Diseases Caused by Bacteria and Phytoplasmas

Citrobacter freundii Causing Ginger (*Zingiber officinale*) Rot in Tangshan, China

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Ginger (*Zingiber officinale* Rosc.) is an important economic crop, and its rhizome can be used as a seasoning agent and traditional medicine in China. During July 2018 and 2019, decay symptoms occurred in the ginger planting area of Tangshan City, Hebei Province, with incidence rates of 15 to 20%. The pathogen infected the rhizomes and leaves. The symptoms included leaf chlorosis and gradual wilting; even the whole plant wilted, and the rhizome became soft and presented light brown maceration. In serious cases, the interior of the rhizome was completely eroded, with gray-white juice overflowing the epidermis and with a foul smell. The rhizome surfaces of ginger plants were disinfected with 1% NaOCl, and colonies were isolated and purified on nutrient agar (NA) solid medium by streaking. Eight isolates were obtained from 15 diseased tissue samples. Further morphological, physiological, and biochemical identification of the pure cultured bacteria was carried out. Three strains of bacteria were picked for further analysis. All of the three strains were gram-negative, short rod-shaped, nonmotile bacillus. Colonies were round and milky yellow, smooth raised, and moist after incubation at 28°C for 24 h on NA. Physiological and biochemical test results showed that

strains were facultatively anaerobic; negative for indole, methyl red, the Voges-Proskauer test, and urease; positive for glucose, sucrose, sorbitol, inositol, mannitol, citrate utilization, hydrogen sulfide production, and gelatin liquefaction. A typical hypersensitive reaction was induced on 12-week-old tobacco (*Nicotiana benthamiana*) leaves, which were inoculated by injecting suspensions of the isolated strain (10^8 CFU/ml) at 25°C after 24 h. These characteristics were consistent with *Citrobacter freundii* (Werkman and Gillen 1932). To further assess the identity of the strains, the genomic DNA was extracted from one bacterium (JXJ4). The partial 16S rRNA region (Lane 1991) and specific *rpoB* and *gyrB* genes (Brady et al. 2013; Mollet et al. 1997) were amplified and sequenced with primers 27F/1492R, CM7/CM31b, and UP1f/UP2r, respectively. The obtained 16S, *rpoB*, and *gyrB* sequences (GenBank accessions MN148645, MN158728, and MW199734) of the isolate showed 99.93, 99.51, and 99.82% identity to the corresponding sequences of *C. freundii* in GenBank (CP024679.1, CP024677.1, and KM509081.1). Maximum likelihood analysis was performed, and the phylogenetic tree clustered with *C. freundii* (MEGAX, bootstrap $n = 1,000$). The pathogenicity of the isolates was tested on ginger plants and rhizome tissue. The bacterial suspensions (10^8 CFU/ml) of three isolates were injected into the basal stem and rhizome center of nine healthy ginger seedlings, respectively, and control groups were treated with sterile water. The inoculated plants were kept in a moist chamber (28°C, 16-h light/8-h dark period), and ginger rhizomes were placed in the incubator (30°C, 16-h light/8-h dark period). Seven days after inoculation, the ginger tubers showed symptoms of decay, and 20 to 25 days later, the ginger plant leaves browned and died. The pathogenicity test was repeated four times, and all controls were healthy. Pathogens were reisolated from symptomatic plants and rhizomes and identified as *C. freundii* based on the morphological, biochemical, and molecular methods described previously, fulfilling Koch's postulates. To our knowledge, this is the first report of ginger rot caused by *C. freundii* in China.

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