Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium aroidearum* Causing Bacterial Soft Rot of Carrot in Taiwan

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Funding: This study was financially supported in part by the Advanced Plant Biotechnology Center from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan. Plant Dis. 105:695, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1824-PDN>. Accepted for publication 23 October 2020.

Carrot (*Daucus carota*) is an important root vegetable planted and consumed worldwide (Stein and Nothnagel 1995). In June 2020, carrots (cv. New Kuroda) showing soft rot symptoms were observed in a 600 sq ft plot located in Pitou, Changhua, Taiwan (23°54'00.9"N, 120°28'37.3"E; with around 400 plants). About 10% of the plants on site had similar symptoms; infected taproot tissues were macerated and emitted a foul odor. In most cases, the peels above the rotten tissues remained intact. Two infected plants were brought to the lab. Macerated tissues were suspended in water and examined under a microscope at 600× (without staining). Rod, motile bacteria were observed in all of the samples, and the bacteria were isolated onto nutrient agar. Three bacterial strains were obtained from two taproots; strain Car1 was isolated from one plant, and strains Car2 and Car3 were isolated from the other. Their colonies were translucent, round, and convex. All isolates could ferment glucose and induce soft rot symptoms on potato tuber slices (Schaad et al. 2001). They were not able to produce indigoidine on yeast dextrose calcium carbonate agar and tested negative for phosphatase activity (Schaad et al. 2001). The 16S rDNA of Car1 to Car3 was amplified using primers 27F/1492R (Lane 1991). Cloning and sequencing of their 16S rDNA (GenBank accession no. MT889640) revealed that their sequences shared 99.9% identity (1,463/1,464 bp) with that of *Pectobacterium aroidearum* CFBP 8168^T (SCRI 109^T; GenBank accession no. NR_159926.1). Multilocus sequence analyses targeting the three isolates' *dnaX*, *leuS*, and *recA* genes

were conducted. The concatenated sequences (1,596 bp) of Car1 to Car3 and those included in a previous work (Portier et al. 2019) were subjected to phylogenetic analysis. The sequences of Car1 to Car3 were identical (GenBank accession nos. MT892671 to MT892673). A maximum-likelihood tree showed that the three isolates belonged to the same clade as *P. aroidearum* CFBP 8168^T (GenBank accession nos. MK516971, MK517115, and MK517259). For the concatenated sequences analyzed, the identity between *P. aroidearum* CFBP 8168^T and our three isolates was 99.4% (1,587/1,596 bp). The pathogenicity of these isolates was determined by inoculating the bacteria into carrot (cv. Xiangyang No.2) taproots. Strains Car1 to Car3 were grown on nutrient agar for 48 h (28°C), and cell suspensions with OD₆₀₀ values of 0.3 (2.4×10^8 CFU/ml; in water) were prepared. The suspensions of each strain (100 µl) were loaded into 200-µl pipette tips. The tips were then pierced into intact carrot taproots (2.4 cm deep), ejected, and left on the plants (one tip per plant). Three taproots were tested for each strain. Tips loaded with 100 µl of water were used for the controls (three replicates). The plants were incubated in a sealed plastic container kept in a growth chamber set at 28°C. After 48 h, all of the inoculated taproots produced soft rot symptoms resembling those observed in the field, and plants in the control group did not. Bacteria were reisolated from macerated tissues of the artificially infected plants and found to share the same *leuS* sequence with Car1 to Car3. Occurrences of carrot soft rot in Taiwan have only been attributed to *Dickeya* spp. (*Erwinia chrysanthemi*) in previous studies (Hsu and Tzeng 1981). The present study is the first report of *P. aroidearum* infecting carrots in Taiwan. The findings may add to our understanding of the diversity of soft rot pathogens affecting carrot production in Taiwan.

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The author(s) declare no conflict of interest.

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Keywords: prokaryotes, vegetables, soft rot bacteria

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