Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Lasiodiplodia theobromae* Causing Leaf Spot on *Aloe vera* var. *chinensis* in Malaysia

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Aloe vera L. var. *chinensis* (Haw.) Berg. (family Asphodelaceae), locally known as 'Lidah Buaya', is an economically important plant as the gel from the leaves possesses anti-inflammatory, antiarthritic, antibacterial, and hypoglycemic properties and is used for cosmetic, pharmaceutical, and healing purposes in Malaysia. In July 2021, irregular black sunken spots (3 to 10 mm in diameter) were observed on Lidah Buaya leaves at the leaf development stage in a field in the Penampang district of Sabah province (N5°56'37.1" E116°04'21.5"). The disease severity was about 30% with 10% incidence. The tissues surrounding the black spots became brown and dry when the plants grew older. No gel was contained in the sunken zones. Symptomatic leaf tissues (5 × 5 mm) were cut from the infected margin, surface sterilized with 75% ethanol for 1 min, washed with 2% NaOCl solution for 1 min, rinsed, and air dried before plating on five potato dextrose agar (PDA) plates (pH 7). Plates were incubated at 25°C for 3 days in the dark. Greyish-white fluffy mycelia were observed, and then became dark gray with age. Dark pigmentation in each plate was produced after a week of incubation at 25°C. A representative isolate Penampang was further characterized morphologically and molecularly. Immature conidia were single-celled, aseptate, ellipsoid and hyaline, measuring 19.4 × 24.5 μm (*n* = 30). Mature conidia were brown, thick-walled, and one-septate with longitudinal striations, 22.5 × 28.3 μm (*n* = 30). Genomic DNA was extracted from fresh mycelia of isolate Penampang based on the extraction method described by Khoo et al. (2022), with additional mechanical disruption

using a micropestle before heating. KOD One PCR master mix (Toyobo, Japan) containing hot-start modified KOD DNA polymerase was used for PCR amplification. The PCR conditions were 94°C for 10 s, 55°C for 5 s, and 72°C for 2 s, for 30 cycles, and initial denaturation of 94°C for 3 min and a final extension step of 72°C for 5 min. The internal transcribed spacer (*ITS*) region of rDNA and tubulin (*TUB*) genes were amplified using ITS1/ITS4 and T10/Bt2b primer sets, respectively (O'Donnell and Cigelnik 1997; White et al. 1990). The products were then sent to Apical Scientific Sdn. Bhd. for sequencing. The generated *ITS* (OK209451) and *TUB* (OL660667) were 100% identical to *L. theobromae* isolate MRR-161 and CPC:27690 (GenBank MW282884 and MT592639, respectively) in BLASTn analysis. Phylogenetic analysis using maximum likelihood based on the combined *ITS* and *TUB* sequences indicated that the isolates formed a supported clade (91% bootstrap value) to the related *L. theobromae*. The morphological and molecular characterization of the fungus matched *L. theobromae* described by Pečenka et al. (2021). Mycelial agar plugs (5-mm diameter) from a 7-day-old PDA culture of the Penampang isolate were placed onto pinpricked leaves of three 2-month-old Lidah Buaya plants. Pinpricked leaves of three 2-month-old Lidah Buaya plants received sterile 5-mm-diameter PDA agar plugs to serve as controls. The inoculated plants were covered with plastic for 48 h and incubated at 25°C. All inoculated leaves developed symptoms as described above 6 to 7 days postinoculation, whereas no symptoms occurred on controls, fulfilling Koch's postulates. The experiments were repeated twice. The reisolated fungus was identical to representative isolate Penampang morphologically and molecularly. *L. theobromae* was reported previously on *A. vera* in Cuba (Urtiaga 1986) and India (Mathur 1979). To our knowledge, this is the first report of *L. theobromae* causing leaf spot on *A. vera* in Malaysia. The occurrence of this disease emphasizes the importance of disease surveillance in the region. Plant disease management strategies need to be established to reduce losses.

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