Diseases Caused by Bacteria and Phytoplasmas

First Report of Preharvest Soft Rot of Peach Fruit (*Prunus persica*) Caused by *Enterobacter mori* in China

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Peach (*Prunus persica* L. Batsch) is one of the major fruit crops of China and a rich source of vital nutrients and fibers. In July 2019, a peach orchard was visited at Pinggu District, Beijing, China. The average temperature and relative humidity at the sampling site were $28 \pm 2^\circ\text{C}$ and $75 \pm 2\%$, respectively. In the orchard, unripe and nearly ripe peach fruits cultivar ‘China Pearl’ were observed with soft brown patches (3 to 4×2 to 3 cm) on the surface. The samples ($n = 25$) were collected based on typical symptoms. When the rotten part of the fruit was pressed, a liquid oozed out of the fruit, emitting an unpleasant odor. The disease incidence of fruit soft rot was 22%. The percent incidence was calculated based on total number of infected fruits divided by total number of fruits examined. Infected fruit tissues were excised into small pieces (5 mm) and surface disinfested with 1% NaClO for 25 s and rinsed twice with sterilized distilled water (SDW). These pieces (four to five pieces per sample) were macerated with 1.5 ml of SDW in a 2-ml tube. Later, 5 μl of supernatant was streaked on Luria Bertani (LB) agar plates and incubated at 28°C for 3 days. Oozing liquid (50 μl) was also inoculated on 100 ml of LB broth and incubated for 24 h,

and 5 μl was streaked on LB agar medium and incubated at 28°C . Purified colonies were obtained by restreaking three times. The isolate formed creamy to light yellowish, irregular, round, rough or smooth-looking colonies on LB medium and pink colonies on eosin methylene blue. The bacterium was rod shaped, measuring 0.8 to 1.4 μm in length and 0.2 to 0.5 μm in width, observed using a scanning electron microscope (Hitachi-SU8010). Morphological characteristics were similar to previously described characteristics of *Enterobacter* spp. (Nishijima et al. 1987; Zhu et al. 2011). Genomic DNA was extracted from all 25 isolates by using a TIANamp Bacteria DNA Kit (Tiangen-Biotech, Beijing, China) according to the manufacturer’s instructions. The 16S ribosomal RNA gene of 25 isolates was amplified by using universal primers 27F (5’-AGAGTTTGATCCTGGCT CAG-3’) and 1492R (5’-CTACGGCTACCTTGTACGA-3’), and *rpoB* gene was amplified with primers F2 (5’-AACCAGTTCCGCGTTGGCCTGG-3’) and R2 (5’-CCTGAACAACACGCTCGGA-3’) (Mollet et al. 1997). Sequences for EPT-1 were submitted to GenBank (accessions MN548761 for 16S rRNA and MN594495 for *rpoB*). Accessions MN548761 and MN594495 had 99.35 and 99.77% sequence identity with *Enterobacter mori* (GenBank accessions KF747680 and GQ406571). A maximum likelihood phylogenetic tree constructed (1,000 replicates) using Mega X (Kumar et al. 2018) indicated that isolate EPT-1 clustered with *E. mori* sequences. To confirm the pathogenicity, medium-sized ($n = 60$) surface-disinfested ripe peach fruits cultivar China Pearl were wound inoculated with 5 μl of suspension of EPT-1 (10^7 CFU/ml), and the control fruits were treated with 5 μl of sterilized water. The fruits were kept in a sterilized plastic box and incubated at 28°C for 7 days at 70% relative humidity, and the pathogenicity test was repeated three times. Symptoms similar to the original fruit samples were observed on all inoculated fruits. The pathogen was reisolated, and the colonies obtained from the oozing liquid were similar to those of infected fruit tissues and identified as *E. mori* based on morphological and sequencing analysis. Previously, *E. mori* has been reported to cause bacterial wilt on *Morus alba* L. in China (Zhu et al. 2011). To our knowledge, this is the first report of *E. mori* causing soft rot of peaches in China.

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