Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium punjabense* Causing Blackleg and Soft Rot on Potato in Hebei and Fujian Province, China

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Funding: Funding was provided by China Agriculture Research System of MOF and MARA and Start Funding of University of Inner Mongolia. Plant Dis. 106:1977, 2022; published online as <https://doi.org/10.1094/PDIS-12-21-2731-PDN>. Accepted for publication 10 January 2022.

Pectobacterium spp. and *Dickeya* spp. cause blackleg and soft rot on potato worldwide (Charkowski 2018). In China, Potato plants (cv. Favorita and Jizhang 8#) with blackleg symptoms (vascular browning of crown stems) were observed in the field in Zhangjiakou, Hebei Province, in 2018 and in Ningde, Fujian Province, in 2019. The disease incidence was around 50 and 10% in Zhangjiakou (5 ha) and Ningde (4 ha), respectively. Diseased plants (three from each site) were collected to isolate the pathogen. Blackleg symptomatic stems were soaked in 75% ethanol for 2 min and rinsed and ground in sterile distilled water. Serial tenfold dilutions of the solution were plated onto a crystal violet pectate agar (CVP) plate (Ge et al. 2018). Two to 3 days after incubation at 28°C, four bacterial colonies in total that digested pectin from the media and developed pits on CVP plates were purified and sequenced for identification using the universal 16S rRNA gene primer set 27F/1492R (Monciardini et al. 2002). Two colony sequences that showed more than 99% sequence identity to *Pectobacterium punjabense* type strain SS95 (MH249622) were submitted to GenBank (accession numbers: OK510280 and MT242589). Additionally, six housekeeping genes, *proA* (OK546205, OK546199), *gyrA* (OK546206, OK546200), *icdA* (OK546207, OK546201), *mdh* (OK546208, OK546202), *gapA* (OK546209, OK546203), and *rpoS* (OK546210, OK546204), from these two isolates were amplified

and sequenced (Ma et al. 2007; Waleron et al. 2008). All strains showed 99 to 100% identity with MH249622^T. Phylogenetic trees based on the 16S rRNA gene sequences and concatenated sequences of the housekeeping genes of the two isolates were constructed using MEGA 6.0 software (Tamura et al. 2013). Koch's postulates were performed on potato seedlings and potato tubers (cv. Favorita) by injecting 100 µl of bacterial suspension of ZRIMU18112 and ZRIMU19122 (10⁵ CFU/ml) or sterile phosphate-buffered solution into the crown area of the stems or the tubers, and keeping them at 100% humidity and 21°C for 1 day. Four days after inoculation, the infected area of the inoculated seedlings rotted and turned black, while the controls were symptomless. Two days after inoculation, the infected tubers rotted and turned black, while the controls were symptomless. Bacterial colonies were reisolated from these symptomatic tissues and identified using the same methods described above. Blackleg on potato plants or soft rot on potato has been reported to be caused by *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliense*, *P. parmentieri*, and *P. polaris* in China (Cao et al. 2021; Wang et al. 2022; Zhao et al. 2018). To our knowledge, this is the first report of blackleg and soft rot of potato caused by *P. punjabense* in China. We believe that this report will draw attention to the management of this pathogen in China.

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The author(s) declare no conflict of interest.

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Keywords: blackleg, *Pectobacterium punjabense*, potato, soft rot

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