Diseases Caused by Bacteria and Phytoplasmas

First Report of Soft Rot of *Aconitum carmichaelii* Caused by *Pectobacterium brasiliense* in China

Hao Zhang,¹ Yang Xu,^{2,3} Dan Zhao,⁴ Yuwen Yang,¹ Tingchang Zhao,¹ Wei Guan,^{1,†} and Tieli Wang^{2,†}

¹ State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

² State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

³ Institute of Healthcare China Academy of Chinese Medical Sciences, Nanchang, China

⁴ Institute of Chinese Medicinal Materials, Mianyang Academy of Agricultural Sciences, Mianyang, China

H. Zhang and Y. Xu contributed equally to this work.

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Aconitum carmichaelii Debx. is a Chinese traditional medicine herb that is widely planted in China. The processed lateral roots of *A. carmichaelii* are known as fuzi and are used for the treatment of pain and inflammation in the joints (Zhou et al. 2015). In July 2019, a high incidence (approximately 50 to 100%) of soft rot of *A. carmichaelii* was observed in several commercial fields in Jiangyou County of Sichuan Province, China. Soft rot brownish lesions developed on infected stems, leading to collapse and wilting of entire plants. From symptomatic plants, the margins between the diseased and healthy areas were cut into pieces (5 × 5 mm), which were surface sterilized using 75% ethanol for 30 s and 2% NaOCl for 1 min, followed by three rinses with sterile water. The sterilized sections were macerated in drops of sterile water, and the extract was streaked onto King's B (KB) agar medium and incubated for 48 h at 30°C. Single colonies that were round, convex and creamy on the plates after 2 days were streaked on KB agar plates. Ten bacterial strains were isolated, and the strain Fuzi915 was chosen for further analyses. The 16S rDNA gene sequence (GenBank accession MZ881946) amplified by primer pair 27F/1492R (Monciardini et al. 2002) showed 99.85% identity to the sequence of *Pectobacterium brasiliense* (syn. *P. carotovorum* subsp.

brasiliense) strain HNP201736 (MN393938.1) and *P. carotovorum* subsp. *carotovorum* strain PJP201706 (MN394020.1), respectively, and also showed 99.78% identity to *P. brasiliense* strain SX309 (CP020350.1). To further identify the Fuzi915 strain, the PCR assay was carried out using primer pairs Y1/Y2, EXPCCF/EXPCCR, and BR1f/L1r (De Boer and Ward 1995; Duarte et al. 2004; Kang et al. 2003), specific to *P. carotovorum*, *P. carotovorum* subsp. *carotovorum*, and *P. carotovorum* subsp. *brasiliense*, respectively. Specific fragments of 434 bp and 322 bp were amplified by the Y1/Y2 and BR1f/L1r primer sets, respectively, but there was no amplification by the EXPCCF/EXPCCR primer set, indicating that the Fuzi915 strain belongs to *P. carotovorum* subsp. *brasiliense* (Onkendi and Moleleki 2014). Additional phylogenetic trees based on two housekeeping genes, *mdh* (MZ892962) and *gapA* (MZ892963), were constructed using the maximum-likelihood method with 1,000 bootstraps. The Fuzi915 strain clustered with all *P. brasiliense* strains including type strain *P. brasiliense* BC1. Further, a pathogenicity test was conducted on healthy *A. carmichaelii* roots and seedlings maintained in a growth chamber at 25°C and 95% humidity. Root inoculation was followed by drenching 10⁷ CFU/ml of the cell suspension of Fuzi915 strain in soil surrounding the *A. carmichaelii* roots. Ten roots were inoculated with the cell suspension while 10 roots were drenching inoculated with sterile water as a negative control. Stem inoculation was followed by injecting 10³ CFU/ml of the cell suspension in the stem of 10 *A. carmichaelii* seedlings, while 10 were injected with sterile water as a negative control. After 5 days, *P. carotovorum* subsp. *brasiliense*-inoculated roots became brown and soft, and *P. carotovorum* subsp. *brasiliense*-inoculated seedlings became wilted and water soaked and started to collapse, similar to symptoms observed in the field. No symptoms were observed on the control plants inoculated with sterile water. The strain was reisolated successfully from symptomatic *A. carmichaelii* and was identified as *P. brasiliense* by using PCR with the same primers to complete Koch's postulates. To our knowledge, this is the first report of the soft rot of *A. carmichaelii* caused by *P. brasiliense* in China.

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[†]Indicates the corresponding authors.

W. Guan; wyngwan@yahoo.com, and T. Wang; wtl82@163.com