AGRIVITA Journal of Agricultural Science. 2022. 44(2): 312-321

AGRIVITA
Journal of Agricultural Science
www.agrivita.ub.ac.id

Description and Pathogenicity of Colletotrichum Species Causing Chili Anthracnose in Yogyakarta, Indonesia

Rosa Chryse Sutomo[1)], Siti Subandiyah[1,2)], Arif Wibowo[2)] and Ani Widiastuti [1,2*)]

[1)] The Graduate School of Universitas Gadjah Mada, Yogyakarta, Indonesia 55281
[2)] Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281

ARTICLE INFO

Keywords:
Appresoria
ChEC3
GADPH
Polyphasic Approach

Article History:
Received: February 14, 2022
Accepted: June 6, 2022

*) Corresponding author:
E-mail:aniwidiastuti@ugm.ac.id

ABSTRACT

Chili anthracnose caused by *Colletotrichum* spp. is a significant disease of chili cultivation in Indonesia. The current taxonomic status of *Colletotrichum* spp. has been rearranged due to a well-developed molecular study. Several species of *Colletotrichum* were re-identified after being analyzed by a polyphasic approach. The polyphasic method in this research combines morpho-cultural and molecular identification using ITS-rDNA and GADPH region. The pathogenicity test would be more reliable in identifying the species complexes of Colletotrichum that are difficult to differentiate. This study applies a polyphasic approach to identify *Colletotrichum* species causing chili anthracnose, especially in fruits collected from chili fields in Yogyakarta, Indonesia, and observe its pathogenicity in wounded and unwounded inoculation. The results of those combination methods showed that isolates collected from chili fields in Yogyakarta were *C. scovillei*. The pathogenicity test revealed that the fungus caused anthracnose disease in wounded and unwounded OR Twist 42 chili fruits. The unwounded infection was confirmed by amplification of the ChEC3 pathogenicity gene 24 h after inoculation, showing that appressoria production may possess an essential role in the unwounded inoculation. This study revealed that *C. scovillei* does not always need a wound for its pathogenicity.

INTRODUCTION

Chili anthracnose caused by *Colletotrichum* spp. is a significant disease of chili cultivation in Indonesia. Several species of *Colletotrichum* are associated with chili anthracnose in Indonesia, especially *C. gloeosporioides and C. acutatum.* (Damm, Cannon, Woudenberg, & Crous, 2012; Ibrahim, Hidayat, & Widodo, 2017; Voorrips, Finkers, Sanjaya, & Groenwold, 2004). Both significantly cause yield loss in chili varying from 20-90%. Disease symptoms on fruits are similar, generally indicated by circular concave spots resembling concentric rings of reddish-brown color, with a light brown core and dark brown to blackish edges. These spots have a diameter of about 2-8 mm. Sometimes the mass of fungal spores is white, pink, or orange to black and is formed from concentric rings on the surface of the injured tissue (Gautam, Avasthi, & Bhadauria, 2012).

Despite its importance, a recent study reveals that the current taxonomic status of *C. gloeosporioides* and *C. acutatum* especially in Southeast Asian regions must be reconfirmed. Many research reported the renewal classification of the *Colletotrichum* spp. species complex, including the *C. acutatum* and *C. gloeosporioides* complexes. This classification update is due to the sequence similarity reaching 99%, especially when molecular identification is only carried out using Internal Transcribed Spacer (ITS) primers (Mongkolporn & Taylor, 2018). Instead of the ITS region as a single molecular marker, multigene areas are needed to confirm the fungal species complex. Some *C. acutatum* collected from Thailand, and Indonesia

ISSN: 0126-0537 Accredited First Grade by Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Decree No: 30/E/KPT/2018

Cite this as: Sutomo, R. C., Subandiyah, S., Wibowo, A. and Widiastuti, A. (2022). Description and pathogenicity of colletotrichum species causing chili anthracnose in Yogyakarta, Indonesia. *AGRIVITA Journal of Agricultural Science, 44*(2), 312-321. http://doi.org/10.17503/agrivita.v44i2.3705

is reported as a species complex and re-identified as *C. scovillei* and *C. nymphaeae* (Damm, Cannon, Woudenberg, & Crous, 2012).

Similarly, *C. gloeosporioides* become a species complex consisting of *C. siamense, C. fructicola,* and *C. asianum* (Sharma & Shenoy, 2014). Therefore, accurately identifying *Colletotrichum* spp. is challenging for plant protection and biosecurity. Prihastuti, Cai, Chen, McKenzie, & Hyde (2009) suggest some housekeeping genes as multigene markers such as Glyceraldehyde 3-phosphate dehydrogenase (GADPH) housekeeping gene. This gene helps separate the *C. scovillei* species, initially known as *C. acutatum*. de Silva, Ades, Crous, & Taylor (2017) report that *C. scovillei* is not detected in Australia, therefore, it has a vital quarantine pest status.

The polyphasic approach, combining cultural morphology, molecular analysis using ITS-rDNA and GADPH region, and pathogenicity test, will be more reliable in identifying species complexes of *Colletotrichum* spp. (Cai et al., 2009; Cannon, Damm, Johnston, & Weir, 2012; Gautam et al., 2022). This method completes the results of morphological identification, which lead to uncertainty, while the molecular approach using ITS primers cannot differentiate the *C. acutatum* complex species (Oo, Lim, Jang, & Oh, 2017).

