

Research Note

A Report of Fungi Associated With the Scarred-Scabbed Symptoms on Maprang (*Bouea macrophylla* Griffith) in Eastern Thailand

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Maprang, commonly known as a marian plum or plum mango (*Bouea macrophylla* Griffith), is an edible tropical fruit belonging to the family Anacardiaceae. It is grown extensively throughout ASEAN countries including Indonesia, Malaysia, Thailand, and the Philippines (Dechsupa *et al.*, 2019; Nguyen *et al.*, 2020). There are three varieties of maprang grown in Thailand, including maprang prieyo (sour maprang), maprang wan (sweet maprang), and mayong chid (sweet maprang with bitter flavor) (Dechsupa *et al.*, 2019).

During the last few years, the scarred-scabbed symptoms on maprang fruits have been observed in Rayong Province, which is located on the coastline of eastern Thailand. Disease symptoms include numerous tiny dark spots and subsequent development of scarred-scabbed areas on the fruits. The scarred-scabbed areas can distort the mature fruits. Therefore, the scarred-scabbed symptoms are aesthetic-related problems that can reduce the value of the fruit in markets. The etiology of the disease symptoms in Rayong Province is unclear. The possibility of the etiology has been ascribed to the wounds created by feeding activities of chili thrips (*Scirtothrips dorsalis* Hood) associated with an anthracnose *Colletotrichum gloeosporioides* Penz. fungus. However, the microbiological study has yet to be identified the cause of the disease.

Thirty samples of diseased fruits were collected in January 2023 from different orchards in Rayong Province, Thailand (Figure 1). Every orchard was routinely sprayed with abamectin, cypermethrin, and profenopos insecticides to control the chili thrips. The scarred-scabbed areas of each fruit sample were cut into 1 × 1 cm fragments, after which the surfaces were sterilized with 0.5% (v/v) of sodium hypochlorite solution for three min, and subsequently rinsed three times in sterilized water, similar to the method used by Chantarasiri *et al.* (2021). The samples were plated on dichloran rose bengal chloramphenicol (DRBC) agar as a fungal selective medium and incubated at 30 °C for seven days in the dark. The emerging fungal mycelia of each colony were inoculated on potato dextrose agar (PDA) and incubated for culture purification under the aforementioned conditions. All isolated fungi were primarily categorized according to their colony and conidia morphology.

Genomic DNA of the representative fungal isolates was extracted from the mycelia using the GF Fungus DNA Extraction Kit (Vivantis, Malaysia), and the internal transcribed spacer (ITS) regions were PCR amplified using ITS1/ITS4 universal primers (White *et al.*, 1990). The PCR was carried out using the OnePCR reaction mixture (Bio-Helix, Taiwan). The conditions of PCR were conducted according to Planonth and Chantarasiri (2022).

The resulting PCR products were nucleotide sequenced by Macrogen Inc. (South Korea) and deposited the sequences in the GenBank database of the National Center for Biotechnology Information (NCBI). The phylogenetic trees were generated by the BIONJ algorithm with 100,000 bootstraps using SeaView software version 5.0.2 (Gouy *et al.*, 2010).

A pathogenicity test for the isolated fungus was done on 20 healthy maprang fruits by spraying method (Chantarasiri *et al.*, 2021). A conidial suspension (10^5 conidia/mL) of the pathogenic fungus was sprayed onto the surface of maprang fruits. All fruits were incubated in sterilized plastic bags at 30 °C for seven days in the dark and observed for the symptoms. All experiments were assayed in triplicate. The pathogenic fungus was isolated from the symptomatic fruits and genetically identified accordingly as aforementioned experiment.

