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Identification and Characterization of A Fungal Isolates Associated with Anthracnose Symptoms on Avocado (*Persea americana* Mill.) from West Sumatra

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ABSTRACT

Anthracnose is one of the major postharvest diseases affecting avocado (*Persea americana* Mill.) and is commonly associated with fungi from the genus *Colletotrichum*. However, other fungal genera may also be associated with anthracnose-like symptoms in avocado fruits. This study aimed to identify fungal isolates associated with anthracnose symptoms on avocado fruits collected from West Sumatra using morphological and molecular approaches. Symptomatic fruits were incubated to induce lesion development, followed by fungal isolation and purification on Potato Dextrose Agar (PDA). Morphological characterization included macroscopic and microscopic observations. Molecular identification was performed using LSU rDNA amplification, sequencing, BLAST analysis, and phylogenetic reconstruction. A single fungal isolate, DTTDL318, was successfully obtained from avocado fruit showing anthracnose symptoms. Morphologically, the isolate exhibited characteristics consistent with the genus *Fusarium*, including white cottony colonies with orange reverse pigmentation, septate hyphae, fusiform conidia with pointed ends, and thick-walled chlamydospores. LSU sequence analysis indicated that the isolate belongs to the *Fusarium solani* species complex (FSSC) Phylogenetic analysis demonstrated that isolate DTTDL318 clustered in the same clade with *Fusarium cf. solani* and *Fusarium waltergamsii* with a bootstrap value of 88%, indicating a close phylogenetic relationship with members of the *Fusarium solani* species complex (FSSC). These findings indicate the presence of a Fusarium isolate associated with anthracnose-like lesions on avocado fruits.

Keywords: Avocado, Anthracnose, *Fusarium*, Morphological, Molecular

ABSTRAK

Antraknosa merupakan salah satu penyakit pascapanen utama yang menyerang alpukat (Persea americana Mill.) dan umumnya dikaitkan dengan jamur dari genus Colletotrichum. Namun, genus jamur lain juga dapat berasosiasi dengan gejala mirip antraknosa pada buah alpukat. Penelitian ini bertujuan untuk mengidentifikasi isolat jamur yang berasosiasi dengan gejala antraknosa pada buah alpukat yang dikoleksi dari Sumatera Barat menggunakan pendekatan morfologi dan molekuler. Buah bergejala diinkubasi untuk menginduksi perkembangan lesi, kemudian dilakukan isolasi dan pemurnian jamur pada media Potato Dextrose Agar (PDA). Karakterisasi morfologi meliputi pengamatan makroskopis dan mikroskopis. Identifikasi molekuler dilakukan melalui amplifikasi gen LSU rDNA, sekuensing, analisis BLAST, dan rekonstruksi filogenetik. Satu isolat jamur, yaitu DTTDL318, berhasil diperoleh dari buah alpukat yang menunjukkan gejala antraknosa. Secara morfologi, isolat tersebut menunjukkan karakteristik yang sesuai dengan genus Fusarium, meliputi koloni putih seperti kapas dengan pigmentasi jingga pada bagian balik koloni, hifa bersekat, konidia berbentuk fusiform dengan ujung meruncing, serta klamidospora berdinding tebal. Analisis sekuens LSU menunjukkan bahwa isolat tersebut termasuk ke dalam kompleks spesies Fusarium solani (Fusarium solani species complex/FSSC). Analisis filogenetik menunjukkan bahwa isolat DTTDL318 berkelompok dalam klad

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yang sama dengan Fusarium cf. solani dan Fusarium waltergamsii dengan nilai bootstrap sebesar 88%, yang mengindikasikan adanya hubungan filogenetik yang dekat dengan anggota kompleks spesies Fusarium solani (FSSC). Temuan ini menunjukkan keberadaan isolat Fusarium yang berasosiasi dengan lesi menyerupai antraknosa pada buah alpukat.

Kata kunci: Alpukat, Antraknosa, Fusarium, Morfologi, Molekuler

INTRODUCTION

Avocado (*Persea americana* Mill.) is a tropical–subtropical fruit crop native to Mexico and Central America that has been cultivated and consumed for more than 8,000 years (Widianti et al., 2022). Avocado fruit is rich in nutrients and bioactive compounds beneficial to health, including the pulp, seed, and peel. The pulp contains carbohydrates, fats, protein, fiber, vitamins (A, C, K, and B-complex), minerals (potassium, magnesium, copper), as well as bioactive compounds such as carotenoids, tocopherols, and phenolics (Marsigit, 2016). Due to its high nutritional value, avocado has become an economically important horticultural commodity with increasing consumer demand. However, postharvest diseases, particularly fungal infections, pose a significant threat to fruit quality, shelf life, and market value, resulting in substantial economic losses.