Chili (*Capsicum anuum*) var OR Twist 42 produced by Oriental Seed Indonesia is able to grow in the lowlands or highlands (Fahrudin, Basunanda, & Purwantoro, 2013). Due to its high production potential, farmers commonly use this variety in Yogyakarta, Indonesia. However, based on the preliminary survey, anthracnose becomes a significant problem in the variety, reducing its productivity and selling value. Therefore, this variety is used in this study.

In the species complex of Colletotrichum, the formation of appressoria in the pathogenicity process is essential. However, information about genes related to the pathogenicity and appressoria formation of every species complex is limited. The CgDN3 gene is reported as an essential gene responsible for the pathogenicity mechanism of *Colletotrichum gloeosporioides*, which initiates hypersensitivity reactions in host plants (Stephenson, Hatfield, Rusu, Maclean, & Manners, 2000). The mechanism of interaction of CgDN3 protein with the host plant during infection is still not clearly understood. However, based on sequence analysis, it is elucidated that some of the CgDN3 gene products are secreted to initiate penetration of pathogens into plasma membrane surface and play a role as molecular suppressors for hypersensitivity reactions (Oku, Shiraishi, & Ouchi, 1987). In several types of Colletotrichum, there is a CgDN3 gene paralogue that also plays a vital role in the infection process, namely ChECs, which have similarity to CgDN3 by 54% (Kleemann et al., 2012). Kleeman et al. (2012) report the function of some CgDN3 paralogues genes and find that ChECs genes and their homology also play a role in the initiation of appressoria formation in some Colletotrichum species, similarly to the CgDN3 gene. ChEC3 is expressed during cell penetration and early appressoria formation and can initiate cell death in host plants. Therefore, the ChEC3 gene is used in this study to check the gene involved in the early pathogenicity test of Colletotrichum sp by gene expression analysis method and RNA extraction from the chili fruits. This study finally aims to identify and characterize the Colletotrichum species causing chili anthracnose on chili fruits collected from fields in Yogyakarta, Indonesia, using a polyphasic approach. It also examines the ability of the pathogen causing chili anthracnose by wounded and unwounded mechanism and confirms the involvement of the ChEC3 gene in the pathogenicity process.

MATERIALS AND METHODS

Sample Collection and Isolation of Colletotrichum Species

Samples were collected from anthracnose-infected chili fruits (*Capsicum anuum*) at chili cultivation fields in Sleman and Bantul Regency, Yogyakarta, Indonesia, in January 2018. The fruit surface was tapped gently with EtOH 95% in the laboratory and dried in sterilized filter paper. The margin between healthy and infected tissues was cut, isolated in potato dextrose agar (PDA) medium, and incubated at room temperature. After 3-4 days, a single spore isolation technique was applied to get pure cultures (Choi, Hyde, & Ho, 1999).

Molecular Identification

Identification of the complex species isolates was obtained by PCR assay using two sets of primers, which were internal transcribed spacer (ITS-rDNA) and glyceraldehyde-3-phosphate dehydrogenase (GADPH) with primers sequences:

ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') - ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Talhinhas, Sreenivasaprasad, Neves-Martins, & Oliveira, 2005); with annealing temperature at 55°C 30s; and GDF1 (5'- GCCGTCAACGACCCCTTCATTGA-3') - GDR1 (5'-GGGTGGAGTCGTACTTGAGCATGT-3') (Peres, Kuramae, Dias, & De Souza, 2002; Prihastuti, Cai, Chen, McKenzie, & Hyde, 2009) with annealing temperature at 60°C 45s. PCR products were sequenced at PT. Genetika Science Indonesia. The sequencing data were compared with all fungal sequences in the NCBI GenBank database and analyzed by CLUSTALW. The phylogenetic tree was inferred using the maximum likelihood method.

Morphological Characterization

Morphological characterization was conducted by observing colony color, shape, conidia size, and appearances of appressoria at seven days old (Than et al., 2008). Appressoria were produced by slide culture technique, by putting spores on PDA medium on the slide culture and were covered by a cover slip. Appressoria made then were investigated under of microscope.

DNA Extraction

Mycelia from each isolate growth in PDA for seven days were scraped from the surface for DNA extraction. Genomic DNA was extracted using a PrepMan™ Ultra Sample Preparation (Thermo Fisher Scientic Inc., UK) according to the manufacturer's instructions. Quality and DNA concentration was estimated in 1.2% agarose gel by comparing band intensity with a 100 bp DNA ladder (Smobio, Taiwan).

Pathogenicity Test

In this study, fungal isolates were tested for their pathogenicity on OR Twist 42 chili fruits (PT. Oriental Seed Indonesia) collected from the commercial field in Sleman, Yogyakarta, using the wounded and unwounded inoculation methods (Lin, Kanchana-udomkan, Jaunet, & Mongkolporn, 2002).

