Diseases Caused by Bacteria and Phytoplasmas

First Report of Rhizome Rot on Ginger (*Zingiber officinale*) Caused by *Enterobacter cloacae* in Shandong Province, China

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Ginger (*Zingiber officinale* Roscoe) is an important crop planted on 60,000 to 70,000 ha every year in Shandong Province, China. In June to July 2019, a bacterial disease was observed on ginger in Shandong Province. Disease incidence was ~15% in each of three fields in Rushan (36°41' to 37°08'N, 121°11' to 121°51'E). Symptoms included leaf chlorosis, basal root and stem rot, rhizome rot, and wilting of foliage. Symptoms were similar to those associated with bacterial wilt of ginger, caused by *Ralstonia solanacearum* (Smith) Smith, a devastating disease of ginger in China (Ren and Fang 1981). Bacterial colonies isolated from infected ginger rhizomes surface disinfested with 1% NaClO were purified on nutrient agar (NA). Eighteen isolates were obtained from 20 diseased tissue samples. Three virulent isolates (SDGR1, SDGR2, and SDGR3) that differed morphologically from strains of *R. solanacearum* were selected for characterization based on morphological, biochemical, and molecular analyses. The colonies were light yellow, circular, smooth, and convex on NA. Individual cells were rod-shaped with peritrichous flagella (Dong and Cai 2001). Isolates were gram negative, facultatively anaerobic, negative for urease, oxidase, and pectate degradation, as well as positive for catalase, lactose, and asparagine utilization (Dong and Cai 2001). These characteristics were consistent with *Enterobacter* spp. (Enterobacteriaceae). For further identification, bacterial DNA of each isolate was extracted from a pure culture grown in liquid nutrient broth medium on a shaker at 28°C for 16 h. The sequence of the 16S rRNA gene was amplified using primers 27F/1492R (Lane 1991). The three isolates had identical 16S rRNA sequences, and the consensus sequence (GenBank MT233300) was analyzed by NCBI BLASTn, revealing 99.8% identity with the sequence

from *Enterobacter cloacae* strain R8-2 (GQ406570) (Wang et al. 2008). Construction of a phylogenetic tree of 16S rRNA sequences using a maximum likelihood analysis showed that the three ginger isolates clustered most closely with *E. cloacae*. The pathogenicity of three isolates was tested on ginger plantlets and immature rhizomes (Nishijima et al. 2004). Fifteen plantlets for each of the three isolates were each injected with 0.3 ml of a bacterial suspension (10^7 to 10^8 CFU/ml) at the stem base. The same number were injected with sterile water as a control. Inoculated plants were placed in a chamber at 90% relative humidity (RH) and 28°C with a photoperiod of 16 h. Rhizomes were surface disinfested in 1% NaClO for 3 min, rinsed in distilled water, and then cut into segments. Fifteen segments for each of the three isolates were injected with 0.2 ml of bacterial suspension (10^7 to 10^8 CFU/ml). Control rhizomes were treated similarly with sterile water. All rhizomes were placed in a chamber at 90% RH and incubated at 30°C in the dark. The pathogenicity tests were conducted three times independently. Brown discoloration, chlorosis, and necrosis of leaves developed 2 to 3 weeks after inoculation of plantlets for all three isolates. Water-soaked, brown, and rotted rhizome tissue developed about 1 week after inoculation of rhizomes for all three isolates. No obvious symptoms were observed on the control plantlets and rhizome segments. The bacterial isolates that caused symptoms on the inoculated plants were reisolated and identified as *E. cloacae* based on the same morphological, biochemical, and molecular analyses. The pathogen was not isolated from the control plantlets and rhizomes. *E. cloacae* has been reported as the cause of ginger rhizome rot in the United States and mulberry wilt in China (Nishijima et al. 2004; Wang et al. 2008). To our knowledge, this is the first report of ginger rhizome rot caused by *E. cloacae* in China. Confirming the existence of this pathogen in this area will be useful to adopt effective field management measures to control this disease on ginger.

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