Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas fragariae* Strain YL19 Causing Crown Infection Pockets in Strawberry in Liaoning Province, China

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Strawberry (*Fragaria × ananassa* Duch.) is an important fruit crop in China. Typical crown infection pocket symptoms were observed on strawberry in Liaoning province, China (121°60'E, 38°90'N) in fall 2017. Disease incidence was ~5 to 10% but could reach 30 to 40% in heavily affected plastic tunnels. Infected plants early displayed water-soaked symptoms on the abaxial leaf surface and subsequently developed reddish-brown stripes and coalesced lesions on the adaxial leaf surface around the main veins. Several 0.3 to 0.8 mm diameter pockets were observed inside the crown tissues after dissection. Diseased plants rarely reached fruiting, were easily broken between the crown tissue and stem, and would eventually die. To identify the causal agent, several surface-disinfested infected main veins and crown tissues were individually ground in sterile water (SW) and plated on sucrose peptone agar (SPA) medium (Hayward 1960) with 10-fold serial dilutions and incubated at 25°C. Yellow colonies grew on the medium at the 10⁻⁴ dilution 7 days after plating in all specimens. The colonies were aerobic, yellow, viscous, smooth, and gram-negative, typical of *Xanthomonas*. To confirm identity, 18 colonies selected randomly were subjected to PCR for the amplification of the *cpn60* (Sahin et al. 2010), *gyrB*, *rpoD*, and *fyuA* (Fargier et al. 2011), respectively. Results showed that the colonies are identical. The *cpn60*, *gyrB*, *rpoD*, and *fyuA* sequences were deposited in GenBank as MT513132.1, MW233896, MW233897, and MW233895, respectively. BLAST searches with sequences of *cpn60*, *gyrB*, *rpoD*, and *fyuA* revealed 97.7, 96.4, 97.8, and 97.3% similarity with the corresponding sequences of *X. fragariae* strain NBC2815 (LT853880.1), respectively. The resulting concatenated data set of *cpn60*-*gyrB*-*rpoD*-*fyuA* was used to build a multilocus sequence analysis by maximum likelihood criteria. The *cpn60*-*gyrB*-*rpoD*-*fyuA* sequences of the isolate from

Liaoning clustered in the clade containing the type strain of *X. fragariae* NBC2815. Thus, the bacterial strain was designated as *X. fragariae* strain YL19. To fulfill Koch's postulates, the bases of leaf petioles of disease-free strawberry plants were syringe-infiltration inoculated with bacterial suspension (2 × 10⁸ CFU) prepared from colonies of YL19 washed from SPA plates. Inoculated and control (treated with SW) plants were placed in a chamber (25/20°C day/night, ≥90% RH, 12-h/12-h photoperiod) for 3 months. After 1 month, water-soaked symptoms were observed in the crown tissues of all YL19-inoculated plants. Two months after inoculation, a significant crown pocket similar to symptoms observed in the field developed on all inoculated plants. No symptoms were observed in control plants. The bacteria were reisolated from symptomatic leaves, petioles, and crowns and confirmed as YL19 by the described morphological and molecular analyses. Pathogenicity tests were conducted three times and the same results obtained. *X. fragariae* usually causes angular leaf spot (ALS), a serious bacterial disease in many strawberry production regions worldwide. Typical symptoms of ALS caused by *X. fragariae* include reddish-brown, irregular spots on the upper leaf surface, with water-soaked lesions along leaf veins. Although ALS caused by *X. fragariae* has been reported in Tianjin and Taiwan province, China (Wang et al. 2017; Wu et al. 2021), there is no report of symptoms of infection pockets on crowns, as caused by YL19. Thus, YL19 is different from the other two *X. fragariae* strains reported in China, or the disease caused by YL19 could be a severe case of ALS and vascular decline or collapse in strawberry (Bradbury 1977). These results showed a previously unreported strain YL19 of *X. fragariae* is the causal agent of crown infection pocket in strawberry in China; it may lead to serious losses to the local strawberry industry. This report will assist in developing management measures for this disease promptly.

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