The wounded inoculation was conducted by small cutting off chili fruit surfaces using a scalpel and then infiltrating 20 µl of conidia suspension (106 conidial/ml) with a syringe. Unwounded inoculation was conducted by infiltrating the conidia suspension directly without any wounds on the upper fruit surfaces. Control fruits were infiltrated with 20 µl of sterilized distilled water onto wounded fruit. All fruits were incubated in a closed sterile box at room temperature for seven days. This experiment was statistically designed as Randomized Complete Block Design (RCBD) with three boxes as a block containing five fruits for each box as fruits replication (a total of 15 fruits for every treatment). The percentage of lesion area was evaluated for seven days by measuring the average length (the longest and the shortest) of the lesion compared to the size of fruit surface times 100%.

Detection of ChEC3 Pathogenicity Gene by Polymerase Chain Reaction (PCR) Method

Detection of the ChEC3 pathogenicity gene was conducted by comparing the amplification of cDNA by PCR method from the wounded and unwounded inoculation on OR Twist 42 chili fruits as the method above. RNA extraction of chili fruits was performed at 0 and 24 hours using RNeasy® Plant Mini Kit (Qiagen, Germany), following the manufacturer's instruction. cDNA reverse transcriptase was conducted using SuperScript™ III (Invitrogen, USA). The primer sequence used in this experiment was pathogenicity gene *ChEC3*F (5'-CCTCCTTCTCGCTCTTCCCT-3') dan *ChEC3*R (5'-GTGTGCTATATTCCACGCCCA-3') (Kleemann et al., 2012). PCR amplification was conducted with 5 minutes at 94°C, 34 cycles of 45 seconds at 94°C, 30 seconds at 55°C,1 minute at 72°C, and a final extention at 7 minutes at 72°C. DNA visualization was performed on 1.2% agarose gel with the target band is 250 bp.

Data Analysis

Data of pathogenicity test was analyzed by Analysis of Variance (ANOVA) with $\alpha = 0.05\%$ and posthoc analysis with Duncan's Multiple Range Test (DMRT) for a significantly different result by using SAS software (SAS Institute, USA).

RESULTS AND DISCUSSION

Anthracnose disease caused by *Colletotrichum* spp. is one of the destructive diseases for chili cultivation, especially during the rainy season. Up to date, several species of Colletotrichum were reported in association with the disease. In Indonesia, *C. gloeosporioides* and *C. acutatum* are two primary causal agents of chili anthracnose. However, a recent issue revealed that the taxonomic status of several species of Colletotrichum needs to confirm. Cannon, Damm,

Johnston, & Weir (2012) and Damm, Cannon, Woudenberg, & Crous (2012) have described that the current taxonomy of *C. gloeosporioides* and *C. acutatum* can not be easily differentiated, especially using morpho-cultural characterization and molecular identification using available primer sets. The current status of *C. gloeosporioides* and *C. acutatum* have become species complex, containing many species in the same clade. It made complex species under *C. gloeosporioides* and *C. acutatum* are revisited to be an improved classification. Novel molecular analysis using polygenes analysis becomes a reliable solution for identifying complex species.

This experiment showed that polygenes markers of ITS region and GADPH housekeeping gene were useful to differ some *Colletotrichum* spp. Based on the concatenated method by combining analysis of DNA sequences using ITS and GADPH gene region, the isolates are identified as *C. scovillei* (Fig. 1), which is in a different line from *C. acutatum* and *C. gloeosporioides*. In the GADPH gene region, the DNA sequences of all isolates had 100% similarities with *C. scovillei* isolate, which are reported by Damm, Cannon, Woudenberg, & Crous (2012). Therefore, it is confirmed that COSLI1016, COBALE1017, and COBAN0118 isolated from chili fruits in Yogyakarta, Indonesia, are *C. scovillei*.

C. scovillei raised its importance in the world as it is written in the CABI's Invasive Species Compendium (CABI, 2021). However, due to its aggressiveness, the distribution is limited in certain areas: East Asia (China, Japan), Shout-east Asia (Indonesia, Philippines), South America, and South Carolina, USA (CABI, 2021; Giacomin et al., 2021; Hsieh et al., 2022). On Chili pepper in Indonesia, it has just reported in Sulawesi except Makasar and West Java (de Silva et al., 2019), Bali (Khalimi, Darmadi, & Suprapta, 2019) and Yogyakarta (Anggrahini, Wibowo, & Subandiyah, 2020). Furthermore, it becomes essential in biosecurity because it has not yet been found in Australia (de Silva, Ades, Crous, & Taylor, 2017). Therefore, proper identification is critical to prevent the broader distribution worldwide and future pathogen management.

