First report of Bacterial Soft Rot and Blackleg on potato caused by

_Pectobacterium parmentieri_ in Hawaii

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_Pectobacterium_ genus comprises numerous soft causing bacteria affecting vegetable, ornamental and fruit crops (Charkowski 2018; Ma et al, 2007). Potato is the major host for several _Pectobacterium_ species including _P. parmentieri_ – a newly described species, formerly known as _P. wasabiae_ (Khayi et al, 2013). During surveys conducted in January 2018 and January 2019 at Oahu, sixty-seven infected potato samples, exhibiting wilting, water-soaked regions with watery ooze, darkened and necrotic basal stem symptoms extending upward (Figure 1A – 1B), were collected. Symptomatic stems and tubers were cut into 0.5 – 1 cm pieces, surface sterilized with 10% sodium hypochlorite solution for 30 sec, followed by three rinses with sterile distilled water. After sterilization, each stem piece was macerated, streaked on CVP medium, and incubated at 26 ± 2°C for 48 h. Colonies, forming pits on the CVP medium (Figure 1C – 1D), were re-streaked on TZC. DNA was isolated from infected plant tissues using the DNeasy Plant Mini Kit (Qiagen, Germantown, MA). Bacterial genomic DNA from purified cultures was isolated using DNeasy Blood and Tissue Kit (Qiagen). End-point PCR was performed using the primers Pec.dnaA-F1 and
Pec.dnaA-R1 (Ahmed et al., 2018) to amplify the \textit{dnaA} gene; PCR products were enzymatically purified by adding 2 µL of ExoSAP-IT. Sequencing was performed at the GENEWIZ facility; both forward and reverse strands were aligned and manually edited using Geneious. The BLASTn results of \textit{dnaA} region displayed 99-100% identity and 100% query cover with all \textit{P. parmentieri} strains sequences in the NCBI GenBank database. Eleven strains originating from the same field (growing multiple potato cultivars) but from different plants, namely PL30 (MN428432), PL32 (MN428433), PL67 (MN428434), PL70 (MN428435), PL71 (MN428436), PL72 (MN428437), PL74 (MN428438), PL75 (MN428439), PL123 (MN428440), PL124 (MN428441) and PL128 (MN428442), were identified as \textit{P. parmentieri}. Three weeks-old potato plants, grown from healthy tubers under temperature-controlled conditions, were artificially inoculated at three consecutive nodes starting from the base of the stem by injecting 100 µL of bacterial inoculum of PL70 (3.56 x $10^8$ CFU/mL) and PL72 (2.78 x $10^8$ CFU/mL) (Figure 1E); control plants were inoculated with 100 µL sterile water – experiment was performed in triplicates with consistent results. After ~72 h, blackleg and stem rot symptoms, resembling those observed in the field, were observed (Figure 1F); no symptoms on control plants were observed. Bacteria from inoculated symptomatic stems were re-isolated on CVP - pits were produced by both strains. To fulfill Koch’s postulates, DNA was isolated, amplified and sequenced for \textit{dnaA} region; BLASTn results showed 100% identity with original \textit{P. parmentieri} strains. Additionally, a phylogenetic analysis based on the \textit{dnaA} gene was conducted with the 11 \textit{P. parmentieri} Hawaiian strains (Figure 2); all these strains were clustered together with other 18 \textit{P. parmentieri} strains of GenBank database, further confirming the identity and genetic relationship of these new \textit{P. parmentieri} strains. This is the first report of the \textit{P. parmentieri}, causing soft rot and blackleg.
The presence of this pathogen along with the previous report of *D. dianthicola* (Boluk and Arif, 2019), outline the importance of exerting proper crop management, and accurate and efficient diagnostics to prevent its spread; it also warrants a state-wide survey for pectolytic bacteria on vegetable crops.

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**Figure 1.** Naturally and artificially inoculated plants showing blackleg and stem rot symptoms. (A-B) Stem rot and blackleg symptoms produced by *Pectobacterium parmentieri* on potato field; (C) *P. parmentieri* PL70 forming pits on CVP media after 48 hours on CVP medium; (D) pits after 72 hours of incubation of *P. parmentieri* strains PL70 and PL72; (E) plant inoculated with *P. parmentieri* strains PL 70 and PL72 at 0 hour post inoculation; (F) plants displaying clear symptoms of blackleg and stem rot at 72 hours post inoculation with *P. parmentieri* strains PL 70 and PL72. Red arrows are highlighting the symptoms observed on naturally and artificially inoculated plants.

**Figure 2.** Phylogenetic analysis. Circular phylogenetic tree based on the *dnaA* gene, alignment of 109 strains covering all *Pectobacterium* species, was determined in MEGA X with a bootstrap test of 1,000 replicates. All positions containing gaps and missing data were eliminated. The evolutionary history was inferred using the Neighbor-Joining method, and *Dickeya dianthicola* IPO 2222 and *D. zeae* EC1 were included as an outgroup. *Pectobacterium* species type strains included in the analysis are indicated with light cyan triangle shapes associated with the names,
whereas, the red squares representing the eleven Hawaiian strains clustered together with the
other 18 *P. parmentieri* strains from different geographic origins. The *dnaA* sequences of all
species utilized in this dendrogram were retrieved from either complete or draft genomes
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References


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