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First identified *Colletotrichum scovillei* causing anthracnose on chili peppers (*Capsicum annuum L.*) from traditional market in Bandar Lampung, Indonesia

Wawan Abdullah Setiawan *, Yulianty, Wahyu Widianningrum, Martha Lulus Lande and Mohammad Kanedi

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia.

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Abstract

Chili peppers (*Capsicum annuum* L.) was one of the vegetables that have good marketing prospects in Indonesia. However, the disease that was often found in chili plants is anthracnose. Anthracnose disease is usually caused by the fungus, Colletotrichum sp. which can attack from the growing period until harvest. The purpose of this study was to isolate and analyze the phylogenetic fungi of Colletotrichum sp. as a pathogen in chili peppers taken from the traditional market in Bandar Lampung, Lampung, Indonesia. We have isolated the Colletotrichum sp. from chili peppers. That isolate was characterized macroscopically and microscopically, namely W1, which has been identified as Colletotrichum sp. W1 was then analyzed phylogenetically using Internal Transcribed Spacer (ITS) sequence. Molecular Evolution Genetics Analysis (MEGA) software version X was used to analyze the sequence that has obtained. W1 isolate was known as *Colletorichum scovillei* which had been deposited in GeneBank with accession number LC577889.

Keywords: Chili peppers; Antrachnose; *Colletotrichum scovillei;* Market

1. Introduction

The price of chili in Indonesia can increase sharply at certain times. This is due to the high public demand for chili [1]. However, despite the uncertain price, chili remains one of the most important vegetable commodities. Chili peppers gives a spicy taste that can increase appetite for spicy food lovers and also gives a natural red color to food [2].

Various factors can affect the quality of chili yields, one of which is disease. The disease that is commonly found in chili plants in Indonesia is anthracnose. This anthracnose disease generally attacks chili at all stages of growth or at harvest and also during storage. The signs of this disease are small black spots on chilies and in mature plants it can cause shoots to die and continue to the bottom of the plant [3]. Anthracnose disease is caused by the fungus Colletotrichum sp. which can reduce the production and quality of red chili as much as 45-60% [4]. Several Colletotrichum species that have previously been found in Bandar Lampung are *Colletotrichum capsici* and *Colletotrichum gloeosporioides* [5]. The study was conducted to determine whether there are other species of Colletotrichum that cause anthracnose on chili peppers besides the two species in Bandar Lampung. The characterization method used was macroscopic, microscopic, and also phylogenetic analysis using Internal Transcribed Spacer (ITS) DNA sequences [6].

* Corresponding author: Wawan Abdullah Setiawan
Departement of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia.

2. Material and methods

2.1. Isolation of *Colletotrichum sp*. from Chili Peppers at Bandar Lampung Traditional Market

Chili peppers samples were obtained from traditonal markets in Bandar Lampung including the "Way Kandis" market, "Pasar Tengah" marekt, "Rajabasa" market, "Tamin" market, and "Pasir Gintung" market. The direct method [7] was used for the isolation of the fungus Colletotrichum sp. The direct method is to take the sick and healthy parts of the chili. The sections were then placed in a petri dish containing sterile Potato Dextrose Agar (PDA) media and then incubated for 7 days at room temperature to observe fungal growth. The growing fungi were isolated by streak plate method. Isolates were observed macroscopically and microscopically. Macroscopically, the observations included colony shape, color, elevation, and the appearance of conidia on PDA. Microscopically, the hyphae, mycelium, and conidiaspores were observed using binocular microscope type Eclipse E100 from Nikon, Boston, USA.

2.2. Phylogenetic Analysis

After obtaining the isolates which were suspected to be *Colletotrichum* sp. macroscopically and microscopically, the isolates were analyzed phylogenetically. Isolates of *Colletotrichum* sp. grown in Potato Dextrose Broth (PDB) medium for 4 days at room temperature on an orbital shaker OS 150 (Wiggen Hauser, Germany). DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (cat. no. A1120, Promega, USA).

