Diseases Caused by Bacteria and Phytoplasmas

First Report of Soft Rot Caused by *Pectobacterium versatile* on *Hydrangea macrophylla* in China

R.-X. Chen, Y.-X. Shi, X.-W. Xie, A.-L. Chai, L. Li,[†] and B.-J. Li[†]

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

R.-X. Chen and Y.-X. Shi contributed equally to this work.

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Hydrangeas (*Hydrangea macrophylla*) are recognized for their abundant, showy inflorescences and colorful sepals (Schreiber et al. 2011). In recent years, the shrubs have been grown as potted plants and for landscaping in China. During the spring of 2021 in a seedling greenhouse located in Tonghai County, Yunnan Province, China (24.12°N, 102.73°E), a severe bacterial disease was observed on 12-month-old potted plants of cv. Enziandom. Symptoms on hydrangeas initially appeared as thick liquid produced on the surface of the infected petiole base and water soaking in the infected stems. With the development of the infection, the basal stem color was dark brown, and the stem base rapidly turned to wet rot. Severely infected leaves exhibited soft rot symptoms, and the presence of soft rot on mature plants decreased ornamental and market value. This disease was found in 40% of 15 surveyed greenhouses, and the disease incidence ranged from 30 to 50% in different regions. To identify the causal agent, diseased tissues from infected plants were surface sterilized using 75% ethanol for 30 s and soaked in sterile distilled water three times. The exudate from sheared tissue was streaked onto nutrient agar (NA) plates, and plates were incubated at 28°C for 24 h (Koike et al. 2002). Four representative bacterial isolates, BXH21032401 to BXH21032404, were selected for further analysis. Colonies on NA were white, small, round, and translucent with smooth edges. All bacterial isolates were gram-negative, rod-shaped, with two to four

circumferential flagella and no entrapment membrane. For physiological and biochemical tests, 96 phenotypes were screened using the BIOLOG GENIII microplate system (Biolog, Hayward, CA, U.S.A.); the test results of four isolated strains were consistent with those reported for *Pectobacterium versatile* (Portier et al. 2019). Total genomic DNA was extracted from bacterial suspensions with the TIANamp Bacteria DNA Kit (TIANGEN, China). PCR amplification assays were conducted with specially designed primers using extracted genomic DNA as a template. All sequences of the PCR products were deposited in GenBank with accession numbers MZ852237 to MZ852240 (16S rRNA), MZ921938 to MZ921941 (*pmrA*), MZ926722 to MZ926725 (*acnA*), MZ926726 to MZ926729 (*icdA*), MZ926730 to MZ926733 (*gapA*), MZ852233 to MZ852236 (*mdh*), MZ926718 to MZ926721 (*mtlD*), MZ921934 to MZ921937 (*pgi*), MZ926734 to MZ926737 (*proA*), and MZ921942 to MZ921945 (*rpoS*). Maximum likelihood phylogenetic trees based on 16S rRNA, *pmrA* sequences, and multilocus sequence analysis of housekeeping genes (*acnA*, *icdA*, *gapA*, *mdh*, *mtlD*, *pgi*, *proA*, and *rpoS*) (Xie et al. 2018) all clustered BXH21032401 to BXH21032404 into a clade containing other confirmed strains of *P. versatile* (Kravchenko et al. 2021). Pathogenicity tests were performed by spraying leaves of healthy 6-month-old potted *H. macrophylla* (cv. Enziandom) plants with a bacterial suspension (20 ml/plant, 10⁸ CFU ml⁻¹). Plants were inoculated with sterile distilled water as a control. Soft rot symptoms appeared on inoculated petioles 5 days after incubation in a growth chamber at 28°C and showed similar symptoms as observed initially in the greenhouse. The strains were reisolated successfully from symptomatic hydrangea to complete Koch's postulates. The pathogenicity test was carried out twice. To our knowledge, this is the first time that *P. versatile* is reported to cause hydrangea soft rot disease in China.

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[†]Indicates the corresponding authors.

L. Li; caulilei@163.com, and B.-J. Li; libaojuivf@163.com