Diseases Caused by Bacteria and Phytoplasmas

Ginger Tuber Rot Caused by *Enterobacter asburiae* in China

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Ginger (*Zingiber officinale* Roscoe) is a popular spice and widely grown commercial crop in China. Tangshan is the main planting area of ginger in Hebei Province, China. In October 2018, a postharvest rot disease was discovered on ginger tubers stored in a cellar (13 to 15°C, 85 to 90% relative humidity) in the Tangshan region. Disease incidence was approximately 20 to 30% of the stored yield (1,000 kg). Symptoms included water-soaking of the rhizome surfaces and rot of the internal tissues. Vascular bundles became dark brown with stenchlike smell. Small square pieces (about 3 to 5 mm²) of tubers were excised from the junction of diseased and healthy tissues ($n = 50$) using a sterile scalpel, sterilized with 75% alcohol for 20 s followed by 0.1% mercuric chloride solution for 30 s, and then washed with sterile water. Samples were macerated in sterilized water and cultured on nutrient agar (NA) at 28°C for 48 h (Fang 1998). Three single colony isolates were recultured on NA. The resulting bacterium was a gram-negative, short, nonmotile bacillus. Colonies were round and milky white, smooth raised, and moist after incubation at 28°C for 24 h on NA. Physiological and biochemical test results showed that the isolate was anaerobic; produced acid from glucose, sucrose, sorbitol, inositol, and mannitol in an oxidative and fermentative manner; was

positive for urease; and was negative for indole, methyl red, the Voges-Proskauer test, gelatin liquefaction, and arginine double hydrolase. A typical hypersensitive reaction was induced on 12-week-old tobacco leaves (*Nicotiana tabacum* L.) that were inoculated by injecting a suspension of the isolated strain (10⁸ CFU/ml) at 25°C after 24 h. According to the physiological and biochemical characteristics of the strain, we identified this bacterium as *Enterobacter asburiae* (Janda et al. 2006). To identify the pathogen, DNA was extracted from bacterial cultures by a bacterial genomic DNA extraction kit (Biomed BMamp Rapid Bacteria DNA Kit; Biomed, Beijing, China), and PCR was performed with the universal 16S rDNA primers 27F/1492R (Lane 1991) and partial specific *rpoB*, *gyrB*, and *atpD* genes (Brady et al. 2013; Mollet et al. 1997). The 16S, *rpoB*, *gyrB*, and *atpD* sequences were deposited in GenBank under accession numbers MK935342, MK928968, MT081000, and MT081001, respectively. BLAST analysis revealed the isolate shared 99, 98, 100, and 100% identity with *E. asburiae* type strain ATCC 35953 in GenBank (CP011863). Maximum likelihood analysis was performed, and the phylogenetic tree clustered with *E. asburiae* (MEGAX, bootstrap $n = 1,000$). Pathogenicity tests were conducted by dropping 20 µl of the bacterial suspension (10⁸ CFU/ml) on a transverse section of the ginger tubers; tubers inoculated with sterile water were the negative control. Ginger tubers were incubated at 28°C for 48 h with plastic wrap to maintain high humidity. Water-soaking of ginger tubers was observed after 16 h, and vascular bundles were rotted about 15 days later, whereas controls were uninfected. The experiments were carried out three times, each time with three replicates. The bacterium was reisolated successfully from the infected ginger and was confirmed to be *E. asburiae* by morphological and molecular characterization. To the best of our knowledge, this is the first report of *E. asburiae* causing rot of ginger tubers in China. This finding will help inform the prevention and management of postharvest diseases of ginger tubers.

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