Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Arcopilus aureus* Causing Leaf Black Spot Disease of *Pseudostellaria heterophylla* in China

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Pseudostellaria heterophylla (family Caryophyllaceae) is a perennial herbaceous plant. Its tuberous roots are highly valued in traditional Chinese medicine. It is mainly cultivated in a geo-authentic production zone located in Guizhou, Anhui, Shandong, and Fujian provinces of China (Zhao et al. 2016). The herb is widely used for treating lung diseases and as a spleen tonic (Pang et al. 2011). A severe leaf black spot disease was observed on *P. heterophylla* in China from 2018 to 2020. Plants displayed water-soaking symptoms in the early stage of infection, then the watery areas turned brown-red and a black mold appeared on the lesions. At a later stage, the leaf spots showed concentric rings surrounded by a yellow halo, and the initial infection site became dry and necrotic. Nine infected plants were collected from three cultivation fields in Shibing County (27°4'21" N, 108°8'0" E), Guizhou Province, on 13 April 2019. The fungus was consistently isolated from symptomatic leaves on potato dextrose agar (PDA) medium according to the method described by Larran et al. (2002). A total of 22 isolates were obtained, including seven isolates of

the genus *Arcopilus* and 15 isolates of the genus *Trichoderma*. The growth rates of isolate MJ2-2b on PDA and oatmeal agar (OA) medium were 3 to 5 mm/day at 25°C. Mycelium of isolate MJ2-2b was dense and yellowish-brown on PDA, while it was sparse and bright red on OA. The mycelium also secreted brownish-red pigment on both PDA and OA. Ascomata, when mature, were water drop and limoniform. Lateral hairs were brown, erect or flexuous, tapering toward the tips. Ascospores, when mature, were grayish-white to gray, limoniform, or fusiform to pyriform. Further, the beta-tubulin gene (*Tub2*) of the fungus was amplified by using primer pairs T1 and TUB4Rd as described by Wang et al. (2016) and subjected to sequencing. NCBI nucleotide BLAST results showed that sequences from seven isolates had a 99.86% identity with *A. aureus* (strain ChL-C, GenBank accession no. MG889987.1). Molecular phylogenetic analysis by maximum likelihood method using MEGA 7 confirmed that the fungal isolate clustered with *A. aureus*. Hence, the causal agent was identified as *A. aureus* based on morphological and molecular characteristics. The sequence was deposited in GenBank (accession no. MW531453). Pathogenicity tests were conducted on 15-day-old tissue-cultured seedlings according to Ghanbary et al. (2018). Leaves of 16 seedlings were inoculated with 1 × 1 mm 5-day-old PDA-grown mycelial plugs of the fungal isolate. The experiment was repeated three times. After 10 days, the inoculated leaves showed the same symptoms observed on plants in the field. The associated fungal pathogen was consistently reisolated from the inoculated seedlings and identified by *Tub2* gene sequencing. At present, there are no reports of *A. aureus* causing disease of plants. To the best of our knowledge, this is the first report of leaf black spot disease on *P. heterophylla* caused by *A. aureus* in China.

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