The isolate was transferred into a 1.5 mL tube. Centrifuged at 10,000 rpm at 20°C for 10 minutes. The supernatant was discarded, plus 300 µL of DNA Rehydration Solution. 20 µL Proteinase-K was added and vortexed using Tissue Lyzer LT (Qiagen, Germany) with oscillations 50 times/second for 3 minutes. Samples were incubated in Cooling – Heating dry block CH-100 (Biosan, Georgia) at 55°C for 20 minutes. Every 5 minutes inverted. The supernatant was transferred to a new 1.5 mL tube. It was centrifuged at 13,000 xg for 5 min at 20°C, after which the supernatant was discarded. Added 350 µL of Nuclei Lysis Solution, then inverted and allowed to stand for 2 minutes. Added 125 µL Protein Precipitation Solution and then homogenized with Bio Vortex V1 (Biosan, Georgia) for 30 seconds and allowed to stand at room temperature for 5 minutes. Incubated on ice for 5 minutes. Centrifuged for 5 min at 13,000 xg. The supernatant was transferred to a new 1.5 ml tube containing 500 µL of isopropanol. Centrifuged at 13,000 xg for 5 min at 20°C. The supernatant was discarded, 500 µL of 75% ethanol was added. The tube then inverted slowly. Centrifuged at 13,000 xg for 2 min at 20°C. The ethanol was carefully removed and then allowed to stand for 15 minutes. The DNA was dissolved with 20 µL of DNA Rehydration Solution and then incubated at 65°C for 15 minutes (tapped gently every 5 minutes, then spinned briefly). DNA then stored at 2 – 8°C. DNA that obtained analyzed for purity and concentration using a Pearl® Nanophotometer (Implen, GMBH, Germany) [8].

After the DNA was successfully extracted, PCR was performed [5]. The total reaction volume for each PCR tube is ± 20 L with the following components: 10 µL NEXproTM e PCR 2X Master Mix, 5,5 µL ddH2O, 4 µL DNA template (500 ng/µL). forward primer: 5'-TCCGTAGGTGAACCTGCGG-3 ' was added 0.25 L and 0.25 L reverse primer: 5′-TCCTCCGCTTATTGATATGC-3′. Amplification was carried out with 35 cycles as follows, denaturation for 30 seconds at 95°C, primer annealing for 60 seconds at 54 °C, and polymerization for 90 seconds at 72 °C. The PCR results were electrophoresed following the protocol of the QIAXCEL ADVANCED apparatus (Qiagen, Germany). The PCR results which indicated the amplification were continued for sequencing using the Sanger method through sequencing services from PT. Genetics. The results of the sequencing were analyzed using Molecular Evolution Genetics Analysis (MEGA) software version X [9].

3. Results and discussion

3.1. Isolation of *Colletotrichum sp*. from Chili Pepperss at Bandar Lampung Traditional Market

Through this study, chili peppers were found that had the characteristics of being attacked by anthracnose (Fig. 1). The sample came from Way Kandis market, Bandar Lampung. According to [10], *Colletotrichum* sp. which causes anthracnose on chili peppers has general characteristics, namely, there are small black spots that are slightly curved and the chilies are still fresh, not dry. On the surface and edges of the peppers there are blackish brown spots which then expand to soft rot, enlarge and lengthen, the collection of black dots consists of a group of seta and fungal conidia and can change color to yellow as if exposed to sunlight [11]. The results of fungal isolation from chili indicated anthracnose according to the characteristics of *Colletotrichum* sp. Macroscopically on petri dishes, it was known that the isolates had white colonies both on the top and bottom views. After day 15, the colonies turned orange and conidia were present (Fig. 2), according to [12].

Figure 1 Visualization of chili peppers suspected of being infected with anthracnose

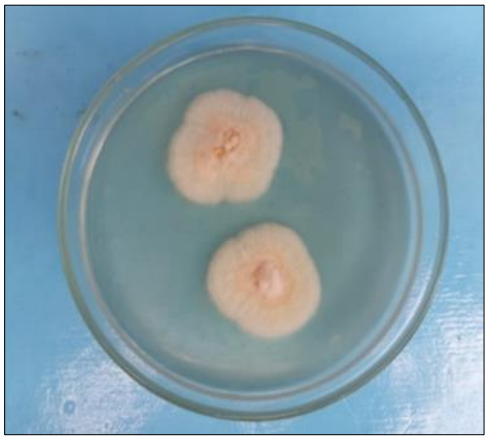

Figure 2 *Colletotrichum* sp. on PDA media aged 15 days

The results of microscopic identification showed that the *Colletotrichum* sp. have insulated and branched hyphae (Fig. 3). These results are in accordance with research by [13], that the genus Colletotrichum have insulated and branched hyphae. They produce transparent and elongated conidia with rounded or tapered ends.

