Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium versatile* Causing Potato Soft Rot in Oregon and Washington

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Potato (*Solanum tuberosum* cv. Russet Norkotah) tubers with symptoms of soft rot were submitted to Oregon State University, Hermiston Agricultural Research and Extension Center Plant Clinic in 2019. One submission in May originated from a 49-ha field with 60 to 70% emergence and seed piece decay (~20% affected) in Umatilla County, Oregon (OR). The second submission, in September, originated from a field in Washington (WA) (size unknown) with 30% soft rot incidence at harvest. We used 31 tubers from the OR submission and 9 tubers from the WA submission to isolate and identify pathogens responsible for the disease symptoms. From each tuber, ~100 mg of tissue at the margin of infection was washed with distilled water (DW), excised, and macerated in 500 µl of sterile DW for 5 min. The resulting solution was streaked on crystal violet pectate (CVP) medium and incubated at 28°C for 24 h. We were able to culture white colonies that formed depressions on CVP plates from some of the tuber samples. One bacterial isolate from OR and one from WA were named JB56A and JB133A, respectively, and preserved in Luria-Bertani (LB) broth with 15% glycerol at -80°C for long-term storage. Genomic DNA was extracted from JB56A and JB133A cultures grown in LB broth overnight at 30°C using the Wizard SV Genomic DNA kit. The partial *dnaX* gene (537 bp) was amplified from genomic DNA of each isolate using primer pair *dnaXf/dnaXr* (Slawiak et al. 2009) and sequenced. These sequences were deposited to NCBI GenBank as

MW930747 (JB56A) and MW930748 (JB133A). BLAST analyses (Altschul et al. 1990) using default parameters indicated that the *dnaX* sequences of JB56A and JB133A were 99.2% (533/537) and 98.7% (530/537) identical to *Pectobacterium versatile* SCC1 (CP021894). A condensed maximum-likelihood (ML) tree was built using the partial *dnaX* sequence of the 2 query strains, 12 *Pectobacterium* reference strains to include all known species of *Pectobacterium*, and 4 *Dickeya* spp. as an outgroup. Our previous study showed that an ML tree with gene *dnaX* is a reliable method to identify *Pectobacterium* and *Dickeya* spp. (Ma et al. 2021). JB56A and JB133A formed a monophyletic clade with *P. versatile* SCC1, confirming their identity. Potato (cv. Upstate Abundance) tuber and stem bioassays (Ma et al. 2018) were conducted twice to assess the pathogenicity of these isolates. Tubers were wounded with a sterile 2-mm-wide wooden applicator stick and 5 µl of culture grown in LB broth overnight (~10⁹ CFU) was pipetted into the wounds. Tubers were incubated at 28°C for 24 h and cut through puncture sites to observe symptoms. Stems of 4- or 5-week-old plants were wounded with a sterile toothpick ~10 cm above the soil line and a smear of JB56A or JB133A grown on LB agar was inserted into the wound using a toothpick and incubated in a greenhouse for 72 h. Positive (*Dickeya dianthicola* ME23) and negative (no bacteria) controls were included in both assays. Tubers and stems exhibited disease symptoms after 24 and 72 h, respectively, following inoculation with JB56A, JB133A, and *D. dianthicola* ME23. No symptoms were observed for negative controls. The identity of bacteria reisolated from the margin of stem lesions was confirmed as *P. versatile* by partial *dnaX* sequence analyses. *P. versatile* was recently described as a distinct species based on whole-genome sequence comparisons (Ma et al. 2021; Portier et al. 2019). In a parallel work, soft rot *Pectobacteriaceae* (*P. atrosepticum* and *P. carotovorum*) were also recovered from other samples, indicating that *P. versatile* was not the only pathogen present in these two fields. In 2018, we isolated *P. versatile* from potato stems with blackleg disease in New York, and a recent study found that *P. versatile* was isolated in the United States from an iris in 1946 (Ma et al. 2021; Portier et al. 2019). However, the geographic distribution and importance of this pathogen in the United States remains largely unknown. To our knowledge, this is the first report of potato soft rot caused by *P. versatile* in OR and WA, two important potato-producing states.

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