Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium punjabense* Causing Potato Soft Rot and Blackleg in Serbia

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Soft rot and blackleg are common diseases of potato (*Solanum tuberosum*) in Serbia. Opportunistic pectinolytic plant pathogens in the genus *Pectobacterium* cause soft rot and wilt diseases by cell wall degradation. They cause major economic losses in potato crops worldwide and are among the top 10 plant pathogenic bacteria (Mansfield et al. 2012). Potato plants ('VR808') with symptoms of wilting, slow growth, stem blackening, and tuber softening were collected from a commercial field in Zobnatica, Serbia, in July 2019 and analyzed. All symptoms occurred in the same field, and ~5% of plants were symptomatic. Isolation was performed from 10 randomly chosen symptomatic plants and tubers. Plant tissue was surface disinfected, and 1-cm sections from the margins of lesions were macerated in sterile distilled water (SDW) for 25 min and streaked on nutrient agar. After 48 h of incubation at 26°C, predominant shiny, cream-colored, round colonies were obtained from all samples. Three representative isolates (MMZKVR1, MMZCVR2, and MMZKVR3) from independent samples were selected randomly for biochemical and pathogenicity tests. Isolates were gram-negative, nonfluorescent facultative anaerobes exhibiting pectinolytic activity on potato tuber slices and hypersensitive response on tobacco leaves. They expressed catalase activity but did not express oxidase or acid phosphatase activity or produce indole. All strains grew at 37°C in 5% NaCl and reduced nitrate. Pathogenicity was tested on healthy 3-week-old potato plants (VR808 and 'Kiebitz') grown in Baltic Tray Substrate (Hawita) in a greenhouse and tubers of the same varieties. Three stems per isolate were inoculated by toothpick piercing (Duarte et al. 2004) using a bacterial suspension (~1×10^8 CFU/ml) and incubated under plastic bags in a greenhouse at 25 ± 2°C. Blackleg symptoms and stem wilting developed within 48 h. No symptoms were observed on plants inoculated with sterile toothpicks dipped in SDW. The pathogen was reisolated from symptomatic plants, fulfilling Koch's postulates, and sequencing of 16S rDNA confirmed the original pathogen. Three tubers per isolate were inoculated by toothpicks dipped in bacterial suspension (~1×10^8 CFU/ml) and placed in a sealed plastic container at 25 ± 2°C. Treatment with SDW was used as a control. Tissue softening around the inoculation point developed within 48 h; no symptoms developed on controls. For molecular analyses, total DNA of isolates was extracted with a DNeasy Plant Mini Kit (Qiagen). The isolates were not detected in diagnostic PCR assays using specific primers Br1F/L1R for the detection of *P. brasiliense* (Duarte et al. 2004) and primers EXPCCF/EXPCCR for *P. carotovorum* subsp. *carotovorum* (Kang et al. 2003). The 16S rDNA PCR amplification was done using the universal PCR primer pair 27F/1492R (Fredriksson et al. 2013) followed by Sanger sequencing (Macrogen Europe BV). BLASTn analysis of sequences (GenBank nos. MZ048661, MZ048662, and MZ157274) revealed 100% query coverage and 100% identity to *P. punjabense* sequences in NCBI (MT242589 and CP038498) isolated from potato in China and Pakistan, respectively (Sarfraz et al. 2018). All three obtained isolates were proposed to belong to *P. punjabense* sp. nov. To further validate identification, MMZCVR2 was used for multilocus sequence analyses of five housekeeping genes: *gyrA*, *recA*, *recN*, *rpoA*, and *rpoS*. The *gyrA* (MZ161817), *recA* (MZ161818), *recN* (MZ161819), *rpoA* (MZ161820), and *rpoS* (MZ161821) sequence analysis had the highest nucleotide identity (99.44 to 100%) with *P. punjabense* strain SS95 (Sarfraz et al. 2018) in the NCBI GenBank database. To our knowledge, this is the first report of blackleg and soft rot caused by *P. punjabense* on potato in Serbia. *P. punjabense* causes soft rot and blackleg disease in potatoes (Sarfraz et al. 2018). Its distribution is poorly known but important because soft rot bacteria are easily transported long distances in latently infected seed tubers and can cause major economic losses.

References:

Duarte, V., et al. 2004. J. Appl. Microbiol. 96:535.
Fredriksson, N. J., et al. 2013. PLoS One 8:e76431.
Kang, H. W., et al. 2003. Plant Pathol. 52:127.
Mansfield, J., et al. 2012. Mol. Plant Pathol. 13:614.
Sarfraz, S., et al. 2018. J. Syst. Evol. 68:3551.

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