Disease Note

Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium brasiliense* Causing Blackleg and Soft Rot of Potato in Argentina

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Blackleg and soft rot diseases represent a major threat to the health of potato (*Solanum tuberosum*) and other vegetable, ornamental, and fruit crops worldwide; their main causal agents are species of *Pectobacterium* and *Dickeya*. In May 2022, 60% of potato plants (cv. Spunta) in a production field in Córdoba, Argentina (31°32′36″S, 64°09′46″W), showed soft rot, blackleg, and wilt. To isolate the causal agent, decayed plant tissues were disinfected in 2% NaClO, macerated in sterile water, and streaked on crystal violet pectate (CVP) medium. Plates were incubated at 28°C for 48 h. Colonies that produced a pit on CVP medium were purified on nutrient agar. Two of the isolates, called 1Aia and 1B, were characterized by tests commonly employed for the identification of pectinolytic bacteria (Schaad et al. 2001). Both produced Gram-negative rods that were facultatively anaerobic, oxidase negative, nonfluorescent on King's B, and resistant to erythromycin and caused soft rot of potato slices. In addition, these isolates did not produce the blue pigment indigoidine and grew on nutrient glucose agar containing 5% NaCl. Phenotypic characteristics of the isolates 1Aia and 1B were compatible with *Pectobacterium* spp. Genomic DNA was extracted using the commercially available Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions for the purification of DNA from Gram-negative bacteria. The isolates were positive in a PCR assay for *Pectobacterium brasiliense* (Duarte et al. 2004). The purified DNA of the isolate 1Aia was used to construct a pooled Illumina library, which was sequenced at the Genomics Unit from the National Institute of Agricultural Technology (INTA, Argentina) by using high-throughput Illumina sequencing technology. Average nucleotide identity (ANI) calculation performed by FastANI v0.1.3 (Jain et al. 2018) showed 96.11% identity between the genome of the type strain LMG 21371 of *P. brasiliense* (accession no. JQOE00000000) and our strain 1Aia (accession no. JAYGXQ000000000). For the pathogenicity test, 3-week-old potato plants (cv. Spunta) planted in pots were infiltrated with 10 µl of a bacterial suspension (1×10^7 CFU/ml) 5 cm above the base of the stem using a sterile syringe. Negative controls were infiltrated with sterile water. The plants were kept under greenhouse conditions and regularly watered. The experiment was performed two times with six plants per treatment. Two days after inoculation, the plants treated with the *P. brasiliense* strain 1Aia or 1B showed necrotic lesions on the stems and tubers and soft rot symptoms, whereas the control plants remained asymptomatic. To fulfill Koch's postulates, bacteria were reisolated from symptomatic plants. Reisolated bacteria, called 1Aia d and 1B d, were confirmed as *P. brasiliense* according to biochemical and PCR results, as outlined above. Also, the % ANI value between the *P. brasiliense* isolates 1Aia and 1Aia d was 99.99% (accession no. JAYGXR000000000). To our knowledge, this is the first report of the occurrence of *P. brasiliense* in Argentina. This pathogen has been observed causing blackleg and tuber soft rot on potato in Brazil (Duarte et al. 2004), the Netherlands (Nunes Leite et al. 2014), Switzerland (de Werra et al. 2015), Russia (Voronina et al. 2019), Serbia (Loc et al. 2022), and the United States (Zhang et al. 2023), among other countries worldwide. Because of the important economic and nutritional value of the crop, the distribution of *P. brasiliense* needs to be investigated and monitored in order to develop effective control strategies.

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