Diseases Caused by Bacteria and Phytoplasmas

Ginger Rhizome Rot Caused by the *Enterobacter cloacae* in Tangshan, China

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Ginger (*Zingiber officinale* Rosc.) is an important economic crop in China; the rhizome tissue, in particular, has a high medicinal value. In July 2019, symptoms of ginger rhizome rot appeared in Tangshan City of Hebei Province, with incidence rates of 15%. The diseased part of the ginger rhizome became soft and presented light-brown maceration. The interior of the rhizome was seriously eroded, leaving only the epidermis, and gray-white, foul-smelling juice overflowed the epidermis. The surface of the ginger rhizome was disinfected with 1% NaOCl, and colonies were isolated and purified on nutrient agar (NA) solid medium by a streak plate method. Eight isolates were obtained from 15 diseased tissue samples. Further morphological, physiological, and biochemical identification of the pure cultured bacteria was carried out. Two isolates of bacteria were picked for further analysis. The bacteria were Gram-negative bacilli, which were milky white with round protuberances on NA solid medium. Physiological and biochemical test results showed that isolates were facultatively anaerobic, with gelatin liquefaction; negative for indole, methyl red, hydrogen sulfide production, and the Voges-Proskauer test (V-P); and positive for D-glucose, sucrose, sorbitol, inositol, mannitol, and citrate utilization. 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 *Enterobacter* spp. To further assess the identity of the species, the genomic DNA was extracted from one bacterium (SDXJ1). The partial 16S rRNA gene and specific *rpoB* and *gyrB* genes were amplified and sequenced with primers 27F/1492R, CM7/CM31b, and UP1f/UP2r (Brady et al. 2013; Lane 1991; Mollet et al. 1997). The obtained 16S and *rpoB* sequences (GenBank accession nos. MK937637, MZ911902, and MZ911901) of the isolate showed 99.33, 99.21, and 99.57% identity to the corresponding sequences of *Enterobacter cloacae* in GenBank (CP055458, AP022228, and AP022519, respectively). Maximum-likelihood analysis was performed, and the phylogenetic tree clustered with *E. cloacae* (MEGAX, bootstrap $n = 1,000$). The pathogenicity of the isolates was tested on ginger plants and rhizomes tissue. The bacterial suspensions (10^8 CFU/ml) of two isolates were injected into the basal stem and rhizome center, respectively, of six healthy ginger seedlings, and control groups were treated with sterile water. The inoculated plants were kept in a moist chamber (28°C, 16 h of light and 8 h of darkness) and ginger rhizomes were placed in the incubator (30°C, 16 h of light and 8 h of darkness). 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. The pathogens of symptomatic plants and ginger rhizomes were studied. They were identified as *E. cloacae* by physiological, biochemical, and molecular biological methods, fulfilling Koch's hypothesis. This is the first report of ginger rhizome rot caused by the *E. cloacae* in Tangshan, China. The research results are of great significance for the prevention and control of the disease. Our laboratory has reported that *Citrobacter freundii* can cause ginger rot (Zhao et al. 2021). Whether the two strains infect alone or together in the field still needs to be further explored.

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The author(s) declare no conflict of interest.

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