

First report of *Pectobacterium wasabiae* causing soft rot of cabbage in Malaysia.

Abstract

Soft rot of cabbage (*Brassica rapa*) occurs sporadically in Malaysia, causing economic damage under the hot and wet Malaysian weather conditions that are suitable for disease development. In June 2011, 27 soft rotting bacteria were isolated from cabbage plants growing in the Cameron Highlands and Johor State in Malaysia where the economic losses exceeded 50% in severely infected fields and greenhouses. Five independent strains were initially identified as *Pectobacterium wasabiae* based on their inability to grow at 37°C, and elicit hypersensitive reaction (HR) on *Nicotiana tabacum* and their ability to utilize raffinose and lactose. These bacterial strains were gram-negative, rod-shaped, N-acetylglucosaminyl transferase, gelatin liquefaction, and OPNG-positive and positive for acid production from D-galactose, lactosemelibiose, raffinose, citrate, and trehalose. All strains were negative for indole production, phosphatase activity, reducing sucrose, and negative for acid production from maltose, sorbitol, inositol, inulin, melezitose, α -methyl-D-glucoside, and D-arabitol. All the strains exhibited pectolytic activity on potato slices. PCR assays were conducted to distinguish *P. wasabiae* from *P. carotovorum* subsp. *brasiliensis*, *P. atrosepticum*, and other *Pectobacterium* species using primers Br1f/L1r (2), Eca1f/Eca2r (1), and EXPCCF/EXPCCR, respectively. DNA from strains did not yield the expected amplicon with the Br1f/L1r and Eca1f/Eca2r, whereas a 550-bp amplicon typical of DNA from *P. wasabiae* was produced with primers EXPCCF/EXPCCR. ITS-RFLP using the restriction enzyme, *Rsa* I, produced similar patterns for the Malaysian strains and the *P. wasabiae* type strain (SCRI488), but differentiated it from *P. carotovora* subsp. *carotovora*, *P. atrosepticum*, *P. carotovorum* subsp. *brasiliensis*, and *Dickeya chrysanthemi* type strains. BLAST analysis of the 16S rRNA DNA sequence (GenBank Accession No. KC445633) showed 99% identity to the 16S rRNA of Pw WPP163. Phylogenetic reconstruction using concatenated DNA sequences of *mdh* and *gapA* from *P. wasabiae* Cc6 (KC484657) and other related taxa (4) clustered Malaysian *P. wasabiae* strains with *P. wasabiae* SCRI488, readily distinguishing it from other closely related species of *Pectobacterium*. Pathogenicity assays were conducted on leaves and stems of four mature cabbage plants for each strain (var. *oleifera*) by injecting 10 μ l of a bacterial suspension (10⁸ CFU/ml) into either stems or leaves, and incubating them in a moist chamber at 80 to 90% relative humidity at 30°C. Water-soaked lesions similar to those observed in the fields and greenhouses were observed 72 h after injection and bacteria with similar characteristics were consistently reisolated. Symptoms were not observed on water-inoculated controls. The pathogenicity test was repeated with similar results. *P. wasabiae* was previously reported to cause soft rot of horseradish in Japan (3). However, to our knowledge, this is the first report of *P. wasabiae* infecting cabbage in Malaysia.

Keyword: Cabbage; Soft rot of cabbage; *Pectobacterium wasabiae*.