Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium parmentieri* Causing Blackleg on Potato in Inner Mongolia, China

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Funding: Funding was provided by China Agriculture Research System (No. CARS-09-P17). Plant Dis. 105:2237, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2502-PDN>. Accepted for publication 19 February 2021.

Blackleg on potato plants (*Solanum tuberosum*) is caused by *Pectobacterium* spp. and *Dickeya* spp. (Charkowski 2018) worldwide. From June to August in both 2018 and 2019, cases of blackleg were investigated in potato-producing areas in Hulunbuir, Ulanqab, and Hohhot in Inner Mongolia, China. The total surveyed field area was about 200 ha. The plants showed typical blackleg symptoms, such as black and stunted stems or curled leaves, and the number of infected plants was counted. The disease showed an incidence of around 8%. Five diseased plants were collected from a 10-ha potato field with approximately 75,000 potato plants (cv. mainly Favorita and Xisen) per hectare. Two-centimeter-long samples of symptomatic stems were removed from the selected plants using a sterile scalpel. The surfaces of the samples were disinfected with 75% ethanol for 2 min. They were then rinsed with sterile distilled water and soaked in 5 ml of sterile distilled water for 30 min. Aliquots of three tenfold dilutions of this solution were plated onto the crystal violet pectate agar (CVP) plate and incubated for 3 days at 28°C (Ge et al. 2018). A single bacterial colony that showed pitting on CVP plates was picked with a toothpick and was streaked onto nutritional agar (She et al. 2013) to obtain pure colonies. Amplification of a 1.4-kb segment containing the 16S rRNA gene was performed on the pure colonies using the universal primer set 27F/1492R (Monciardini et al. 2002). The amplicons were sequenced and submitted to GenBank nucleotide BLAST analysis. The 16S rRNA gene sequences of four isolates (GenBank accession nos.

MN626412, MN626449, MN625916, and MT235556) showed more than 99% sequence identity to *Pectobacterium parmentieri* type strain RNS 08-42-1A (NR_153752.1). Six housekeeping genes of these four isolates were amplified and sequenced: *proA* (MT427753 to MT427756), *gyrA* (MT427757 to MT427760), *icdA* (MT427761 to MT427764), *mdh* (MT427765 to MT427768), *gapA* (MT427769 to MT427772), and *rpoS* (MT427773 to MT427776) (Ma et al. 2007; Waleron et al. 2008). All sequences showed 99 to 100% sequence identity with *P. parmentieri* strains. Phylogenetic trees were constructed by multilocus sequence analysis using MEGA 6.0 software (Tamura et al. 2013). The samples were tested against Koch's postulates on potato seedlings (cv. Favorita) by injecting 100 µl of bacterial suspension (10⁷ CFU/ml) or sterile phosphate-buffered solution into the stems 2 cm above the base (Ge et al. 2018). The seedlings were incubated at 21°C and 80% humidity (She et al. 2013). Three to 5 days after inoculation, only infected seedlings showed similar symptoms as those observed in the field: the infected area turned black and rotten. Bacterial colonies isolated from these symptomatic seedlings were identified using the same methods described above and were identified as inoculated *P. parmentieri* strains. Blackleg on potato plants has been reported to be caused by *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum*, and *P. carotovorum* subsp. *brasiliense* in China (Zhao et al. 2018). To our knowledge, this is the first report of blackleg of potato caused by *P. parmentieri* in Inner Mongolia, China. We believe that this report will draw attention to the identification of this pathogen, which is essential to disease management.

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The author(s) declare no conflict of interest.

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Keywords: *Solanum tuberosum*, blackleg, *Pectobacterium parmentieri*

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