeosporioides*, and *C. acutatum* clades, and the *C. siamense* sub-clade, respectively, in order to test their pathogenicity. Clearly visible anthracnose symptoms developed 5 days after inoculating with *C. acutatum* and 7 days after inoculating with *C. capsici*, *C. gloeosporioides* and *C. siamense* (Fig. 7). The anthracnose symptoms typically appeared as sunken lesions at the inoculation area. The symptoms caused by *C. gloeosporioides* showed black sunken lesions (Fig. 7a). The necrotic spots of *C. siamense* were orange and surrounded with brown-black necrotic tissue (Fig. 7b). The symptom lesions of *C. acutatum* and *C. capsici* produced similar sunken lesions but fruiting structures on the center of the lesions were orange and black in color, respectively (Fig. 7c and d).

Discussion

Analysis of phylogeny of partial actin (*ACT*) gene and combined dataset (ITS + *ACT*) was compared with the phylogeny of ITS region. The phylogram showed that phylogenies could separate the collected isolates into three main groups, Cc, Cg, and Ca.

In the analysis of *Colletotrichum* isolates from strawberry in Korea (Nam et al. 2013), *C. fruticola* which was included in the *C. gloeosporioides* complex was clearly distinguished from the Cg group by using a combination of ITS, partial actin, and GAPDH genes in analysis. This study suggested that the phylogenetic analysis with combined genes was useful for an accurate identification of different species of *Colletotrichum*. In the classification of *Rhizoctonia* spp. by SNPs, the use of ß-actin gene could display more allele discriminations than rDNA-ITS, while the use of rDNA-ITS sequences was not sufficient to distinguish the isolates within the subgroups (Wei et al. 2014).

In this study, the combined nucleotide sequence data (ITS+ *ACT*) and ITS sequence clearly divided the *C. gloeosporioides* group into two molecular groups which were correlated to the colony characteristics. The colony characteristic of the first group was white to light gray mycelia with orange conidia mass. In the first sub-group isolate, CN4 was closely related to *C. siamense* (ICMP 18575) with 73% and 89% bootstrap support in the combined analysis and ITS region analysis, respectively. *C. siamense* was reported in *Capsicum annuum* in Thailand by Weir et al. (2012). This species is a member of the *C. gloeosporioides* species complex. Meetum et al. (2015) reported that *C. siamense* was found in dragon fruits in western and central parts of Thailand, and the pathogen was identified based on morphological characteristics and ITS nucleotide sequence. In the second sub-group, Cg, the colony characteristic was gray to dark gray mycelium with an orange conidial mass. The isolate members in this group were found

Fig. 7 Pathogenicity test of representative isolates of each *Colletotrichum* species showing the disease symptoms on chili fruits. **a** *C. gloeosporioides* isolate NAN1603, **b** *C. siamense* isolate CN4, **c** *C. acutatum* isolate NAN1103 and **d** *C. capsici* isolate (CC9.4)

Table 1 *Colletotrichum* isolates from different locations in 12 provinces of Thailand in the years 2010–2015

