Diseases Caused by Bacteria and Phytoplasmas

First Report of Stalk Bacterial Soft Rot of Sugarcane Caused by *Dickeya zeae* in China

Yanchang Yang,¹ Ziting Yao,¹ Muqing Zhang,^{1,2,3} Chengwu Zou,^{1,2,3,†} and Baoshan Chen^{1,2,3,†}

¹Guangxi Key Laboratory for Sugarcane Biology and College of Agriculture, Guangxi University, Nanning 530005, China

²State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, Guangxi University, Nanning 530005, China

³Ministry and Province Co-sponsored Center of Collaborative Innovation for Sugarcane Industry, Nanning 530005, China

Y. C. Yang and Z. T. Yao contributed equally to this work.

Funding: Funding was provided by the National Natural Science Foundation of China (31960519) and the Guangxi Postdoctoral Science Foundation of China (BH2018065). The funding bodies provided only the funds and did not have any role in the design of the study, sample collection, data analysis, interpretation, and writing of the manuscript. Plant Dis. 105:1188, 2021; published online as <https://doi.org/10.1094/PDIS-10-20-2234-PDN>. Accepted for publication 3 December 2020.

In late September 2019, seven stalks of ~1,400 stalks of sugarcane cultivar Zhongzhe 1 exhibited soft rot symptoms in a trial plot in Beihai city, Guangxi province, China. Symptoms included scorched and collapsed leaves, maceration of stalks, and sour-smelling exudates from the stalks. Severely diseased stalks had collapsed and were dead. Internal stalk fragments of 5 × 5 mm were collected at the junction of healthy and diseased tissue after surface sterilization of stalks with 70% ethanol for 1 min and three times rinsing with sterile distilled water (SDW). Stalk fragments were placed on Luria-Bertani agar medium (1% w/v tryptone, 0.5% w/v yeast extract, 1% w/v NaCl, 1% w/v agar, pH 7.0), and plates were put in an incubator at 30°C for 48 h. Four types of bacterial colonies were obtained, and small and white colonies with irregular margins were the most dominant. A single colony of each type was diluted in SDW, and aliquots of each suspension were streaked on fresh medium plates to obtain pure cultures. Ten 8-week-old stalks (11th leaf stage) of sugarcane plants, which derived from cuttings of symptomless Zhongzhe 1, were inoculated by injection of 300 µl of bacterial suspension (3.5×10^8 CFU/ml) into the stalks. Another 10 stalks were injected with pure water as a control. Inoculated plants were kept in a greenhouse at 25 to 37°C. Among the four types of bacteria, only strain BH9 induced symptoms identical to those of diseased canes observed in the field. Elongated water-soaked lesions were

observed around the inoculation sites 3 days postinoculation. Five of the 10 BH9-inoculated plants had collapsed 2 days later. Water-soaked stalks had a sour smell similar to the field-diseased plants. Eight days postinoculation, all BH9-inoculated plants exhibited symptoms, but control plants remained symptomless up to 30 days after inoculation. Uniform white colonies with irregular margins were isolated from the inoculated stalks that developed soft rot, and these bacteria caused stalk soft rot symptoms when inoculated to a new batch of 10 healthy plants. The 16S rRNA gene of strain BH9 was amplified by PCR with primer pair fD2/rP1, and the amplicons from three independent colonies were sequenced. The sequences of the three amplicons were identical (MT723897). BLAST alignments of the 16S rDNA sequence from BH9 with GenBank revealed that BH9 belonged to the genus *Dickeya* (98.5% identity between *D. zeae* BH9 and *D. zeae* EC1). Further PCR assays and sequencing of three genes, DNA polymerase III gamma subunit gene *dnaX* with primers *dnaXf/dnaXr*, DNA gyrase gene *gyrB* with primers *gyrBf1/gyrBr1*, and recombinase A gene *recA* with primers *recAf/recAr*, were performed to identify the species within the genus *Dickeya* (Zhang et al. 2014). BH9 sequences of these genes (MT723898 to MT723900) had highest identity (97.5, 97.6, and 97.7%, respectively) with those from *D. zeae* EC1 (CP006929.1). To determine the evolutionary relationship of BH9 to other *Dickeya* species and strains, a phylogenetic analysis was performed using *dnaX*, *gyrB*, and *recA* sequences. BH9 clustered with *D. zeae* strains and formed a lineage distinguishable from other *Dickeya* species. Among the closest strains, *D. zeae* NCPB3531 (CM001980.1) was isolated from potato and *D. zeae* CSL RW192 (CM001972.1) from river water (Pritchard et al. 2013). Consequently, strain BH9 was identified as *D. zeae*. This bacterial species has been reported to cause soft rot in rice (Pu et al. 2012), banana (Zhang et al. 2014), maize (Martinez-Cisneros et al. 2014), and clivia (Hu et al. 2018). To the best of our knowledge, this is the first report of a bacterial stalk rot caused by *D. zeae* in sugarcane. In fact, low incidence of *D. zeae*-caused stalk soft rot was recently found in sugarcane fields in Fusui County, about 150 km north to Beihai. Given the potential threat of this disease to the local sugarcane industry, the mode of transmission, cultivar resistance, and measures to control the disease should be investigated.

References:

- Hu, M., et al. 2018. BMC Microbiol. 18:136.
- Martinez-Cisneros, B. A., et al. 2014. Plant Dis. 98:1267.
- Pritchard, L., et al. 2013. Genome Announc. 1:e00978-13.
- Pu, X. M., et al. 2012. Plant Dis. 96:1818.
- Zhang, J. X., et al. 2014. Plant Dis. 98:436.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: sugarcane, stalk bacterial soft rot, *Dickeya zeae*

[†]Indicates the corresponding authors.

E-mail: zouchengwu@gxu.edu.cn (C. W. Z.) and B. S. Chen; chenyaaj@gxu.edu.cn