Diseases Caused by Bacteria and Phytoplasmas

First Report of New Bacterial Leaf Blight of Rice Caused by *Pantoea ananatis* in Southeast China

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In autumn 2020, leaf blight was observed on rice (*Oryza sativa* L., variety Zhongzao39, Yongyou9, Yongyou12, Yongyou15, Yongyou18, Yongyou1540, Zhongzheyu8, Jiafengyou2, Xiangliangyou900, and Jiyu351) in the fields of 17 towns in Zhejiang and Jiangxi Provinces, China. The disease incidence was 45 to 60%. Initially, water-soaked, linear, light brown lesions emerged in the upper blades of the leaves and then spread down to leaf margins, which ultimately caused leaf curling and blight during the booting-harvest stage. The disease symptoms were assumed to be caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), the pathogen of rice bacterial blight. Sixty-three isolates were obtained from the collected diseased leaves as previously described (Hou et al. 2020). All isolates showed circular, smooth-margined, yellow colonies when cultured on peptone sugar agar medium for 24 h at 28°C. The cells were all gram-negative and rod-shaped with three to six peritrichous flagella; positive for catalase, indole, glucose fermentation, and citrate utilization; and negative for oxidase, alkaline, phenylalanine deaminase, urease, and nitrate reductase reactions. 16S rRNA gene sequence analysis from the six isolates (FY43, JH31, JH99, TZ20, TZ39, and TZ68) revealed that the amplified fragments shared 98% similarity with *Pantoea ananatis* type strain LMG 2665^T (GenBank JFZU01). To further verify *P. ananatis* identity of these isolates, fragments of three house-keeping genes including *gyrB*, *leuS*, and *rpoB* from the six isolates were

amplified and sequenced, which showed the highest homology to LMG 2665^T with a sequence similarity of 95 to 100%. Primers (Brady et al. 2008) and GenBank accession numbers of gene sequences from the six isolates are listed in the supplementary materials. Phylogenetic analysis of *gyrB*, *leuS*, and *rpoB* concatenated sequences indicated that the six isolates were clustered in a stable branch with *P. ananatis*. Based on the above morphological, physiological, biochemical, and molecular data, the isolates are identified as *P. ananatis*. For pathogenicity tests, bacterial suspension at 10⁸ CFU/ml was inoculated into flag leaves of rice (cv. Zhongzao39) at the late booting stage using the clipping method. Water was used as a negative control. The clipped leaves displayed water-soaked lesions at 3 to 5 days after inoculation (DAI), and then the lesion spread downward and turned light brown. At about 14 DAI, blight was shown with similar symptoms to those samples collected from the rice field of Zhejiang and Jiangxi provinces. In contrast, the control plants remained healthy and symptomless. The same *P. ananatis* was reisolated in the inoculated rice plants, fulfilling Koch's postulates. In the past decade, *P. ananatis* has been reported to cause grain discoloration in Hangzhou, China (Yan et al. 2010) and induce leaf blight as a companion of *Enterobacter asburiae* in Sichuan province, China (Xue et al. 2021). Nevertheless, to the best of our knowledge, this is the first report of *P. ananatis* as the causative agent of rice leaf blight in southeast China. This study raises the alarm that the emerging rice bacterial leaf blight in southeast China might be caused by a new pathogen *P. ananatis*, instead of *Xoo* as traditionally assumed. Further, the differences of occurrence, spread, and control between two rice bacterial leaf blight diseases caused by *P. ananatis* and *Xoo*, respectively, need to be determined in the future.

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