Species	Isolate code	Location	Region	Collection year	Accession no.	
					Act	ITS
C.acutatum	BK94_3	Bangkok	C	2010	LC170514	LC169648
C.acutatum	Tr3	Trat	E	2011	LC170509	LC169643
C.acutatum	KSS503	Nakhon Ratchasima	NE	2012	LC170512	LC169646
C.acutatum	NAN1103	Nan	N	2013	LC170496	LC169629
C.acutatum	NR1	Nakhon Ratchasima	NE	2014	LC170519	LC169653
C.acutatum	NR3	Nakhon Ratchasima	NE	2014	LC170520	LC169655
C.acutatum	NR5	Nakhon Ratchasima	NE	2014	LC170521	LC169656
C.acutatum	NR6	Nakhon Ratchasima	NE	2014	LC170522	LC169657
C.acutatum	NR7	Nakhon Ratchasima	NE	2014	LC170523	LC169658
C.acutatum	NR8	Nakhon Ratchasima	NE	2014	LC170524	LC169659
C.acutatum	NR9	Nakhon Ratchasima	NE	2014	LC170525	LC169660
C.acutatum	NR10	Nakhon Ratchasima	NE	2014	LC170526	LC169661
C.acutatum	NR12	Nakhon Ratchasima	NE	2014	LC170527	LC169662
C.acutatum	NR13	Nakhon Ratchasima	NE	2014	LC170528	LC170541
C.acutatum	NR14	Nakhon Ratchasima	NE	2014	LC170529	LC169663
C.acutatum	PR249	Phrae	N	2015	LC170530	LC169664
C.acutatum	PR250	Phrae	N	2015	LC170531	LC169665
C.acutatum	PR251	Phrae	N	2015	LC170532	LC169666
C.acutatum	PR253	Phrae	N	2015	LC170533	LC169667
C.acutatum	PR254_1	Phrae	N	2015	LC170534	LC169668
C.acutatum	PR259	Phrae	N	2015	LC170536	LC169669
C.acutatum	PR262	Phrae	N	2015	LC170538	LC169670
C.acutatum	PR264	Phrae	N	2015	LC170539	LC169671
C.acutatum	PR266	Phrae	N	2015	LC170540	LC169672
C.capsici	CC9–4	Bangkok	C	2010	LC170499	LC169632
C.capsici	CN2	Chainat	C	2012	LC170500	LC169633
C.capsici	KSS706	Nakhon Ratchasima	NE	2012	LC170511	LC169645
C.capsici	PHB1–3	Phetchabun	W	2012	LC170518	LC169652
C.capsici	PHB74	Phetchabun	W	2012	LC177749	LC177750
C.capsici	KJN002	Kanchanaburi	W	2013	LC170517	LC169651
C.capsici	NAN1305	NAN	N	2013	LC170501	LC169635
C.capsici	NAN2101_1	NAN	N	2013	LC170502	LC169636
C.capsici	PSN1001_1	Phitsanulok	N	2013	LC170508	LC169642
C.capsici	CC1201_1	Bangkok	C	2014	LC170505	LC169639
C.capsici	CC1702	Bangkok	C	2014	LC170507	LC169641
C.capsici	Ccm	Chiang Mai	N	2014	LC170515	LC169649
C.capsici	PR254	Phrae	N	2015	LC170535	LC152972
C.capsici	PR261	Phrae	N	2015	LC170537	LC169673
C.gloeosporoides	KSS1	Nakhon Ratchasima	NE	2012	LC170510	LC169644
C.gloeosporoides	KSS2_2	Nakhon Ratchasima	NE	2012	LC170497	LC169630
C.gloeosporoides	KSS3_2	Nakhon Ratchasima	NE	2012	LC170493	LC169626
C.gloeosporoides	NAN1603	Nan	N	2013	LC170495	LC169628
C.gloeosporoides	NAN2002_2	Nan	N	2013	LC170494	LC169627
C.gloeosporoides	NAN2202_1	Nan	N	2013	LC170504	LC169638
C.siamense	KPS2	Nakhon Pathom	W	2010	LC170506	LC169640
C.siamense	PU2	Loei	NE	2012	LC170516	LC169650
C.siamense	KSS104_1	Nakhon Ratchasima	NE	2012	LC170498	LC169631
C.siamense	CN4	Chainat	C	2012	LC170513	LC169647

in various areas of the country where they could cause a serious problem in chili and other crops.

In our phylogenetic analysis, members of the *C. capsici* group were divided into two sub-groups with 100% bootstrap support. The phylogenetic analysis of ITS-DNA sequences showed the relationship between each group and sample collecting areas. Isolate KJN002, the only isolate from the western area of the country, was distinguished from the other *C. capsici* isolates. Based on the phylogenetic analysis of a partial actin and the combined dataset, the isolate members in the first sub-group were from all collection areas. In the other group, the samples were mainly from northern part of Thailand, and no isolate was obtained from the western part of the country. The morphological characteristics of the isolate members of both sub-groups were not different.

ITS region phylogenetic analysis of *C. acutatum* indicated that all isolates in this group were formed in a monophyletic group, whereas the analysis of partial actin and combined data

exhibited some variation. In the analysis, the *C. scovilli* (CBS 120708) was used as a reference nucleotide sequence. The colony characteristics of isolate members in the *C. acutatum* group were similar, and some isolates produced fusiform conidia.

Based on these results, the use of ITS region phylogenetic analysis was not able to distinguish a group of isolates based on the collecting locations. Otherwise, combined (*ACT* and ITS) sequence analysis, as indicated in the Cc clade of the tree, was successful to discriminate this group. However, the discrimination in Cc clade did not correlate with morphological characteristics, but it was shown to be related to the sample collecting area. In many previous studies, the ITS region had been broadly used to define the *Colletotrichum* species due to its ability to separate isolates belonging to different species. However, in the work of Crouch et al. (2009), in which the group of *Colletotrichum* isolates with falcate conidia were distinguished from other closely related species, the ITS region alone was inadequate to separate them. Application of multi-gene or multi-locus phylogenetic analyses with ITS sequences was required for reliable analysis (Du et al. 2005). The use of the actin region, the coding sequence, has been suggested in fungal phylogenetic analysis. This gene encodes conserved actin protein found in all eukaryotic cells and a single copy in the majority of fungi tested. The gene has been used to study evolutionary relationships. In addition, it is easy to amplify and align the sequences (Daniel and Meyer 2003; Reeb et al. 2004). This is good for studying the relationships and variation in the species. In the study of Du et al. (2005), using mating-type gene sequences for resolving the *Colletotrichum* species complex, indicated that MAT1–2 mating type sequences provided many more alignable variable sites than rDNA regions, and the ITS2 sequence has low complexity.