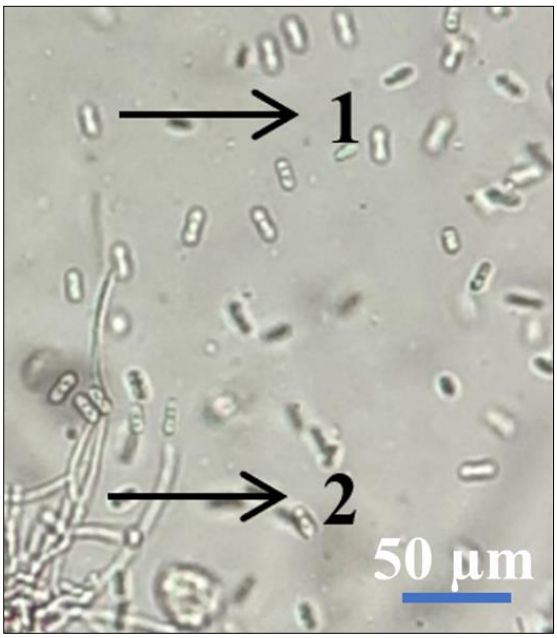

Figure 3 Isolates of *Colletotrichum* sp. microscopically. 1 = conidia. 2 = hyphae: branched and insulated

After DNA extraction, the purity and concentration of DNA from *Colletotrichum* sp. W1 which is presented in Table 1. The DNA can be continued to the PCR stage [14]. The electrophoresis of sample W1 (Fig. 4) where there is a DNA band with a range of ± 600 bp.

Table 1 DNA purity and concentration of *Colletotrichum* sp. W1

Sample	Purity (A_{260}/A_{280})	Concentration (A_{260}/A_{230})
W1	2,030	134 ng/µL

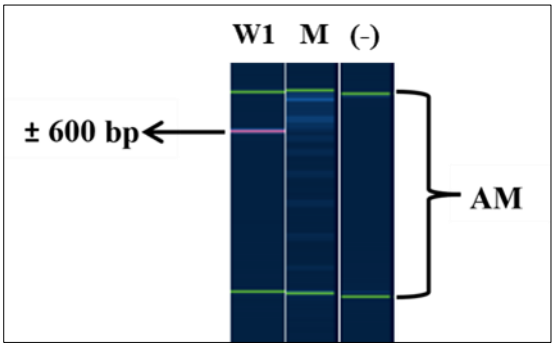

Figure 4 Visualization of PCR Results from W1 isolate. S = W1 isolate, M = size marker 100 bp – 2.500 bp (QIAxcel DNA Size Markers, Qiagen, Germany), (-) = negative control (ddH_2O), AM = alignment marker (QIAxcel DNA Alignment Markers Qiagen, Germany)

The samples that showed the amplified band were then continued for sequencing using the Sanger method. Sequencing was carried out using sequencing analysis services from PT. Genetika Sciences. It is known that the sequence of bases of the sequencing amplicons is as follows:

>W1

ATACCTAACCGTTGCTTCGGCGGGCAGGGGAAGCCTCTCGCGGGCCTCCCCTCCCGGCGC

CGGCCCCCACCACGGGGACGGGGCGCCCGCCGGAGGAAACCAAACTCTATTTACACGACG

TCTCTTCTGAGTGGCACAAGCAAATAATTAAAACTTTTAACAACGGATCTCTTGGTTCTG

GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT

CATCGAATCTTTGAACGCACATTGCGCTCGCCAGCATTCTGGCGAGCATGCCTGTTCGAG

CGTCATTTCAACCCTCAAGCACCGCTTGGTTTTGGGGCCCCACGGCACACGTGGGCCCTT

AAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTAACGTCTCGCACTGG

GATCCGGAGGGACTCTTGCCGTAAAACCCCCAAATTTTTTACAGGTTGACCTCGGATCAG

GTAGGAATACCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAAGATCATTACTGAGTT

ACGCTCT

Phylogenetic tree results using Molecular Evolution Genetics Analysis (MEGA) version X software [8] has shown that isolate W1 was *Colletotrichum scovillei* (Fig. 5). Through MEGA X, it was known that the genetic distance between the isolate and its closest branch, *Colletotrichum scovillei* LD351 (code access LC488857.1) was 0.