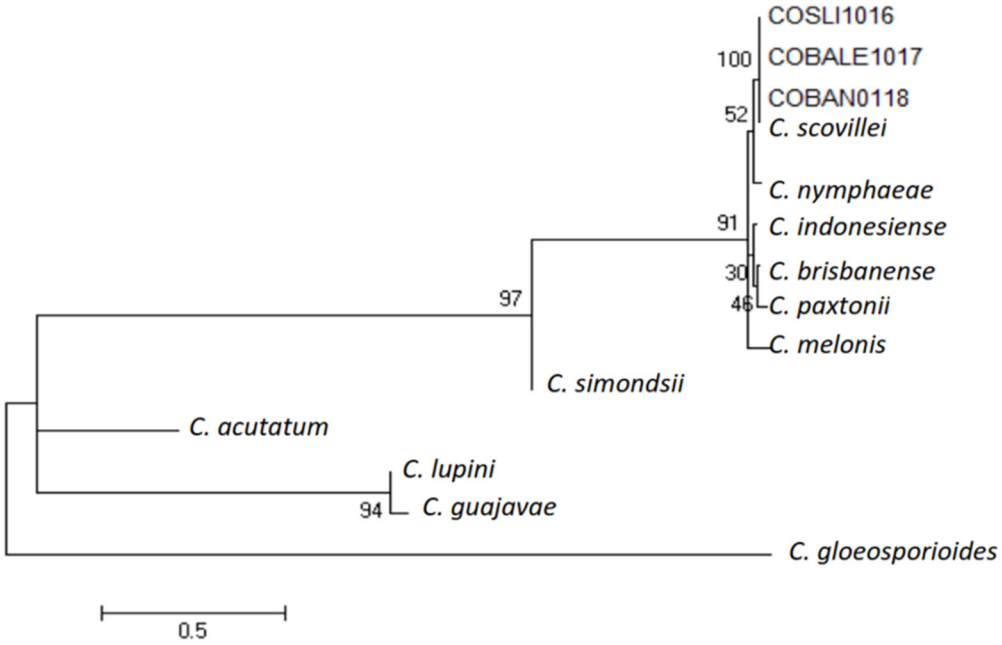

Fig. 1. Phylogenetic tree generated by a maximum likelihood analysis of a combined dataset of internal transcribed spacer (ITS) and glyceraldehyde-3-phosphate dehydrogenase (GADPH) gene sequences of *Colletotrichum scovillei* and those of other related *Colletotrichum* spp. within the *Colletotrichum acutatum* species complex by Damm, Cannon, Woudenberg, & Crous (2012). Number beside each branch represent bootstrap values obtained after a bootstrap test with 1.000 replications. Bar indicates the number of nucleotide substitutions. *Colletotrichum gloeosporioides* served as the out-group

Molecular identification using ITS primer sets alone cannot differentiate the *C. acutatum* complex species. The ITS locus known has lower variability compared to the GADPH region. Sreenivasaprasad & Talhinhas (2005) report that the ITS region is only used to distinguish Colletotrichum species complex, while analysis of the partial GAPDH gene region sequences identifies species within the species complex (de Silva, Ades, Crous, & Taylor, 2017). PCR amplification using multigene primer sets was conducted to confirm the complex species level. PCR amplification of the ITS-rDNA region produced an approximately 576-bp fragment of *Colletotrichum scovillei* using ITS1/ITS4 primer sets. From the BLAST search, the ITS region of all *C. scovillei* isolates had 100% similarities to exact ITS sequences of *C. scovillei, C. acutatum*, and *C. nymphaeae.* Talhinhas & Baroncelli (2021) report that *C. acutatum* and *C. nymphaeae* species are species under *C. acutatum* species complex. Therefore, ITS primer sets could not differentiate the *C. acutatum* complex species. Thus in this research, additional primer sets from GADPH housekeeping gene were combined with ITS primers to confirm the real fungal identity.

Three isolates were obtained from infected fruits collected from Sleman Regency, COSLI1016 and COBALE1017; and an isolate from Bantul regency COBAN0118. Based on colony color, they formed grey to dark grey cottony with dense pale grey aerial mycelium (Fig. 2A and Fig. 2B). Although the earlier colonies showed a few bright orange colors, finally, they showed conidial masses with grey and white bottom color. Conidia produced were hyaline, smooth-walled, aseptate, straight, cylindrical to fusiform, slightly acute, or one end round (Fig. 2C). Based on conidia measurement, the size varied about 11.55–18.5 × 3.0–4.5 µm. Sexual morphology was not observed. Acervuli and chlamydospores were also not observed and there were no setae formed. Appressoria were single or grouped, ovoid to ellipsoidal, dark brown, and measuring about 6.5–10 × 4.2–6.5 µm (Fig. 2D). Morphological characterization could not be used to identify the *Colletotrichum* spp. as this genus has various complex species that cannot be differentiated from their morphology.

