Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Hollow Stalk Caused by *Dickeya chrysanthemi* (syn. *Erwinia chrysanthemi*) on Tobacco in Nanping, China

W. Lin,^{1,2} J. Y. Liu,² Y. X. Feng,³ S. C. Zhang,¹ Q. Zhao,¹ Q. Lin,² and J. Wang^{1,†}

¹ Tobacco Research Institute of Chinese Academy of Agricultural Science, Qingdao, China

² Nanping Tobacco Company of Fujian Province, Nanping, China

³ China Tobacco Hebei Industrial Co., Ltd., Shijiazhuang, China

Funding: Funding was provided by the Fujian Agricultural Science and Technology Application Study (grant no. 201735070024072) and the Agricultural Science and Technology Innovation Program (ASTIP-TRIC04). Plant Dis. 104:3248, 2020; published online as <https://doi.org/10.1094/PDIS-06-20-1305-PDN>. Accepted for publication 16 July 2020.

Tobacco, *Nicotiana tabacum* L., family Solanaceae, is grown for its leaves (mature period). It originated in the Mesoamericas but today is cultivated globally. China produces about one-third of the world's supply of tobacco leaves and is the largest tobacco producer in the world (Hu et al. 2007). In the early summer of 2019, after tobacco was topped, typical tobacco hollow stalk disease symptoms were observed in 2 to 5% of the fields in Shaowu and Jianyang counties of Nanping, Fujian province, China. Symptoms consisted of lesions extending from the point of topping downward, resulting in a black hollow stem (harvest period). The symptoms were similar to tobacco hollow stalk disease caused by *Pectobacterium* species. Four infected stems of different tobacco plants were collected from Nanping (N 27°37'48", E 118°2'24") for isolation. Small sections from the stems were surface sterilized with 1% sodium hypochlorite for 3 min, rinsed three times in sterile distilled water (SDW), and then macerated in 400 µl of SDW. The suspensions were then streaked on nutrient agar and incubated at 28°C in darkness. After 2 days, two predominant isolates that produced circular, convex, small (<1 mm) colonies without pigmentation were purified. The Biolog Gen III Microplates system was then used to identify the putative pathogenic bacteria based on the results of the biochemical assays read by the plate reader and its associated software and database. After culturing for 48 and 60 h, Biolog analysis identified the two isolates as *Dickeya chrysanthemi* (SIM = 0.516, DIST = 7.224; and SIM = 0.589, DIST = 5.984). To confirm its identification, the

16S rDNA gene from strain NPEc1 was amplified with the general primer 27F/1492R (Frank et al. 2008). A BLASTn search in GenBank of this sequence (1,465 bp, accession no. MN640862) showed over 99% identity to the 16S rDNA of *D. chrysanthemi* (CP 001655). Partial sequences of housekeeping gene DNA polymerase III, subunits gamma and tau (dnaX), and the recombinase A (recA) gene were also analyzed according to the method of Suharjo et al. (2014). The 511-bp dnaX sequence (MT597420) and 736-bp recA sequence (MT597421) respectively matched 98.22 and 96.19% with *D. chrysanthemi* (syn. *Erwinia chrysanthemi*) strains CFBP 2048 (JX434939) and ICMP 5703 (DQ859873). For pathogenicity testing, six tobacco seedlings of the 45-day-old cultivar Yunyan 87 were planted in nutriculture at room temperature. After 3 days, approximately 20 µl of each bacterial suspension (10⁷ CFU/ml) of isolates NPEc1 and NPEc2 was injected into the leaf axils of three tobacco stems. As a control, similar seedlings were inoculated with SDW. Twelve hours after inoculation, symptoms of water-soaked decay appeared in the injected leaf axils. After 2 days, these symptoms developed into a severe rot similar to that of naturally infected plants. In contrast, the controls were symptomless. The bacterium was isolated from the rotten tissues and demonstrated a similar identity to the inoculants by the Biolog automated microbial identification system comparison, thus fulfilling Koch's postulates. The inoculation was repeated twice. On the basis of morphological, Biolog Gen III, and molecular characteristics and pathogenicity, the causal agent of a hollow stalk rot on tobacco in Nanping was identified as *D. chrysanthemi*. Previously, this pathogen was reported on tobacco in Cuba by Pérez (1981) and more recently isolated from poinsettia stems in China (Rungnapha et al. 2008). To our knowledge, this is the first report of *D. chrysanthemi* causing a hollow stalk disease of tobacco in China. Reference cultures have been deposited in CGMCC (no. MT211276). Further spread of the pathogen may pose a threat to tobacco production in Nanping and other regions in China.

References:

- Frank, J., et al. 2008. Appl. Environ. Microbiol. 74:461.
- Hu, T., et al. 2007. Int. J. Public Policy 2:235.
- Pérez, R. L. 1981. Cienc. Tec. Agric., Prot. Plant. 4:79.
- Rungnapha, K., et al. 2008. Plant Dis. 92:1135.
- Suharjo, R., et al. 2014. J. Gen. Plant Pathol. 80:237.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: tobacco, *Dickeya chrysanthemi*, tobacco hollow stalk

[†]Indicates the corresponding author.
E-mail: wjing323@163.com (J.W.)