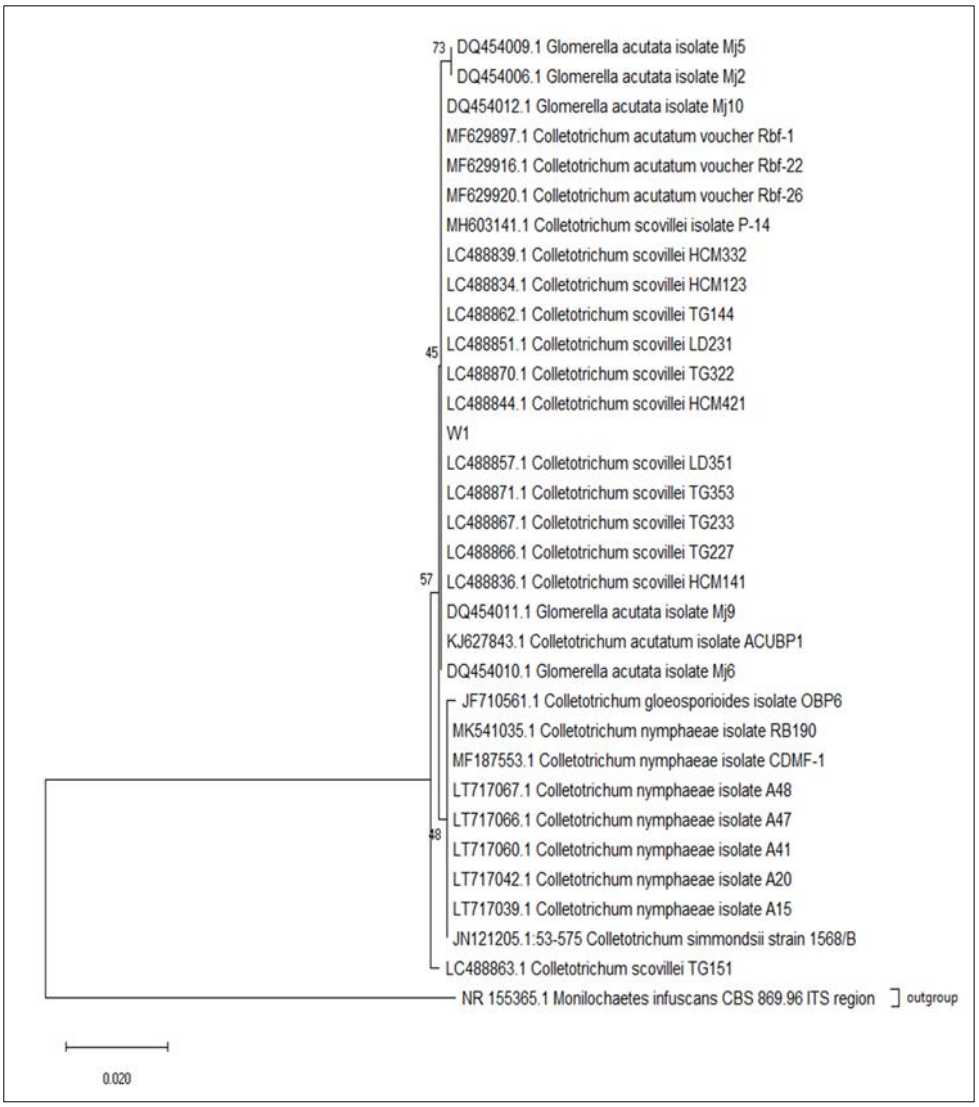

Figure 1 Phylogenetic tree using Neighbor – Joining method and Kimura 2 – Parameter model [15]. *Monilochaetes infusicans* CBS 869.96 ITS region (Accession number NR 155365) was used as an outgroup [16]. Bootstrap values (1000 resamples) are given in percent at the nodes of the tree.

4. Conclusion

Through this research, it is first report that the fungus *Colletotrichum scovillei* has been found as the cause of chili anthracnose at traditional markets in Bandar Lampung, Lampung, Indonesia. The sequencing results have been deposited in Gene Bank as *Colletotrichum scovillei* W1 with accession number LC577889.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No potential conflict of interest relevant to this article was reported.

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