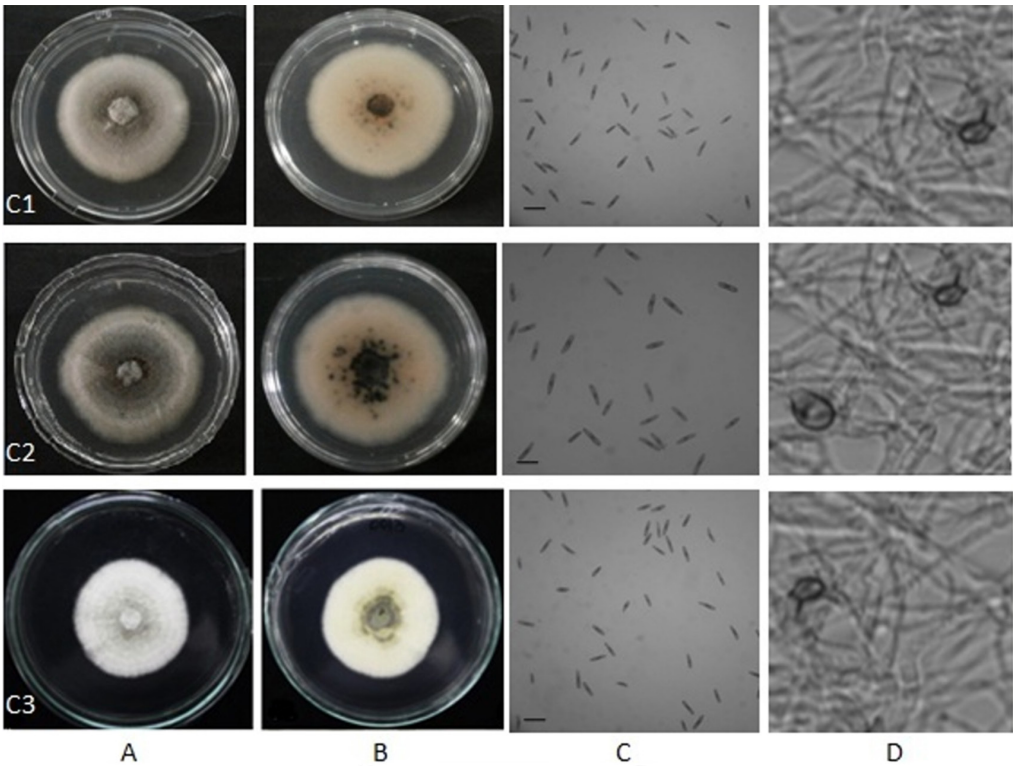

Fig. 2. Variation of cultural characters of *Colletotrichum scovillei* isolates. (C1) COSLI1016; (C2) COBALE1017; (C3) COBAN0118 on PDA 7 days after inoculation, (A) upper; (B) reverse; (C) conidia (bars = 10 µm); (D) appresoria

In the recent genomics and big data era, morphological characterization was conducted to support the molecular result due to the difficulty of distinguishing the fungal species based on the morphological characters, especially the fungi with species complex such as *Colletotrichum* spp. Based on some reports, *C. scovillei* is one of the Colletotrichum species causing chili anthracnose disease previously reported in Japan, Brazil, China, and Korea (Caires et al., 2014; Kanto et. al., 2014; Oo, Lim, Jang, & Oh, 2017; Zhao et al., 2016). Anggrahini, Wibowo, & Subandiyah (2020) and Khalimi, Darmadi, & Suprapta (2019) report that *C. scovillei* have also been collected from Indonesia. Conidia of *C. scovillei* were hyaline, smooth-walled, aseptate, straight, cylindrical to fusiform with both ends slightly acute or one end round, 9–14.5–18–19.5 × 3.5–4.5 µm. Appressoria were single or grouped, ovoid to ellipsoidal, medium or dark brown color, and measured at 6.3 ± 1.2 × 5.6 ± 0.8 µm (Damm, Cannon, Woudenberg, & Crous, 2012). Based on morphology identification, observed conidia and appressoria were included in this range to support the molecular identification result.

Detached fruit assay revealed that all isolates of *C. scovillei* were pathogenic to OR Twist 42 chili fruits via both also wounded unwounded methods (Fig. 3, Table 1). In wounded inoculation, anthracnose lesions grow faster and are earlier visible than unwound in all inoculated fruits (Fig. 3B-3D). Three days after inoculation, anthracnose symptoms have appeared on the surface of inoculated fruits from a wounded method. While in an unwounded way, the sign seems to start five days after inoculation (Fig. 3E-3H). Wounded inoculation showed to accelerate the disease progression. Results in Table 1 show that in the unwounded inoculation, there is no significant difference in the percentage of lesion area among the three isolates. However, COBALE1017 shows a significantly higher lesion area in the wounded inoculation than the two other isolates.

Fig. 3. Pathogenicity of anthracnose on OR Twist 42 chili fruits after *Colletotrichum scovillei* inoculation. Symptom on wounded inoculation after 7 days (A-D) and unwounded inoculation (E-H); control fruits (A,E); COSLI1016 (B, F); COBALE1017(C, G); COBAN0118 (D,H)

Results of the pathogenicity test showed that the wounded inoculation produced a higher disease incidence than the unwounded method. It indicated that the wound accelerated *C. scovillei* to infect chili. However, in unwounded inoculation, *C. scovillei* isolates had no significantly different. Therefore, it noted that *C. scovillei* did not always need any wound for its infection. The result of pathogenicity test indicated that all of *C. scovillei* isolates were pathogenic to OR Twist 42 in both wounded and unwounded inoculation. The result corresponded to the previous study that severe anthracnose pathogen-induced the symptom without requiring open entry points such as a wound. It also showed that OR Twist 42 was a susceptible host for *C. scovillei*. On the other hand, it is noticed that in the wounded inoculation, COBALE1017 produced the highest percentage of lesions significantly different from the other isolates. It showed that fungi could possess different aggressiveness in their infection process among one species, which is very important to study further for their genetic variety and pathogenicity.

