Diseases Caused by Bacteria and Phytoplasmas

First Report of *Pantoea stewartii* subsp. *stewartii* Causing Bacterial Leaf Wilt of Sugarcane in China

Dong Cui,¹ Mei-Ting Huang,¹ Chun-Yu Hu,¹ Jun-Bo Su,² Ling-Hong Lin,³ Talha Javed,¹ Zu-Hu Deng,¹ and San-Ji Gao^{1,†}

¹ National Engineering Research Center for Sugarcane, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

² South Subtropical Crops Research Institute, Chinese Academy of Tropical Agricultural Science, Zhanjiang, Guangdong 524091, China

³ Institute of Plant Protection, Fujian Academy of Agricultural Science, Fuzhou, Fujian 350013, China

D. Cui and M.-T. Huang contributed equally to this work.

Funding: This work was supported by an Earmark Fund from the China Agriculture Research System (CARS-170302). Plant Dis. 105:1190, 2021; published online as <https://doi.org/10.1094/PDIS-09-20-2015-PDN>. Accepted for publication 27 October 2020.

The pathogen of *Pantoea stewartii* subsp. *stewartii* (*Pss*), which is the causal agent of Stewart's bacterial wilt of corn, also infects numerous experimental hosts of graminaceous plants (Pepper et al. 1967; Wang et al. 2012). However, little is known about this pathogen naturally infecting sugarcane. In 2017, we observed some sugarcane cultivars showing leaf blade bleaching at the disease initiation stage, which further resulted in development of blight and necrotic lesions in Zhanjiang, Guangdong province of China. To diagnose this putative disease, five symptomatic leaf samples were collected from different sugarcane cultivars. The *Pss* was found to infect these samples using nested PCR with *Pss*-specific outer primers PS1/PS4 and inner primers Ps2r/Ps3r that targeted at the 16S rRNA gene of this pathogen (Wang et al. 2009). The expected 262-bp fragments from positive samples were amplified, cloned, and sequenced (GenBank accession nos. MW015795 to MW015799). BLASTN analysis revealed that these isolates had more than 99.5% nucleotide identity (222 bp out of 262 bp) with each other and with *Pss* strains (ATCC 8199 and DC283) as well as *P. stewartii* subsp. *indologenes* strains (SR2-12 and LMG 2632) after sequences were trimmed at the 5'- and 3'-terminal of inner primer sequences. In addition, these leaf samples were surface sterilized with 75% alcohol followed by being macerated and chopped in sterile water. Upon

plating on solid nutrient agar medium at 28°C for 24 to 36 h, the bacterial colonies exhibited yellow color with circular, convex, smooth, and translucent edges. Straight rods and nonencapsulated cells were detected under transmission electron microscopy. Moreover, an identical colony termed as PSCN1 was isolated from sugarcane cultivar YZ08-1095 and was further confirmed by the PCR with universal primer pair 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWTGT ACAAGGC-3') that targeted at bacterial 16S rRNA gene (Marchesi et al. 1998). A 1,362-bp DNA fragment sequence was obtained from PSCN1 strain and deposited in GenBank (accession no. MW015767). Sequence analysis showed that PSCN1 shared 99.9 to 100% nucleotide identity (1,315 bp out of 1,362 bp) with the two reference strains of *Pss* (ATCC 8199 and DC283) after sequences were trimmed at the 5'- and 3'-terminal of primer sequences. According to Koch's postulates, pathogenicity testing was carried out on YZ08-1095 plants with three to five fully developed leaves inoculated with the suspended cells (10⁸ cells/ml) of PSCN1 strain by cutting one-third of leaves before spraying with a suspension. Control plants were mock-inoculated with serial liquid nutrient agar medium. Two independent experiments were performed for the pathogenicity assay, and more than 28 plants of YZ08-1095 were used in each treatment. Plants were cultured in a growth chamber at 28°C and 60% humidity under a 16-h light/8-h dark photoperiod. Leaves inoculated by the PSCN1 initially showed bleached, blight, and wilting symptoms on leaf edges at 7 days postinoculation (dpi), which were similar to those symptoms observed in the fields. Control plants remained asymptomatic. The average incidence of diseased plants was 51.9% at 21 dpi. The bacteria were subsequently reisolated from diseased leaves, and yielded colonies were completely identical to PSCN1. Taken together, our data provide valuable information for diagnosis and controlling this disease in sugarcane.

References:

- Marchesi, J. R., et al. 1998. Appl. Environ. Microbiol. 64:795.
Pepper, E. H., 1967. Stewart's bacterial wilt of corn. Monograph No. 4. American Phytopathological Society, Heffernan Press, Inc., Worcester, MA.
Wang, X., et al. 2012. Mol. Microbiol. 83:195.
Wang, Y., et al. 2009. Acta Phytopathol. Sin. 39:368.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: pathogen identification, *Pantoea stewartii* subsp. *stewartii*, *Saccharum* spp. hybrids, Stewart's wilt

[†]Indicates the corresponding author.
E-mail: S.-J. Gao; gaosanji@fafu.edu.cn