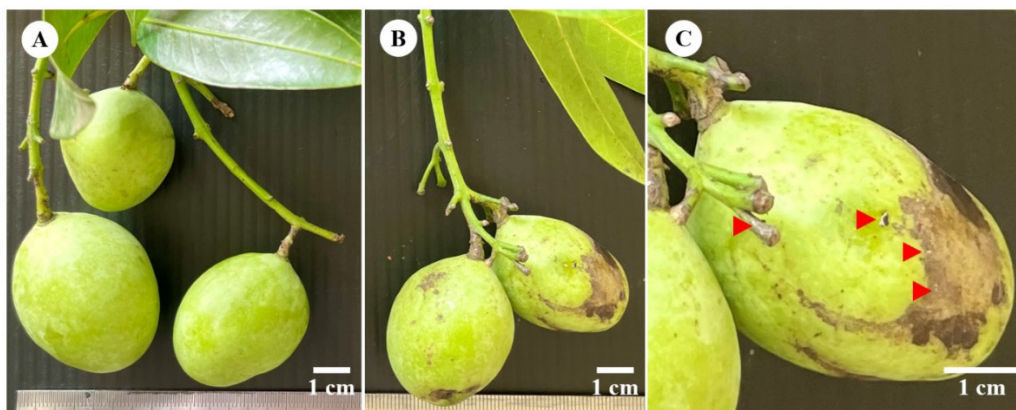


Fig. 1. A. Healthy unripe maprang fruits; B. Scarred-scabbed symptoms on unripe maprang fruits collected from Rayong Province, Thailand; C. Small fungal colony dwelled on the scarred-scabbed areas and stalks of maprang fruits (red arrows).

The fungi ($n=33$) were isolated from the thirty fruit samples and morphologically categorized. The first fungal group ($n=30$) was isolated from every fruit sample. The morphology of the first fungal group was a white colony with greenish-grey velvety powder and often secreted red-colored droplets after three incubation days (Figure 2A). Conidiophores were brush-shaped with spherical to subglobose conidia (Figure 2B). Therefore, they were identified as *Penicillium* sp. based on their morphological characteristics.

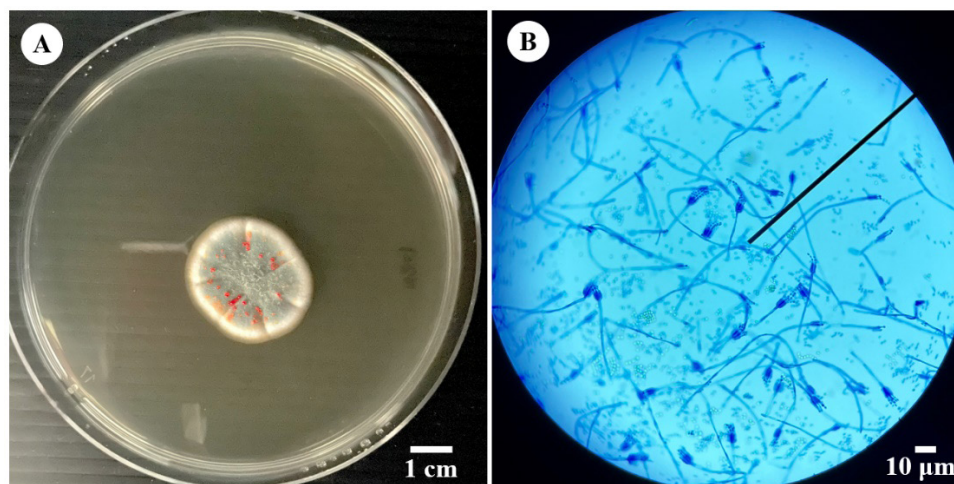


Fig. 2. A. Colony morphology of a representative *Penicillium* isolate cultured on potato dextrose agar (PDA) at 30 °C for seven days; B. Mycelia and conidiophores of a representative *Penicillium* isolate stained by lactophenol cotton blue.

The genomic DNA of a representative fungal isolate (isolate M201) was extracted from the mycelia and PCR amplified. The nucleotide alignment analysis using the BLASTn program showed that this fungus was closely related to *Penicillium sclerotiorum* (OW986441.1) with 99% coverage and 100% identity. The resulting E value was zero. Phylogenetic tree analysis using SeaView software identified the fungal isolate as *P. sclerotiorum* (Figure 3). The nucleotide sequence of *P. sclerotiorum* strain M201 was deposited in the GenBank database under the accession number OQ689633.

