Diseases Caused by Bacteria and Phytoplasmas

First Report of Rhizome Rot Caused by *Pectobacterium brasiliense* on Ginger in China

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In August 2020, ginger (*Zingiber officinale*) rhizomes (cv. Mianjiang) showing soft rot symptoms were observed in a field in Tayang Village, Fengrun District, Tangshan, Hebei Province (North China). The disease incidence in that field (15 ha in size) was more than 20%. Symptomatic rhizomes (brown and water-soaked) were surface-sterilized in 75% ethanol for 60 s, followed by three successive rinses with sterile distilled water. Rhizomes were cut into pieces ca. 0.5 cm in length that were soaked in 500 µl of 0.9% saline for 20 min. Aliquots (20 µl) of three tenfold dilutions of the tissue specimen soaking solution were plated onto lysogeny broth (LB) medium and incubated at 28°C for 24 h. Five single colonies were picked from each LB plate and restreaked three times for purity. Endophytic bacteria were also isolated from asymptomatic rhizomes as a control. The bacterial gDNA was extracted using the EasyPure Bacteria Genomic DNA Kit (TransGen Biotech, Beijing, China). The 16S rDNA region was amplified by PCR using the universal primer pair 27F/1492R (Weisburg et al. 1991) and sequenced. The results of BLASTn against NCBI nr of the 16S rDNA amplicons suggested that most isolates (8/10) obtained from the rotten rhizomes belonged to the genus *Pectobacterium*, and a few isolates (2/10) were *Enterobacter* spp. Only

Enterobacter spp. were isolated from asymptomatic rhizomes. Since all *Pectobacterium* isolates showed identical 16S rDNA sequences, only two isolates were selected for further analysis. *Pectobacterium* isolates TS20HJ1 and TS20HJ2 (MZ853520, MZ853521) represent isolates from two plant individuals. To determine the species of the rhizome rot *Pectobacterium* isolates, multilocus sequence analysis (MLSA) was performed with five housekeeping genes: *acnA*, *icdA*, *mdh*, *proA*, and *rpoS* (MZ994717 to MZ994726) (Ma et al. 2007; Waleron et al. 2008), and a phylogenetic tree was reconstructed using RAxML v8.2.12 (github.com/stamatak/standard-RAxML). No sequence variation was observed at any MLSA locus between the two isolates. The results of phylogenetic analysis showed that the ginger rhizome isolates clustered with *Pectobacterium brasiliense* type strain IBSBF1692^T (Duarte et al. 2004; Nabhan et al. 2012). Ginger seedlings (cv. Mianjiang) were inoculated with the isolate TS20HJ1 by injecting 10 µl of bacterial suspension (10⁸ CFU·ml⁻¹) into the rhizomes or 10 µl of 0.9% saline solution as a control. The seedlings were grown at 28°C and 50% relative humidity. Ten days after inoculation, only the bacteria-inoculated rhizomes showed disease symptoms resembling those observed in the field. Bacterial colonies were obtained from the infected rhizomes and were identified with MLSA gene sequencing, fulfilling Koch's postulates. *P. brasiliense* causes soft rot of a wide range of economically important crops (Oulghazi et al. 2021). To our knowledge, this is the first report of *P. brasiliense* causing rhizome rot of ginger in China. The rhizome rot caused 20 to 25% yield loss on average in the Tangshan region in 2020, which poses a significant threat to local ginger farming. Further research on epidemiology and disease management options is needed.

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The author(s) declare no conflict of interest.

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