Amplifying the ChEC3 gene in both wounded and unwounded infection at 24 h post-inoculation (Fig. 4) confirms that *C. scovillei* probably produced appressorium for initial condition as a physical structure to penetrate fruit cell walls and continue its infection process. In both unwounded inoculation, amplifying the ChEC3 pathogenicity gene at 24 hours post-inoculation showed that *C. scovillei* could produce appressorium as a physical weapon to initiate cell wall penetration and infection. However, it is still unclear why this gene was amplified in the wounded inoculation at 24 hours.

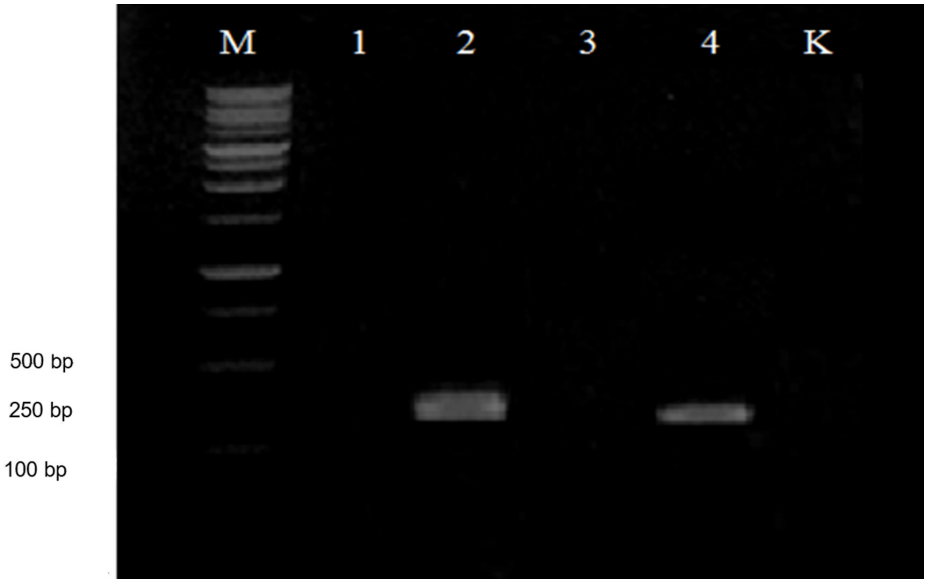

Fig. 4. ChEC3 gene amplification on chili fruits in wounded and unwounded inoculation. Ladder no. 1 and 2: Wounded inoculation, harvested at 0 h (1) and 24 h (2) post inoculation; Ladder no. 3 and 4: Unwounded inoculation, harvested at 0 h (1) and 24 h (2) post inoculation; K: Non-inoculated fruits, harvested at 24 h. M: 100 bp marker ladder

Table 1. Percentage of infection area after inoculation by wounded and unwounded method

Isolates	Subdistrict, Regency	Percentage of infection area (%)	
		Wounded	Unwounded
COSL11016	Kalasan, Sleman	14,6667b	14.6667a
COBAN0118	Bantul, Bantul	14,7333b	14.7333a
COBALE1017	Turi, Sleman	17,8000a	14.7333a

Remarks: [a] Means with the same letters showed not significantly different in every treatment at 0.05 significant level (Duncan Multiple Range Test)

Pathogenicity gene ChEC3 was one of the candidate genes related to appressorium initiation (Kleemann et al., 2012). Detection of the CheC3 pathogenicity gene showed that the CheC3 gene was amplified at 24 hours after inoculation both in wounded and unwounded inoculation. The cDNA band was thicker in the wounded inoculation, indicating that the wound helped fungi to easier infect and transcribe more CheC3 genes into the extracted RNA. Colletotrichum species are well-known plant pathogens, most of which have a 'hemibiotrophic' lifestyle combining a primary, symptomless biotrophic phase with a later necrotrophic phase associated with severe symptoms. It is assumed that appressoria are essential in the penetration hyphae of the biotrophic stage (Kleemann et al., 2012). This study also revealed that the CheC3 gene is possibly involved in *C. scovillei* early infection of pathogenicity. Further research of pathogenicity gene expression in chili-anthracnose is needed to comprehend the plant-pathogen molecular interaction during pathogenicity and disease process.

CONCLUSION AND SUGGESTION

Three isolates collected from chili fields in Yogyakarta, Indonesia, COSLI1016, COBALE1017, and COBAN0118, were identified as *C. scovillei* by ITS and GADPH gene markers. The pathogenicity test against OR Twist 42 chili fruits revealed that *C. scovillei* did not need a wound for its infection and anthracnose disease development. ChEC3 pathogenicity gene probably plays an important role in the initial infection and appressoria formation of *C. scovillei* which needs further comprehensive examination.

ACKNOWLEDGEMENT

This report was a part of master thesis research conducted by Rosa Chryse Sutomo under supervising of the co-authors. Research was financially supported by ACIAR grant (ACIAR-HORT/2009/056) from Australia Government. Authors expressed our sincere gratitude to Prof. Paul Taylor (Univ. of Melbourne, Australia) as expert of Colletotrichum for valuable discussion and technical advice under UGM World Class Professor Project 2017.