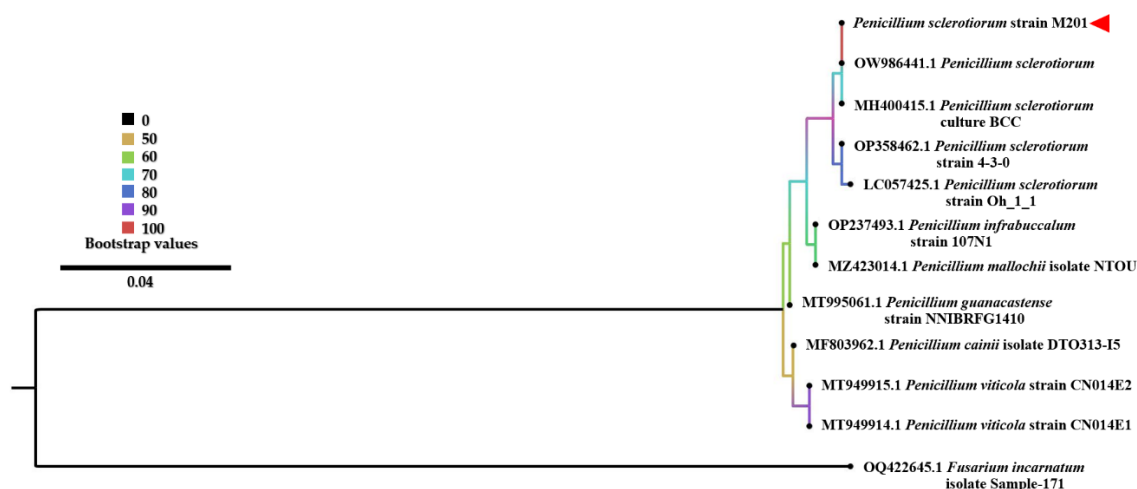


Fig. 3. Phylogenetic tree of *P. sclerotiorum* strain M201 shown using the BIONJ algorithm with 100,000 bootstrap replications. The phylogenetic tree was generated by SeaView software.

The second group ($n=1$) was a white colony with convex elevation fungus. The fungal colony secreted yellow-colored droplets after five incubation days (Figure 4A) and did not produce conidia on the PDA (Figure 4B). This fungus was genetically identified as *Aspergillus flavipes* (strain M101) and deposited the nucleotide sequence in the GenBank database under the accession number OQ689660. A phylogenetic tree of *A. flavipes* is shown in Figure 5. The third group ($n=2$) was a white cottony colony (Figure 6A) and produced slightly curved macroconidia (Figure 6B). They were genetically identified as *Fusarium incarnatum* (strain M502) and deposited the nucleotide sequence in the GenBank database under the accession number OQ689634. A phylogenetic tree of *F. incarnatum* is shown in Figure 7. The fungal pathogen should be observed from every symptomatic sample. Therefore, these three fungal isolates were not selected for further studies in this work.

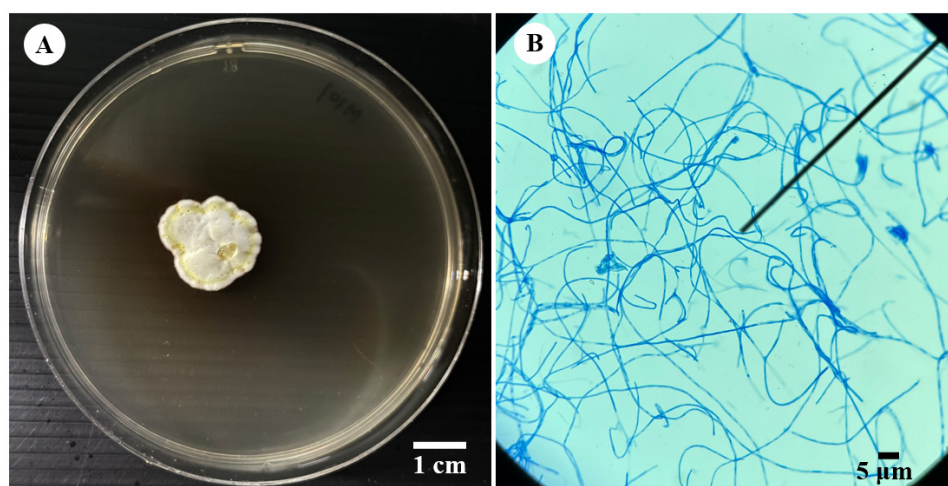


Fig. 4. A. Colony morphology of *Aspergillus flavipes* cultured on potato dextrose agar (PDA) at 30°C for seven days; B. Mycelia stained by lactophenol cotton blue.