Anthracnose is one of the major diseases affecting avocado. This disease is airborne and can infect various plant types, including trees, fruits, grasses, and ornamental plants (Mayadianti et al., 2020). Anthracnose damages leaves, stems, and fruits during both preharvest and postharvest stages (Jayawardena et al., 2021). In avocado, infection may occur on leaves, flowers, and fruits, especially under wet and humid environmental conditions that favor pathogen development. On fruits, symptoms are characterized by circular dark brown to black moist lesions, followed by sunken necrotic spots and soft rot that may exude liquid. Orange conidial masses and hyphal growth on the fruit surface further deteriorate the external appearance, reducing commercial quality (Kwon et al., 2020).

Although anthracnose in avocado is commonly associated with fungi from the genus *Colletotrichum*, several studies have also reported the presence of species from the genus *Fusarium* associated with avocado fruit decay. As plant pathogens, *Fusarium* species infect both aboveground and belowground plant tissues, functioning as either primary or secondary pathogens. These fungi produce conidia that can disperse through air currents, rain splash, and irrigation water. In addition, chlamydospores produced by some species can persist in soil and plant debris for extended periods, serving as long-term inoculum sources (Zakaria, 2023). Their efficient dispersal mechanisms and high environmental persistence enable *Fusarium* species to adapt to diverse hosts and plant tissues, including fruit tissues.

Nevertheless, reports regarding the involvement of Fusarium spp. in avocado fruit disease symptoms remain limited and have not been extensively explored. Most previous studies on avocado anthracnose have focused primarily on Colletotrichum species, whereas information regarding the diversity, taxonomic identity, and phylogenetic relationships of Fusarium isolates associated with anthracnose-like lesions is still scarce. Consequently, the contribution of Fusarium spp. to avocado fruit disease development remains poorly understood. Therefore, identifying fungi associated with anthracnose symptoms in avocado fruits is essential to broaden understanding of postharvest fungal diversity and their potential contribution to disease development.

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MATERIALS AND METHODS

Time and Place

This study was conducted from December 2025 to March 2026 at the Sumatra Biota Research Laboratory, Andalas University, Padang, Indonesia. A survey method was used for sample collection, while laboratory analyses were conducted for morphological characterization and genetic profiling of fungal isolates. Field sampling was carried out using a purposive sampling technique.

Sample Collection

Mature avocado fruits were collected from Limo Kaum District, Tanah Datar Regency, West Sumatra. The fruit samples were cleaned from debris using sterile gauze and sprayed with 70% alcohol over the entire fruit surface. The fruits were then placed in clean plastic containers lined with moist tissue paper, sealed, and incubated for approximately 7 days at room temperature until anthracnose-like lesions appeared.

Isolation and Purification

Incubated fruits showing expanding dark brown to black lesions were selected for fungal isolation. The fruit surface was sterilized by wiping the peel with tissue moistened with 70% alcohol. Fruit peel sections (±0.5 cm^2) were excised from the boundary between healthy and infected tissues, then inoculated onto Petri dishes containing Potato Dextrose Agar (PDA) medium and incubated for 7 days at room temperature. The obtained fungal isolates were subsequently purified on fresh PDA medium for further analysis (Huda et al., 2019).

Morphological Characterization

Morphological characterization included both macroscopic and microscopic observations. Macroscopic characteristics were assessed visually by examining colony shape, colony surface, and colony color. Microscopic observations focused on hyphal morphology, conidial shape, and conidial size. Microscopic examination was performed using a trinocular microscope at 40× magnification.

Molecular Characterization

DNA was extracted from fungal mycelia grown in Potato Dextrose Broth (PDB) for 5–7 days at room temperature using the Quick-DNA Fungal/Bacterial Miniprep Kit according to the manufacturer's protocol. Approximately 50 mg of fungal biomass was transferred into a lysis tube containing DNA Shield, homogenized, and centrifuged. The supernatant was transferred, mixed with MagBinding Buffer, and incubated. Samples were washed, incubated, and DNA was eluted using DNA Elution Buffer. The extracted DNA was used as the PCR template (Sukapiring et al., 2024).

DNA amplification was performed by PCR in a total reaction volume of 25 µL consisting of GoTaq Green Master Mix, nuclease-free water, 1 µL each of LSU2 forward primer and LSU9 reverse primers, and 2 µL of DNA template. PCR conditions included initial denaturation at 95°C for 90 s, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 90 s, and a final extension at 72°C for 5 min (Hidayat et al., 2016).

PCR products were visualized by electrophoresis on 1.2% agarose gel. Samples and DNA marker were loaded into gel wells and electrophoresed at 100 V for 30 min. The gel was stained with ethidium bromide, rinsed, and visualized using a gel documentation system (Hasyyati et al., 2017).

