Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Rot Caused by *Pantoea endophytica* on Tobacco in Liuyang, China

Naila Ilyas,^{1,2} Yingjie Yang,¹ Wanfeng Liu,³ Xiaoxu Li,³ Wenxuan Pu,³ Raghvendra Pratap Singh,^{4,†} and Yiqiang Li^{1,†}

¹ Chinese Academy of Agricultural Sciences Institute of Tobacco Research, Qingdao, Shandong, China, 266100

² Graduate School of Chinese Academy of Agricultural Sciences, Beijing 100081, China

³ China Tobacco Hunan Industrial Co., Ltd., Changsha, China, 410300

⁴ Department of Research & Development, Biotechnology, Uttaranchal University, Dehradun 248007, India

N. Ilyas and Y. J. Yang contributed equally to this article.

Funding: Funding was provided by Chinese Academy of Agricultural Sciences (ASTIP-TRIC07). Plant Dis. 105:4147, 2021; published online as <https://doi.org/10.1094/PDIS-04-21-0737-PDN>. Accepted for publication 7 June 2021.

Tobacco, *Nicotiana tabacum* L., is produced largely in China (~1/3 of the global market). In the monsoon summer of 2020, tobacco plant petioles, where axillary buds were removed, became black-rotten, and thick ooze appeared when squeezed. Lesions encompassed more than half of petiole circumference. Ten tobacco fields (100 plants/field) were investigated in Liuyang, China, and 5% disease severity was found in each infected field. Six infected stalk leaves of different tobacco plants were sampled from a severely infected field in Liuyang (N28°21', E113°52') and were surface sterilized (1% sodium hypochlorite for 3 min), rinsed thrice in sterile distilled water, ground, and streaked on Luria-Bertani agar (LBA). After 24 h at 28°C, circular and convex colonies appeared. One hundred colonies from 10 plates were picked, amplified, and sequenced with the primers 16S-27F/16S-1492R by colony PCR (Lane et al. 1991). The 16S rRNA sequences from 100 colonies were assembled and fell into two sequences, similar to either *Leclercia* sp. (86%) or *Pantoea* sp. (14%). Identification and homology search was done by BLASTn analysis against NCBI and the EzBio Cloud database (Yoon et al. 2017a). The *Pantoea* isolate HN-23 (1,408 bp, MW405831) and the other 16S sequence of 13 *Pantoea* showed 99.57%

identity to the type strain *Pantoea endophytica* 596^T (PJRT0100022) based on the EzBio Cloud database to identify novel bacteria. Colonies of HN-23 were smooth, translucent, convex with entire margin on LBA, and 1 and 3 mm (diameter), white to yellow, after 24 and 48 h, respectively, but white on nutrient agar. Phenotyping of HN-23 (S-1) was performed using API 20E and API ZYM system (bioMérieux, France) and found identical to *P. endophytica* 596^T (Gao et al. 2019). The draft genome of HN-23 (size 4.96 Mbp, total scaffold 79, Scaf N50 218,098 bp, and Scaf N90 61,041 bp) was studied by Illumina sequencing (JAFLWX000000000) and was found to have 98.24% nucleotide identity with the genome of *P. endophytica* type strain 596. Average nucleotide identity (ANI) values were calculated using the Ortho ANI algorithm (Yoon et al. 2017b). HN-23 had 83.89 and 83.65% ANI with *P. rodasii* LMG26273^T and *P. dispersa* CCUG25232^T, respectively (S-2). Six tobacco seedlings (cultivar K326, 30 cm height plants grown at greenhouse at 28°C and 70 to 80% humidity) were injected by 20 µl of culture (10⁹ CFU/ml) of HN-23 and three with dominant species *Leclercia* sp. HN-7, which were then reisolated from infected tissues. Pathogenic tissue extract and sterile water were also used as positive and negative control, respectively, and experiments were performed in triplicate. After 20 h, symptoms of water-soaked decay appeared in the injected leaf axils. After 2 days, a severe rot developed. The controls remained symptomless. The bacterium was then isolated from the rotten tissues, and identity was confirmed by 16S rDNA sequencing, thus fulfilling Koch's postulates. This species was also reported as an endophyte to be isolated from root, stem, and leaf of maize planted in diverse parts of China and identified as *P. endophytica*. To our knowledge, this is the first report of *P. endophytica* as a plant pathogen, which was first isolated from tobacco planted in southern China.

References:

- Gao, J. L., et al. 2019. Syst. Appl. Microbiol. 42:488.
- Lane, D. J., et al. 1991. Page 115 in: Nucleic Acid Techniques in Bacterial Systematics. Wiley, New York, NY.
- Yoon, S. H., et al. 2017a. Antonie van Leeuwenhoek 110:1281.
- Yoon, S. H., et al. 2017b. Inter. J. Sys. Evol. Microbiol. 67:1613.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: prokaryotes, field crops, pathogen detection, pathogen diversity

[†]Indicates the corresponding authors.

R. P. Singh; rpsingh@uttaranchaluniversity.ac.in, and
Y. Q. Li; liyiqiang@caas.cn