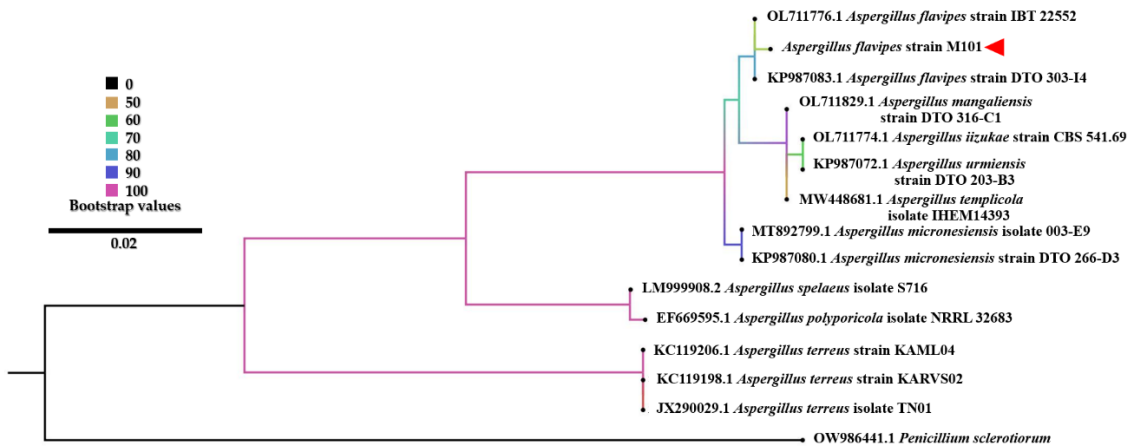


Fig. 5. Phylogenetic tree of *A. flavipes* strain M101 shown using the BIONJ algorithm with 100,000 bootstrap replications. The phylogenetic tree was generated by SeaView software.

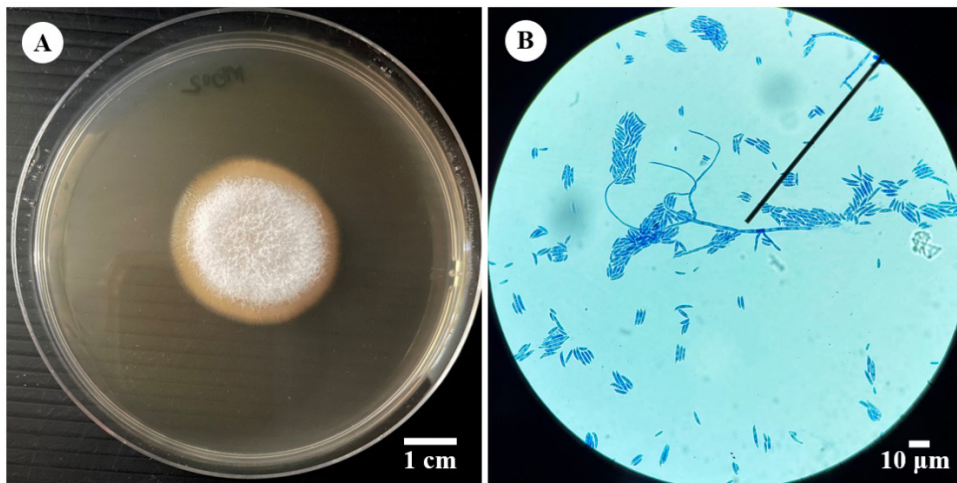


Fig. 6. A. Colony morphology of *Fusarium incarnatum* cultured on potato dextrose agar (PDA) at 30°C for seven days; B. Mycelia and its slightly curved macroconidia stained by lactophenol cotton blue.