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PCR products were purified and sequenced using the Sanger sequencing method. Sequence data were edited and analyzed using MEGA software. Sequence identity was confirmed using BLASTn against the GenBank database. Phylogenetic analysis was performed using the Neighbor-Joining method with bootstrap testing of 1000 replications (Haque et al., 2020).

Data Analysis

Data analysis was conducted descriptively based on the morphological and molecular characteristics of fungal isolates associated with anthracnose symptoms on avocado fruit (*Persea americana* Mill.) collected from West Sumatra.

RESULTS AND DISCUSSION

A single fungal isolate was successfully obtained from avocado fruit (*Persea americana* Mill.) showing anthracnose symptoms, indicating its occurrence in tissues showing anthracnose-like symptoms. Colonies grown on Potato Dextrose Agar (PDA) exhibited macroscopic characteristics including a white colony surface with an orange reverse side, cottony texture, smooth to slightly filamentous margins, and a circular colony shape with relatively symmetrical radial growth. The morphological characteristics of isolate DTTDL318 are shown in Figure 1.

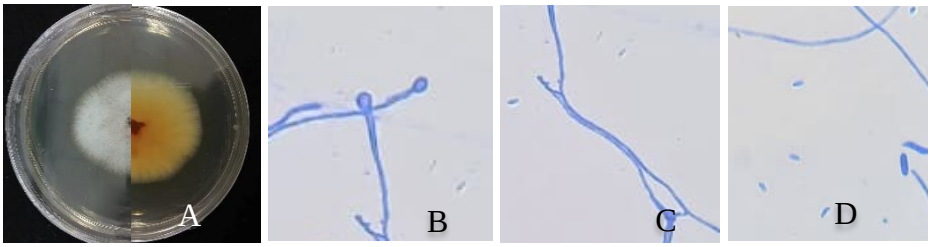

Figure 1. Morphology of isolate DTTDL318: (a) colony on PDA medium, (b) chlamydospores, (c) hyphae, and (d) conidia.

Microscopic observation revealed the presence of septate hyphae. The isolate produced elongated to curved (fusiform) conidia with pointed ends. Chlamydospores were also observed as thick-walled spherical structures. In accordance with James et al. (2022), the morphological characteristics of the isolate were consistent with the genus *Fusarium*, characterized by white colonies that change over time, septate hyphae, oval to reniform microconidia, and sickle-shaped macroconidia with several septa. Furthermore, the presence of thick-walled globose chlamydospores further supports that the isolate belongs to the *Fusarium* group, although morphological characteristics alone are insufficient for species-level identification.

Molecular identification based on LSU rDNA sequences showed that isolate DTTDL318 had a fragment length of approximately ±830 bp (Figure 2). The DNA quantification results (Table 1) showed that the DNA concentration of isolate DTTDL318 was 48.3 ng/µL with a purity ratio (A260/280) of 1.94, indicating good DNA quality suitable for PCR amplification.

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Table 1. DNA quantification using NanoDrop

Sample	Nucleic Acid Conc.	Unit	260/280
DTTDL318	48.3	ng/µl	1.94

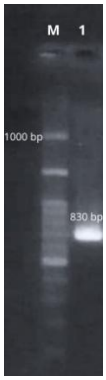

Figure 2. Electrophoresis results of isolate DTTDL318.

The obtained sequence was analyzed using BLAST against the NCBI database. The BLAST results (Table 2) showed 100% query coverage, 100% identity, and an E-value of 0.0 with *Fusarium cf. solani.* This high level of similarity indicates that the isolate belongs to the genus *Fusarium* and has a very close phylogenetic relationship with the *Fusarium solani* species complex (FSSC).

Table 2. BLAST results of isolate DTTDL318.

Sampel	Accession No.	Description	Max score	Total score	Query coverage	E value	Max ident
DTTDL318	MG.189917.1	*Fusarium cf. solani*	1264	1264	100%	0,0	100,00%

The *Fusarium solani* species complex (FSSC) is a group of filamentous fungi widely distributed worldwide and commonly associated with many economically important agricultural crops.In general, FSSC has a broad host range, encompassing more than 100 plant species (Sabahi et al., 2023). The presence of *Fusarium* in this study is consistent with previous reports indicating that this genus is frequently associated with various plants, including avocado. Several species such as *F. solani*, *F. equiseti*, and *F. oxysporum* have been reported in association with avocado plants showing symptoms such as chlorosis, necrosis, and growth disorders (Medina et al., 2026; Ajmal et al., 2022).

The obtained LSU sequences were aligned with reference sequences retrieved from the NCBI database using multiple sequence alignment. A phylogenetic tree was constructed using the Neighbor-Joining method with bootstrap analysis to assess branch support. The phylogenetic analysis showed that isolate DTTDL318 clustered within the same clade as *Fusarium cf. solani* and *Fusarium waltergamsii*, with a bootstrap value of 88%, indicating a close genetic relationship, as shown in Figure 3.

