Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Leaf Spot of *Cucurbita pepo* Caused by *Erwinia persicina* in China

L. Li, H. L. Li, Y. X. Shi, A. L. Chai, X. W. Xie,[†] and B. J. Li[‡]

Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

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In February 2020, the common symptoms of water-soaked spots on *Cucurbita pepo* L. cotyledons were observed in Guangrao county in Shandong province, China. Field investigation showed that 40% of the *C. pepo* cotyledons in an area of approximately 0.8 ha were infected. The disease resulted in a severe loss in seedling production. Samples of *C. pepo* with water-soaked leaf spots were collected and prepared for pathogen analysis. Symptomatic cotyledon tissue was surface disinfested in 75% ethanol for 30 s and then rinsed three times in sterilized water. Bacteria were released in sterile water in a Petri dish for 2 min by cutting symptomatic tissue into small sections and stirring the plant tissue mixture fully. The diffusate was streaked onto plates containing nutrient agar (NA), and plates were incubated at 28°C for 2 days. Three representative isolates were purified eventually from each of the plates. Colonies on NA were small, round, and with smooth margins. All bacterial isolates were characterized as gram-negative, white to cream color, and pink pigment was formed on the plates over long-term culture. The isolates were positive for catalase, Voges-Proskauer, potato rot, methyl red, acetoin production, nitrate utilization, citrate utilization, and acid production from maltose, glucose, melizitose, sucrose, D-arabinose, D-trehalose, cellobiose, lactose, raffinose, mannitol, D-sorbitol, melibiose, and xylitol. KOH production was demonstrated according to strand formation within the potassium hydroxide test (Suslow et al.

1982). Isolates were negative for oxidase, arginine dihydrolase, phenylalanine deaminase, gelatinase, esculine, indole production, and H₂S production. Total genomic DNA was extracted from isolate XHL2002230201 with a TIANamp Bacteria DNA Kit (Tiangen). Universal primers 27F and 1492R (Monciardini et al. 2002) were used in PCR to amplify a 1,307-bp DNA fragment of the 16S rRNA region for molecular identification. Furthermore, four additional housekeeping genes (*gyrB*, *atpD*, *rho*, and *rpoS*) were selected and amplified using specially designed primers. The amplification products of 16S rRNA were sequenced and submitted to GenBank under accession number MT568607.1. Sequence analysis showed 99% similarity to *Erwinia persicina* strains B57 (LM651373.1) and B64 (CI789_17875) by BLAST search in the GenBank database (Cho et al. 2019; Gálvez et al. 2015). A phylogenetic tree was constructed, and the taxonomic position of strain XHL2002230201 was determined from the multilocus sequence analysis on 16S rRNA and the other four housekeeping genes with *E. persicina* and not with other closely related *Erwinia* species. Pathogenicity tests and reisolation and reidentification of the bacteria were performed to confirm the isolate and fulfill Koch's postulates. The strain XHL2002230201 suspensions (10⁸ CFU/ml) were spray inoculated onto 15 *C. pepo* seedlings with two true leaves, and the same number of control plants were inoculated with water. Experiments were repeated three times. All inoculated plants were kept in a moist chamber placed in a greenhouse at 28°C. Initial symptoms were observed on leaves of inoculated plants at 5 days postinoculation, whereas no symptoms appeared on the plants inoculated with sterile distilled water. Based on the morphological and biochemical characters, phylogenetic analysis, and Koch's verification, the bacterial original isolates were identified as *E. persicina*. To our knowledge, this is the first report of *E. persicina* causing leaf spot disease on *C. pepo* in China.

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[†]Indicates the corresponding author. X. W. Xie; xiexuewen@caas.cn and B. J. Li; libaojuivf@163.com