Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Leaf Spot Disease on *Pueraria montana* var. *thomsonii* Caused by *Robbsia andropogonis* in China

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Pueraria montana var. *thomsonii* (*Pmt*) belongs to the Leguminosae and is widely distributed in China, Laos, Thailand, Myanmar, Bhutan, and other Asian countries. The plant is called “fenge” in China, and its root is widely used in medicine and food. In recent years, an unknown leaf spot disease of *Pmt* has occurred in Gaoming, Zhaoqing, and Yunfu districts of Guangdong Province in China, where 1,600 ha of *Pmt* plants were affected. The incidence rate on plants was more than 80% and led to 10 to 15% death of *Pmt* plants in Gaoming district. In the early stage of the disease, radiating, water-soaking lesions appeared between the main veins and side veins of *Pmt* leaves. After the spread of the lesions, they formed short, brown strips with yellow haloes around them, which led to leaf shedding, plant death, and decline of production. To isolate bacteria, diseased leaves were surface sterilized with 0.6% sodium hypochlorite solution for 30 s, followed by three consecutive rinses in distilled water. The leaves were aseptically macerated, and the macerate streaked on PDA medium. Whitish to dull white, mucoid, raised, round, and translucent colonies were obtained. All isolates were gram-negative and had a single, polar, sheathed flagellum. Sequences (approx. 1,458 bp each) of the 16S rRNA gene amplified from five isolates (FG2, FG3, FG9, FG12, and FG17) using primer pair 27F/1492R (Lane et al. 1991) (GenBank accession nos. OL677034, OL677351, OL677352, OL677353, and OL677354, respectively) shared 99.93% sequence identity with that of *Robbsia andropogonis* (synonym: *Burkholderia andropogonis*) (Lopes-Santos et al. 2017) type strain LMG2129 (NR104960.1). The specific 410-bp and 704-bp target fragments

were also amplified from isolates using *R. andropogonis*-specific primers Pf/Pr (Bagsic et al. 1995) and LJ23f/LJ24R (Duan et al. 2009). The four housekeeping genes *atpD*, *lepA*, *gyrB*, and *rpoD* were partially sequenced for FG9 isolates using primers *atpD*-F3/*atpD*-R3, *lepA*-F2/*lepA*-R, LJ23f/LJ24R, and LJ25f/LJ26r (Duan et al. 2009; Estrada-de los Santos et al. 2013), respectively. Multilocus sequence analyses confirmed the isolates from *Pmt* as *R. andropogonis*. Physiological and biochemical tests revealed the isolates are negative for oxidase, arginine dihydrolase, saccharose, and betaine, and positive for sorbitol, lactose, and galactose (Gillis et al. 1995; Lopes-Santos et al. 2017). In addition, all isolates caused a hypersensitive reaction on leaves of *Nicotiana benthamiana* and were pathogenic to some crops, including maize (*Zea mays*), sorghum (*Sorghum bicolor*), carnation (*Dianthus caryophyllus*), common bean (*Phaseolus vulgaris*), and tomato. Five isolates (FG2, FG3, FG9, FG12, and FG17) pathogenicity was tested twice with three replications per isolate. Two young leaves each of 3-month-old *Pmt* plants grown in a greenhouse were sprayed with a bacterial suspension at 10⁸ CFU/ml, then the inoculated leaves were covered individually with plastic bags for 24 h and incubated at 100% relative humidity with 16 h of daylight at 30°C and 8 h of darkness at 22°C in a greenhouse. Radiating, water-soaked lesions with yellow haloes were observed between the main veins and side veins of *Pmt* leaves 5 days after inoculation and were similar to those caused by *R. andropogonis* in the field. Koch's postulates were fulfilled by reisolating bacteria from typical lesions on inoculated plants, and the reisolated bacteria were identical to the inoculated ones. To our knowledge, this is the first report of *R. andropogonis* on *Pueraria montana* var. *thomsonii* in China.

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