Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pectobacterium brasiliense* Causing Soft Rot on Mizuna (*Brassica rapa* var. *japonica*) in the United States

Diksha Klair,¹ Joshua Silva,² Dario Arizala,¹ Gamze Boluk,¹ Shefali Dobhal,¹ Amjad A. Ahmad,² Jensen Uyeda,² Anne M. Alvarez,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. The strains are stored and maintained by the support of the National Science Foundation (NSF-CSBR grant no. DBI-1561663). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. Plant Dis. 105:4149, 2021; published online as <https://doi.org/10.1094/PDIS-03-21-0644-PDN>. Accepted for publication 7 May 2021.

Mizuna (*Brassica rapa* var. *japonica*), a member of family Brassicaceae, is a leafy vegetable having phenolic and other compounds beneficial to human health, such as natural antioxidants (Khanam et al. 2012). In October 2020, a field of mizuna (variety Early) on Oahu Island was observed having 20 to 30% diseased plants. Four randomly selected infected mizuna plants, showing the symptoms of wilt and stem rot, were collected and isolations were made to determine the pathogen. Small sections of infected stems were cut, surface sterilized with 0.6% sodium hypochlorite solution for 30 s, followed by three consecutive rinses in distilled water. The tissues were macerated in a sterile 1.5 ml centrifuge tube containing 100 µl sterile water. Macerated tissues were streaked onto crystal violet pectate medium (CVP) (Hélias et al. 2011) and incubated at 26 ± 2°C for 48 h. Isolated bacterial colonies that formed pits on the CVP plates were restreaked onto dextrose peptone agar: peptone (10 g/liter), dextrose (5 g/liter), and agar (17 g/liter) (DPA—without tetrazolium chloride; Norman and Alvarez 1989) to obtain purified colonies for DNA isolation using DNeasy Blood and Tissue Kit (Qiagen, Germantown, MA). The two housekeeping genes (*dnaA* and *gapA*) were amplified and sequenced following the protocols used by Dobhal et al. (2020) and Boluk et al. (2020), for identity confirmation and phylogenetic analysis. Cleaned PCR products were sent to the

GENEWIZ facility (Genewiz, La Jolla, CA) for sequencing of sense and anti-sense strands. The obtained sequences were aligned, manually edited, and consensus sequences were analyzed with BLASTn using the GenBank nucleotide and genome databases for identity confirmation. The BLASTn results demonstrated 100% query coverage of all four strains (PL248–PL251) and showed 100% identity of PL248 and PL249 and 99% identity of PL250 and PL251 with *Pectobacterium brasiliense*. All the sequences were submitted to GenBank under the following accession numbers: *dnaA* gene MW560271–MW560274 (PL248–PL251); *gapA* gene MW560275–MW560278 (PL248–PL251). Pathogenicity was assessed by artificially inoculating 100 µl bacterial suspension of each strain (PL248, 1.12 × 10⁸ CFU/ml; PL249, 1.32 × 10⁸ CFU/ml; PL 250, 1.2 × 10⁸ CFU/ml; and PL251, 1.15 × 10⁸ CFU/ml) onto 4-week-old mizuna (variety Leafy Asian Greens) plants in three replicates, using sterile pipette tips, which was stabbed into the stem halfway and wrapped with Parafilm. The inoculated plants were maintained under controlled greenhouse conditions. As negative controls, three plants were inoculated with 100 µl distilled water. Soft rot and wilt symptoms were observed 24 h post inoculation. No symptoms were observed on control plants. All four strains were reisolated from the inoculated plants and confirmed as *P. brasiliense* based on resequencing of the *dnaA* region and 100% homology with the sequences of original strain. In the phylogenetic tree, based on two housekeeping genes (*dnaA* and *gapA*), the bacterial strains from mizuna grouped with other *P. brasiliense* retrieved from GenBank. To our knowledge, this is the first report of *P. brasiliense* infecting mizuna plants in Hawaii or in the U.S.A. This is important because this species is one of the most aggressive pectolytic pathogens in the genus *Pectobacterium*. Understanding the diversity of different pectolytic phytopathogens is essential to formulating risk mitigation strategies as *P. brasiliense* could potentially pose a threat to additional vegetable crops, especially the cruciferous vegetables (Arizala and Arif 2019; Klair et al. 2021).

References:

- Arizala, D., and Arif, M. 2019. Pathogens 8:247.
- 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.
- Khanam, U. K. S., et al. 2012. J. Funct. Foods 4:979.
- Klair, D., et al. 2021. Plant Dis. 105:2011.
- Norman, D., and Alvarez, A. 1989. Plant Dis. 73:654.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: pectinolytic bacteria, mizuna soft rot, *Pectobacterium*, plant bacteria, vegetable crop

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