In this study, the phylogenetic analyses using combined sequences of the ACT gene and ITS region were successful to resolve the species complex of *C. gloeosporioides*, the isolates of Thailand, since distinct phylogenetic groups were shown to be associated with their recognizable morphology groups. However, this combined multilocus analysis partially supported the morphological distinct groups in *C. capsici* and *C. acutatum*. Therefore, other DNA barcodes need to be further investigated.

In summary, this study demonstrates that four *Colletotrichum* species can cause chili anthracnose in Thailand, based on morphological characteristics, DNA sequence data of 48 *Colletotrichum* isolates from anthracnose lesions on chili fruit, and additional pathogenicity tests (Table 1).

Acknowledgements In this work, we wish to acknowledge the financial support from the Thailand Research Fund (TRF) through the Royal Golden Jubilee (RGJ) Ph.D. program (Grant No. PHD/0158/2551). The authors acknowledge the Department of Plant Pathology, Kasetsart University for laboratory facilities and the cooperation of the many Thailand chili growers who participated in this study.

References

Alexander PJ, Rajanikanth G, Bacon CD, Bailey CD (2007) Recovery of plant DNA using a reciprocating and silica-based columns. Mol Ecol Resour 7:5–9

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91:553–556

Crouch JA, Clarke BB, Hillman BI (2009) What is the value of ITS sequence data in *Colletotrichum* systematics and species diagnosis? A case study using the falcate-spored graminicolous *Colletotrichum* group. Mycologia 101:648–656

Daniel HM, Meyer W (2003) Evolution of ribosomal RNA and actin gene sequences for the identification of ascomycetous yeasts. Int. J. Food Microbiol 86:61–78

Du M, Schardl CL, Nuckles EM, Vaillancourt LJ (2005) Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexs. Mycologia 97(3):641–658

Hong JK, Hwang BK (1998) Influence of inoculum density, wetness duration, plant age, inoculation method, and cultivar resistance on infection of pepper plants by *Colletotrichum coccodes*. Plant Dis 82: 1079–1083

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Ivery MLL, Diaz CN, Miller SA (2004) Identification and management of *Colletotrichum acutatum* on immature bell peppers. Plant Dis 88: 1198–1204

Johnston PR, Jones D (1997) Relationship among *Colletotrichum* isolates from fruit rot assessed using rDNA sequences. Mycologia 89:420–430

Kim JT, Park SY, Choi W, Lee YH, Kim HT (2008) Characterization of *Colletotrichum* isolates causing anthracnose of pepper in Korea. Plant Pathol J 24:17–23

Manandhar JB, Hartman GL, Wang TC (1995) Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Dis 79:380–383

Meetum P, Leksomboon C, Kanjanamaneesathian M (2015) First report of *Colletotrichum aenigma* and *C.simense*, the causal agents of anthracnose disease of dragon fruit in Thailand. J. Plant Pathol 97(2): 391–403

Nam MH, Park MS, Lee HD, Yu SH (2013) Taxonomic re-evaluation of *Colletotrichum gloeosporioides* isolated from strawberry in Korea. Plant Pathol J 29:317–322

Reeb V, Lutzoni F, Roux C (2004) Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. Mol Phylogenet Evol 32:1036–1060

Sharma PN, Sharma M, Sharma OP, Pathania A (2005) Morphological pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chilli in the subtropical region of north-western India. J Phytopathol 153(4):232–237

Sutton BC (1980) The Coelomycetes: fungi imperfect with pycnidia acervuli and stromata. Commonwealth Mycological Institute, Kew

Sutton BC (1992) The genus *Glomorella* and its anamorph *Colletotrichum*. In: Bailey JA, Jeger MJ (eds) *Colletotrichum*: biology pathology and control. CAB International, Wallingford, pp 1–26

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum

likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Than PP, Jeewon R, Hyde KD, Pongsupasamit S, Mongkolporn O, Taylor PWJ (2008) Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chili (*Capsicum* spp.) in Thailand. Plant Pathol 57:562–572

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Wei Y, Boa J, Cao H, Zhai J, Jantasuriyarat C, Zuo S, Pan X, Wang H, Zhou B (2014) Haplotype variation and phylogeography of *Rhizoctonia solani* AG1-IA strains based on rDNA5.8S-ITS and ß-actin gene sequence analyses. Mycol Progress 13:247–255

Weir BS, Johnston PR, Damm U (2012) The *Colletotrichum gloeosporioides* species complex. Stud Mycol 73:113–180

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sininsky JJ, White TJ (eds) PCR Protocols. Academic, San Diego, pp 315–322