REFERENCES

Anggrahini, D. S., Wibowo, A., & Subandiyah, S. (2020). Morphological and molecular identification of *Colletotrichum* spp. associated with chili anthracnose disease in Yogyakarta Region. *Jurnal Perlindungan Tanaman Indonesia, 24*(2), 161–174. https://doi.org/10.22146/jpti.58955

CABI. (2021). *Colletotrichum scovillei*. Invasive Species Compendium Datasheet. Retrieved from https://www.cabi.org/isc/datasheet/121928#toDistributionMaps

Cai, L., Hyde, K. D., Taylor, P. W. J., Weir, B. S., Waller, J. M., Abang, M. M., ... Johnston, P. R. (2009). A polyphasic approach for studying *Colletotrichum*. *Fungal Diversity, 39*, 183–204. Retrieved from https://www.fungaldiversity.org/fdp/sfdp/FD39-8.pdf

Caires, N. P, Pinho, D. B., Souza, J. S. C., Silva, M. A., Lisboa, D. O., Pereira, O. L, & Furtado, G. Q. (2014). First report of anthracnose on pepper fruit caused by *Colletotrichum scovillei* in Brazil. *Plant Disease, 98*(10), 1437. https://doi.org/10.1094/PDIS-04-14-0426-PDN

Cannon, P. F., Damm, U., Johnston, P. R., & Weir, B. S. (2012). *Colletotrichum* –current status and future directions. *Studies in Mycology, 73*(1), 181–213. https://doi.org/10.3114%2Fsim0014

Choi, Y.-W., Hyde, K. D., & Ho, W. W. H. (1999). Single spore isolation of fungi. *Fungal Diversity, 3*, 29–38. Retrieved from https://fungaldiversity.org/fdp/sfdp/FD_3_29-38.pdf

Damm, U., Cannon, P. F., Woudenberg, J. H. C., & Crous, P. W. (2012). The *Colletotrichum acutatum* species complex. *Studies in Mycology, 73*, 37–113. https://doi.org/10.3114/sim0010

de Silva, D. D., Ades, P. K., Crous, P. W., & Taylor, P. W. J. (2017). *Colletotrichum* species associated with chili anthracnose in Australia. *Plant Pathology, 66*(2), 254–267. https://doi.org/10.1111/ppa.12572

de Silva, D. D., Groenewald, J. Z., Crous, P. W., Ades, P. K., Nasruddin A., Mongkolporn, O., & Taylor, P. W. J. (2019). Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. *IMA Fungus, 10*, 8. https://doi.org/10.1186/s43008-019-0001-y

Fahrudin, Basunanda, P., & Purwantoro, A. (2013). True-to-description test of six cultivars of chilli pepper

(*Capsicum annuum* L.). *Ilmu Pertanian, 16*(2), 12-27.

Gautam, A. K., Avasthi, S., & Bhadauria, R. (2012). First report of *Colletotrichum gloeosporioides* on *Pedilanthus tithymaloides* in India. *Journal on New Biological Reports, 1*(1), 3-5. Retrieved from https://www.cabi.org/ISC/abstract/20133068970

Gautam, A. K., Verma, R. K., Avasthi, S., Sushma, Bohra, Y., Devadatha, B., … Suwannarach, N. (2022). Current insight into traditional and modern methods in fungal diversity estimates. *Journal of Fungi, 8*(3), 226. https://doi.org/10.3390/jof8030226

Giacomin, R. M., de Fátima Ruas C., Baba V. Y., De Godoy, S. M., Sudré, C. P., dos Santos Bento C., … Gonçalves, L. S. A. (2021). Phenotypic, molecular and pathogenic characterization of *Colletotrichum scovillei* infecting *Capsicum* species in Rio de Janeiro, Brazil. *PeerJ, 9*, e10782. https://doi.org/10.7717%2Fpeerj.10782

Hsieh, D.-K., Chuang, S.-C., Chen, C.-Y., Chao, Y.-T., Jade Lu, M.-Y., Lee, M.-H., & Shih, M.-C. (2022). Comparative genomics of three *Colletotrichum scovillei* strains and genetic analysis revealed genes involved in fungal growth and virulence on chili pepper. *Frontiers in Microbiology, 13*, 818291. https://doi.org/10.3389/fmicb.2022.818291

Ibrahim, R., Hidayat, S. H., & Widodo. (2017). Keragaman morfologi, genetika, dan patogenisitas *Colletotrichum acutatum* penyebab antraknosa cabai di Jawa dan Sumatera. *Jurnal Fitopatologi Indonesia, 13*(1), 9-16. Retrieved from https://journal.ipb.ac.id/index.php/jfiti/article/view/16359/12022

Kanto, T., Uematsu, S., Tsukamoto, T, Moriwaki, J., Yamagishi, N., Usami, T., & Sato, T. (2014). Anthracnose of sweet pepper caused by *Colletotrichum scovillei* in Japan. *Journal of General Plant Pathology, 80*, 73–78. https://doi.org/10.1007/s10327-013-0496-9