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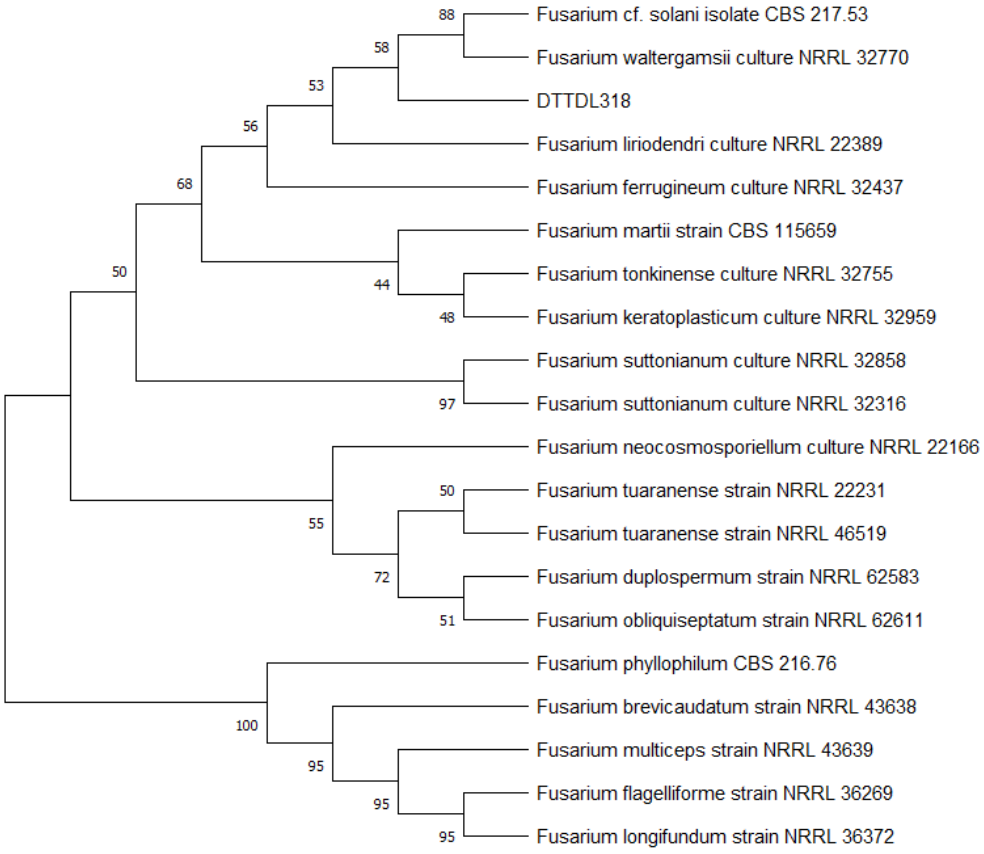

Figure 3. Phylogenetic tree of isolate DTTDL318.

Neighbor-Joining phylogenetic tree based on LSU rDNA sequences showing the relationship of isolate DTTDL318 with selected *Fusarium* species. Bootstrap values (>50%) based on 1000 replicates are shown .This clustering indicates that the isolate has a close phylogenetic relationship with members of the *Fusarium solani* species complex (FSSC). The close positioning of the isolate with several species within the same clade suggests a strong evolutionary relationship and further supports its identification as belonging to the genus *Fusarium*.The presence of *Fusarium* in this study is consistent with previous reports showing that this genus is commonly associated with avocado plants. *Fusarium solani* and *Fusarium oxysporum* have been identified from samples collected in avocado plantations in Antalya (Çalış et al., 2024) and have been reported in association with various diseases in tropical fruit crops. Both species are widely distributed in tropical soils and are frequently linked to vascular wilt diseases. In addition, *Fusarium solani* has been reported to infect roots and stem bark of avocado trees in Lebanon (Abi Saad et al., 2022), indicating that this group has a high adaptability to different plant tissues. These findings support the present study, where isolate DTTDL318, belonging to the *Fusarium solani* species complex (FSSC), indicates that the genus *Fusarium* is not only associated with vegetative tissues but can also colonize avocado fruit tissues.

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CONCLUSION

Isolate DTTDL318 associated with anthracnose symptoms on avocado fruit was identified as *Fusarium* based on morphological characteristics and LSU rDNA analysis, showing 100% similarity *to Fusarium cf. solani*. Phylogenetic analysis revealed that isolate DTTDL318 clustered within the same clade as *Fusarium cf. solani* and *Fusarium waltergamsii* with a bootstrap value of 88%, indicating a close phylogenetic relationship with members of the *Fusarium solani* species complex (FSSC).

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