Diseases Caused by Bacteria and Phytoplasmas

First Report of Strawberry Crown Rot Caused by *Xanthomonas fragariae* in China

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Strawberry (*Fragaria × ananassa* Duch.) is a fruit with great economic importance and is widely cultivated in the world. From 2019 to 2020, a serious crown rot disease was sporadically observed in several strawberry cultivars including ‘Zhang Ji’, ‘Hong Yan’, and ‘Yue Xiu’ in Shanghai, China. Initially, water-soaked rot appeared in the inner tissue of the strawberry crown, and then progressed into browning and hollowing symptoms accompanied by yellow discolorations of young leaves. To isolate and identify the causal agent, small pieces of tissue taken from 10 diseased crowns were sterilized by 70% alcohol. The cut-up pieces were macerated and serially diluted. The dilutions were placed on nutrient agar (NA) medium. After incubation at 25°C for 4 to 5 days, the yellow bacterial colonies were tiny and were streaked on a NA plate for purification. The colonies were yellow, mucoid,

and smooth-margined, and five independent representative colonies were used for further confirmation. To confirm the species identity of the bacteria, genomic DNA was extracted from the five representative isolates, and the 16S rRNA gene was amplified and sequenced using universal primers 27F/1492R. The 16S rRNA sequence was deposited in GenBank (MW725235) and showed 99% nucleotide similarity with *Xanthomonas fragariae* strain LMG 708 (NR_026318). The isolate’s identity was further confirmed by *X. fragariae*-specific primers XF9/XF12 (Roberts et al. 1996). All five isolates could be detected by XF9/XF12 primer. To confirm Koch’s postulates, five healthy strawberry plants were placed in 1,000-ml glass beakers by submerging the cutting wound in 50 ml of bacterial suspension (10⁸ CFU/ml). Five additional strawberry plants treated with sterilized water served as a control. The beakers containing inoculated plants were sealed with plastic film at 25°C. Water-soaked rot appeared on internal tissue of the crown, similar to those observed in the field, within 10 to 12 days after inoculation, whereas the control samples remained healthy. The bacteria were reisolated from rot of inoculated crowns and were confirmed by *X. fragariae*-specific primers XF9/XF12. *X. fragariae* has been reported to cause angular leaf spot on strawberry in China (Wang et al. 2017; Wu et al. 2021). It was also found that *X. fragariae* could systematically infect crown tissue (Mahuku and Goodwin 1997; Milholland et al. 1996). To our knowledge, this is the first report of *X. fragariae* causing strawberry crown rot in China. This report increased our understanding of *X. fragariae* and showed that the spread of this disease might seriously threaten the development of the strawberry industry in the future.

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