Diseases Caused by Bacteria and Phytoplasmas

First Report of a Leaf Spot Disease of Tobacco Caused by *Pseudomonas cichorii* in China

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Tobacco (*Nicotiana tabacum* L.), one of the chief commercial crops, is widely cultivated worldwide. In June 2020 and 2021, an unknown bacterial leaf spot on tobacco was found in Hezhou and Hechi City, Guangxi, China. In all, 30% of the tobacco plants were affected and the rate of diseased leaves reached about 10% in the field under high temperature and rainfall. The disease mainly damaged the middle and top leaves of tobacco plants at the vigorous growing stage. The initial symptoms were water-soaked spots on the front half of a leaf, which then expanded into circular to irregular spots with a yellow halo at the edge. The spots mostly appeared dark brown at high air humidity while yellow-brown at low humidity and exhibited a concentric pattern. In severe cases, the lesions coalesced and the whole leaf was densely covered with lesions, resulting in the loss of baking value. A bacterium was consistently isolated from diseased leaf tissues on nutrient agar (NA). Growth on NA was predominantly grayish-white, circular bacterial colonies with smooth margins, and the bacterium was rod-shaped, Gram-negative, and fluorescent on King's B medium. Seven isolates (ND04A to ND04C and

ZSXF02 to ZSXF05) were selected for molecular identification and pathogenicity tests. Genomic DNA of the bacterium was extracted and the house-keeping gene *encoding citrate synthase (cts)* was amplified with the primers cts-Fs/cts-Rs (forward primer cts-Fs: 5'-CCCGTCGAGCTGCCAATWCTGA-3' and reverse primer cts-Rs: 5'-ATCTCGCACGGSGTRTTGAACATC-3') (Berge et al. 2014; Sarkar et al. 2004). The 409-bp *cts* gene sequences were deposited in the GenBank database for seven isolates (accession nos. OK105110 to OK105116). Sequences of seven isolates shared 100% identity with several *Pseudomonas cichorii* strains within the GenBank database (accession nos. KY940268 and KY940271), and the phylogenetic tree of *cts* genes of the seven isolates clustered with the phylogroup 11 of *P. syringae* (accession nos. KJ877799 and KJ878111), which was classified as *P. cichorii*. To satisfy Koch's postulates, a pathogenicity test was performed by using a needle to dip a suspension of the bacterium (10^8 CFU/ml) and pricking three holes in the tobacco leaf. The control plants' leaves were pricked with sterile water. Three leaves of each tobacco plant were inoculated, and the test was repeated three times. All plants were placed in transparent plastic boxes and incubated in a greenhouse at $25 \pm 3^\circ\text{C}$. The water-soaked spots appeared 24 h after inoculation and quickly expanded through leaf veins. Three days after inoculation, all the inoculated leaves showed symptoms similar to those observed in the field. Control plants remained healthy. Only *P. cichorii* was successfully reisolated from the lesions, confirming Koch's postulates. *P. cichorii* can infect eggplant, lettuce, tomato, and other crops, and has a wide range of hosts (Timilsina et al. 2017; Wali et al. 2015). To our knowledge, this is the first report of *P. cichorii* causing leaf spot on tobacco in China.

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