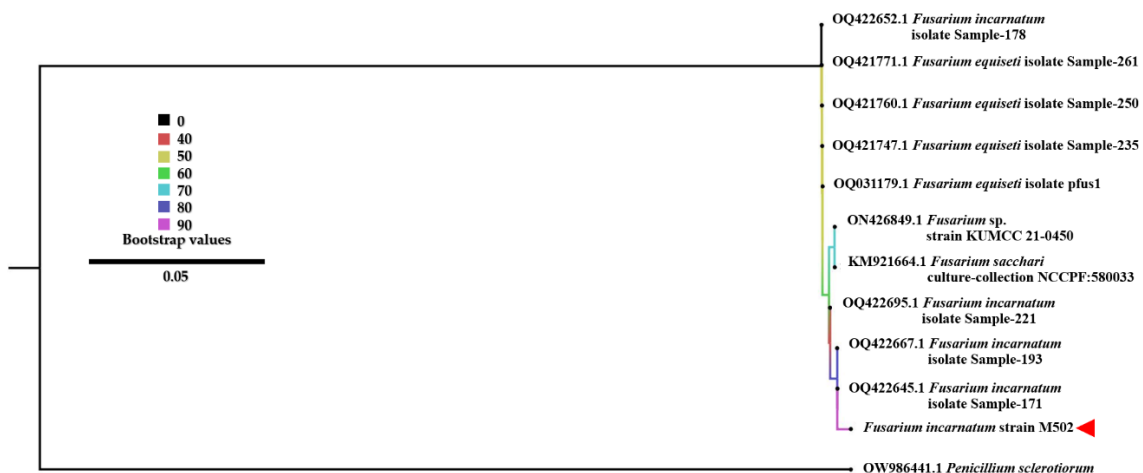


Fig. 7. Phylogenetic tree of *F. incarnatum* strain M502 shown using the BIONJ algorithm with 100,000 bootstrap replications. The phylogenetic tree was generated by SeaView software.

A pathogenicity test was performed by the *P. sclerotiorum* strain M201. The representative fungal isolate was able to induce disease symptoms on the inoculated fruit surfaces such as tiny dark spots (Figure 8). The fungus was re-isolated from the inoculated fruits and genetically identified.

The results showed that the fungus was genetically identified as *P. sclerotiorum*, thus fulfilling Koch's postulates. To the best of our knowledge, this is the first microbiological report of *P. sclerotiorum* causing the scarred-scabbed symptoms on maprang in Thailand. Previous reports described the *P. sclerotiorum* causing postharvest disease of pomegranate (*Punica granatum* cv. Mollar de Elche) in Spain (Palou *et al.*, 2013) and leaf spot disease of black mangrove (*Aegiceras corniculatum*) in the coastline of south China (Deng *et al.*, 2022).



Fig. 8. Pathogenicity test of *P. sclerotiorum* strain M201 on maprang fruits: A. control fruit; B. inoculated fruit after seven days incubation.

To date, chili thrips and anthracnose *C. gloeosporioides* are believed to be the cause of the scarred-scabbed symptoms on maprang in eastern Thailand. This study described the *P. sclerotiorum* causing the disease symptoms. Misidentification of plant pathogens could result in the use of inappropriate methods to control and prevent the disease.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Chantarasiri, A., Boontanom, P., Siriprom, W. & Kongsriprapan, S. 2021. First report of *Geotrichum candidum* causing sour rot of longkong fruits (*Lansium domesticum*) in Southern Thailand. *New Disease Report*, 43: e12016. <https://doi.org/10.1002/ndr2.12016>
- Dechsupa, N., Kantapan, J., Tungjai, M. & Intorasoot, S. 2019. Maprang "*Bouea macrophylla* Griffith" seeds: proximate composition, HPLC fingerprint, and antioxidation, anticancer and antimicrobial properties of ethanolic seed extracts. *Heliyon*, 5: e02052. <https://doi.org/10.1016/j.heliyon.2019.e02052>
- Deng, X.D., Yuan, Y.Q., Zheng, L.J., Zeng, H.L., Kang, Z.Q., Qiu, Q. & Wang, X.R. 2022. First report of leaf spot caused by *Penicillium sclerotiorum* on *Aegiceras corniculatum* in China. *Plant Disease*, 106: 2257. <https://doi.org/10.1094/PDIS-03-21-0663-PDN>
- Gouy, M., Guindon, S. & Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, 27: 221-224. <https://doi.org/10.1093/molbev/msp259>
- Nguyen, N.H., Nguyen, T.T., Ma, P.C., Ta, Q.T.H., Duong, T.H. & Vo, V.G. 2020. Potential antimicrobial and anticancer activities of an ethanolic extract from *Bouea macrophylla*. *Molecules*, 25: 1996. <https://doi.org/10.3390/molecules25081996>
- Palou, L., Taberner, V., Guardado, A., Río, M. & Montesinos-Herrero, C. 2013. Incidence and etiology of postharvest fungal diseases of pomegranate (*Punica granatum* cv. Mollar de Elche) in Spain. *Phytopathologia Mediterranea*, 52: 478-489.
- Planonth, S., Chantarasiri, A. 2022. The oleaginous yeast *Pichia manshurica* isolated from *Lansium domesticum* fruit in Thailand and its fatty acid composition of single cell oil. *Biodiversitas*, 23: 801-

809. <https://doi.org/10.13057/biodiv/d230226>

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (Eds.). Academic Press, San Diego, CA, USA. pp. 315-322. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>