Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Soft Rot Disease on Pak Choi (*Brassica rapa* subsp. *chinensis*) Caused by *Pectobacterium brasiliense* in the United States

Diksha Klair,¹ Gamze Boluk,¹ Joshua Silva,² Dario Arizala,¹ Shefali Dobhal,¹ and Mohammad Arif^{1,†}

¹ Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, Honolulu, HI 96822

² Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, Honolulu, HI 96822

Funding: This work was supported by the USDA National Institute of Food and Agriculture, Hatch project 9038H, managed by the College of Tropical Agriculture and Human Resources. Research was also supported by NIGMS of the National Institutes of Health under award number P20GM125508. Plant Dis. 105:2011, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1854-PDN>. Accepted for publication 3 March 2021.

Pak choi (*Brassica rapa* subsp. *chinensis*) is an important vegetable crop native to China, known for high water content and low caloric value, containing high quality of protein, carbohydrates, fiber, vitamins, minerals, and secondary plant metabolites (Acikgoz 2016). A pak choi field (8,000 sq. ft.) on Oahu, Hawaii, was visited in May 2020. About 10% plants were infected and showed characteristic symptoms of soft rot, wet lesions, macerated infected stem, and necrotic leaves, leading to the suspecting of one of the most devastating bacterial pathogens within genus *Pectobacterium* (Arizala and Arif 2019; Arizala et al. 2020; Boluk et al. 2020; Li et al. 2019). Four infected plants were collected from the field, and stems were surface sterilized with 0.6% sodium hypochlorite solution for 30 s, followed by three consecutive rinses in distilled water. The stems were aseptically macerated, streaked on crystal violet pectate medium (CVP) (Hélias et al. 2011), and incubated for 48 h at 26 ± 2°C. The peculiar morphological characteristic of pectolytic bacterial pathogen, forming pits on CVP, was observed (Meng et al. 2017). Purification of bacterial colonies were done by restreaking of a single colony on dextrose peptone agar (DPA, without tetrazolium chloride; Norman and Alvarez 1989). DNA was isolated from bacterial cultures using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, MA). Molecular identification of four strains (PL243 to PL246) was performed by the sequencing region of the housekeeping gene *dnaA* (chromosomal replication initiation protein) using Pec. *dnaA*-F1/R1 primer set (Dobhal et al. 2020). The amplified PCR product was enzymatically cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA)

and sent for sequencing at the Genewiz facility (La Jolla, CA) using both forward and reverse primers. The *dnaA* gene sequences were aligned using Geneious and manually edited to remove the errors. The consensus sequences were analyzed with the NCBI BLASTn tool and were deposited in NCBI GenBank under the accession numbers MT899920 to MT899923. The NCBI BLASTn report indicated that all the sequences shared 99 to 100% identity and query cover with *Pectobacterium brasiliense* accession numbers MN544627 to MN544629. A phylogenetic analysis, using Geneious, was performed with the *dnaA* sequences representing different *Pectobacterium* spp., and all strains grouped within the clade of *P. brasiliense* (Arizala et al. 2020). A pathogenicity assay was carried out in three replications on pak choi grown in pots containing commercial pot mixture and maintained in the controlled greenhouse (temperature 26 to 30°C; relative humidity 50 to 58%). Three-week-old plant stems were artificially inoculated with 100 µl of bacterial suspension of PL243 (1.3×10^8 CFU/ml), PL244 (1.2×10^8 CFU/ml), PL 245 (1.2×10^8 CFU/ml), and PL246 (1.1×10^8 CFU/ml); control plants were inoculated with 100 µl of distilled water. Two days after inoculation, soft rot and wilting symptoms similar to the ones observed in the field developed for all four strains tested. Bacteria were successfully reisolated from the inoculated plants; DNA was isolated, amplified, sequenced for the *dnaA* region, and analyzed for 100% homology with original strains, to fulfill Koch's postulates. Based on the molecular characteristics reisolates were identical to the original strains. To the best of our knowledge, this is the first report of *P. brasiliense* on pak choi in the United States. Recent reports indicated that the pathogen could potentially pose a threat to cruciferous crops, therefore highlighting a need to conduct a state-wide survey for pectinolytic bacteria and to implement better management strategies to combat the vegetable crop losses.

References:

- Acikgoz, F. E. 2016. Adv. Crop Sci. Technol. 4:233.
- Arizala, D., and Arif, M. 2019. Pathogens 8:247.
- Arizala, D., et al. 2020. Plant Dis. 104:2515.
- Boluk, G., et al. 2020. Plant Dis. 104:2721.
- Dobhal, S., et al. 2020. J. Appl. Microbiol. 128:1703.
- Hélias, V., et al. 2011. Plant Pathol. 61:339.
- Li, L., et al. 2019. BMC Genomics 20:486.
- Meng, X., et al. 2017. Plant Dis. 101:279.
- Norman, D., and Alvarez, A. 1989. Plant Dis. 73:654.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: pectinolytic bacteria, bacterial soft rot, pak choi, *Pectobacterium*, *Brassica*

[†]Indicates the corresponding author.
M. Arif; arif@hawaii.edu