Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas fragariae* Causing Angular Leaf Spot on Strawberry (*Fragaria × ananassa*) in Taiwan

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Angular leaf spot of strawberry, considered an A2 quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO 2019), is an important bacterial disease in many regions. Since 2017, symptoms similar to angular leaf spot were observed in several strawberry cultivars including ‘Taoyuan No. 1’ and ‘Xiang-Shui’. Early symptoms were angular, water-soaked lesions on the abaxial leaf surface, and later, reddish-brown irregular spots and coalesced lesions developed on the adaxial surface. In the humid conditions, sticky bacterial ooze exuding from lesions was observed. To isolate the causal agent, leaves showing water-soaked lesions were surface sterilized, cut into small pieces, and soaked in 5 ml of sterile water for at least 15 min. The supernatant from the cut-up pieces was serially diluted, followed by spreading on sucrose peptone agar (SPA) (Hayward 1960). After incubating at 20°C for 4 to 5 days, single colonies grown on SPA were transferred to a new SPA plate and cultured at 20°C until colonies appeared. The yellow, glossy, and mucoid colonies, which resembled the colony morphology of *Xanthomonas fragariae*, were selected as candidates for further confirmation. First, bacterial DNA of four candidate isolates, B001, B003, and B005 from Miaoli County and B004 from Taoyuan City, was PCR amplified with *X. fragariae*-specific primers: XF9/XF12 (Roberts et al. 1996) and 245A/B and 295A/B (Pooler et al. 1996). All four isolates could be detected by XF9/XF12 primer.

Furthermore, isolates B003 and B004 could be detected by both 245A/B and 295A/B primers, whereas B001 and B005 could be detected by 295A/B only. Next, DNA gyrase subunit B (*gyrB*) was PCR amplified with the primers XgyrB1F/XgyrB1R (Young et al. 2008). The *gyrB* sequences of these four isolates were deposited in GenBank with accession numbers MT754942 to MT754945. The *gyrB* phylogenetic tree was constructed based on Bayesian inference analysis and maximum likelihood analysis. The *gyrB* sequences of the four isolates from Taiwan clustered in the clade containing the type strain of *X. fragariae* ICMP5715, indicating that they belong to *X. fragariae*. B001 and B005 formed a subgroup separated from B003 and B004, suggesting genetic differences between these isolates. To fulfill Koch’s postulates, the abaxial surfaces of strawberry leaves were syringe infiltrated (Silva et al. 2017) or wound inoculated (Wang et al. 2017) with bacterial suspensions (final OD₆₀₀ = 1.0 to 2.0) prepared from colonies of B001 and B003 washed from SPA plates. Inoculated plants were enclosed in a plastic bag (>90% relative humidity) at 25/20°C (day/night) under a 12-h/12-h photoperiod. After 7 to 14 days, water-soaked lesions similar to those observed in the field were developed on all inoculated leaves. The bacteria were successfully reisolated from lesions of inoculated leaves and confirmed by specific primers XF9/XF12, 245A/B, and 295A/B. We also found that the disease commonly occurs in the strawberry fields/nurseries with sprinkler irrigation during winter or early spring and was particularly serious in the windward side or near a riverside. To our knowledge, this is the first report of *X. fragariae* causing angular leaf spot on strawberry in Taiwan. Currently, the disease only occurs severely in certain regions, but establishment of effective management strategies will be needed to prevent spreading of this disease and potential economic loss in the future.

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