Khalimi, K., Darmadi, A. A. K., & Suprapta, D. N. (2019). First report on the prevalence of *Colletotrichum scovillei* associated with anthracnose on chili pepper in Bali, Indonesia. International Journal of Agriculture and Biology, 22(2), 363-368. Retrieved from http://www.fspublishers.org/published_papers/30385_23%20doi%2015.1072%20IJAB-19-0064%20(6)%20363-368.pdf

Kleemann, J., Rincon-Rivera, L. J., Takahara, H., Neumann, U., van Themaat, E. V. L., van der Does, H. C., … O'Connell, R. J. (2012). Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. *PLoS Pathogens, 8*(8), e1002643. https://doi.org/10.1371/journal.ppat.1002643

Lin, Q., Kanchana-udomkan, C., Jaunet, T., & Mongkolporn, O. (2002). Genetic analysis of the resistance to pepper anthracnose caused by *Colletotrichum capsici*. *Thai Journal of Agricultural Science, 35*(3), 259-264. Retrieved from https://agris.fao.org/agris-search/search.do?recordID=TH2001003551

Mongkolporn, O., & Taylor, P. W. J. (2018). Chili anthracnose: Colletotrichum taxonomy and pathogeniciy: Review. *Plant Pathology, 67*, 1255-1263. https://doi.org/10.1111/ppa.12850

Oku, H., Shiraishi, T., & Ouchi, S. (1987). Role of specific suppressors in pathogenicity of *Mycosphaerella species*. In S. Nishimura, C. P. Vance, & N. Doke (Eds.), *Molecular Determinants of Plant Diseases* (pp. 145-156). SpringerVerlag; Tokyo: Japan Scientific Societies Press. Retrieved from https://www.researchgate.net/publication/292740814_Role_of_specific_suppressors_in_pathogenesis_of_Mycosphaerella_species

Oo, M. M., Lim, G.-T., Jang, H.-A., & Oh, S.-K. (2017). Characterization and pathogenicity of new record of anthracnose on various chili varieties caused by *Colletotrichum scovillei* in Korea. *Mycobiology, 45*(3), 184-191. https://doi.org/10.5941%2FMYCO.2017.45.3.184

Peres, N. A. R., Kuramae, E. E., Dias, M. S. C., & De Souza, N. L. (2002). Identification and characterization of *Colletotrichum* spp. affecting fruit after harvest in Brazil. *Journal of Phytopathology, 150*(3), 128-134. https://doi.org/10.1046/j.1439-0434.2002.00732.x

Prihastuti, H., Cai, L., Chen, H., McKenzie, E. H. C., & Hyde, K. D. (2009). Characterization of *Colletotrichum* species associated with coffee berries in northern Thailand. *Fungal Diversity, 39*, 89-109. Retrieved from https://www.fungaldiversity.org/fdp/sfdp/FD39-4-E.pdf

Sharma, G., & Shenoy, B. D. (2014). *Colletotrichum fructicola* and *C. siamense* are involved in chilli anthracnose in India. *Archives of Phytopathology and Plant Protection, 47*(10), 1179–1194. https://doi.org/10.1080/03235408.2013.833749

Sreenivasaprasad, S., & Talhinhas, P. (2005). Genotypic and phenotypic diversity in

Colletotrichum acutatum, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. *Molecular Plant Pathology, 6*(4), 361–378. https://doi.org/10.1111/j.1364-3703.2005.00291.x

Stephenson, S.-A., Hatfield, J., Rusu, A. G., Maclean, D. J., & Manners, J. M. (2000). *CgDN3*: An essential pathogenicity gene of *Colletotrichum gloeosporioides* necessary to avert a hypersensitive-like response in the host *Stylosanthes guianensis*. *Molecular Plant-Microbe Interactions, 13*(9), 929-941. https://doi.org/10.1094/MPMI.2000.13.9.929

Talhinhas, P., & Baroncelli, R. (2021). *Colletotrichum* species and complexes: geographic distribution, host range and conservation status. *Fungal Diversity, 110*, 109–198. https://doi.org/10.1007/s13225-021-00491-9

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J., & Oliveira, H. (2005). Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology, 71*(6), 2987-2998. https://doi.org/10.1128%2FAEM.71.6.2987-2998.2005

Than, P. P., Jeewon, R., Hyde, K. D., Pongsupasamit, S., Mongkolporn, O., & Taylor, P. W. J. (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology, 57*(3), 562-572. https://doi.org/10.1111/j.1365-3059.2007.01782.x

Voorrips, R. E., Finkers, R., Sanjaya, L., & Groenwold, R. (2004). QTL mapping of anthracnose (*Colletotrichum* spp.) resistance in a cross between *Capsicum annuum* and *C. chinense*. *Theoretical and Applied Genetics, 109*, 1275–1282. https://doi.org/10.1007/s00122-004-1738-1

Zhao, W., Wang, T., Chen, Q. Q, Chi, Y. K, Swe, T. M, & Qi, R. D. (2016). First report of *Colletotrichum scovillei* causing anthracnose fruit rot on pepper in Anhui Province, China. *Plant Disease, 100*(10), 2168. https://doi.org/10.1094/PDIS-04-16-0443-PDN