Diseases Caused by Bacteria and Phytoplasmas

First Report of *Stenotrophomonas maltophilia* Causing Root Soft Rot of Sanqi (*Panax notoginseng*) in China

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Funding: The study was supported by the National Key Research and Development Program of China (2017YFD0201604), the Fang Rongxiang Academician Workstation of Yunnan Province (2018IC077), and the Open Research Program of State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan (2021KF010). Plant Dis. 106:755, 2022; published online as <https://doi.org/10.1094/PDIS-07-21-1353-PDN>. Accepted for publication 17 August 2021.

Sanqi (*Panax notoginseng* [Burk.] F. H. Chen) is a traditional Chinese medicinal plant with a long planting cycle of 2 to 3 years that makes it vulnerable to root diseases caused by several pathogens, including *Fusarium solani*, *Alternaria panax*, *Phytophthora cactorum*, and *Pseudomonas* sp. In April 2019, root soft rot samples of sanqi were collected from a plantation site in Songming, southwest of China. Typical symptoms included root softening and necrosis, yellow leaf, and stem wilting. Ten diseased roots samples were collected and sterilized with 0.1% HgCl₂ for 1 min followed by 75% ethanol for 2 min and then rinsed thrice with sterile water. Sterilized roots were cut into small pieces of 5 × 5 mm and cultured on nutrient agar medium for 48 h at 28°C. From the root cultures, a total of 13 bacterial strains were obtained. Three strains (SM2-5, SM2-13, and SM2-14) were selected for further study. These three strains were gram-negative, short rod-shaped (1 to 2 × 0.5 to 1 µm), non-spore-forming, and had polar tufted flagella, as observed under a transmission electron microscope. Also, the strains were positive for oxidase, beta-galactosidase, arginine dihydrolase, and lysine decarboxylase and were negative for amylase and urease tested by biochemical methods (Wang 2017). To further determine the pathogenic species, genomic DNA of these three strains was extracted using a Genomic DNA Kit (Tsing Ke, Beijing, China) to PCR amplify 16S rDNA using universal primers 27F/1492R (Wang 2017). Also, *Stenotrophomonas maltophilia* 23S rDNA specific primers SM1/SM4 (Whitby et al. 2000) were used for PCR amplification to confirm the species. 16S rDNA sequence analysis showed that SM 2-5 (GenBank accession

no. MW555227), SM 2-13 (GenBank MW555228), and SM 2-14 (GenBank MW555229) shared the highest identity (>99.9%) with the *S. maltophilia* strains (GenBank MT323142, MH669295, and MN826555). Furthermore, 23S rDNA sequence analysis of SM 2-5 (GenBank MZ707732), SM 2-13 (GenBank MZ645941), and SM 2-14 (GenBank MZ707733) revealed high identity (>99.8%) with the *S. maltophilia* species. 16S and 23S rDNA phylogenetic analysis (Mega 6.06) using the neighbor-joining method with 1,000 bootstrap replicates revealed the three strains clustering with the other *S. maltophilia* strains. Therefore, based on morphology, metabolic profile, and sequence analysis, the three strains were identified as *S. maltophilia*. To test pathogenicity, the strains were grown in nutrient broth medium for 48 h at 28°C until bacterial suspension reached OD₆₀₀ ≈ 1.0 (2.0 × 10⁹ CFU/ml). Then, healthy roots of 1-year-old sanqi plants, prewashed with sterilized water and poked with a sterilized needle, were soaked in bacterial suspension (2.0 × 10⁹ CFU/ml) of the three strains separately for inoculation (10 min). Sterilized water treatment was used as a control. Subsequently, bacteria-inoculated plants were planted in sterile soil pots and cultured in a greenhouse at 28°C with a shading rate of 70%. Each treatment group included three plants with three replicates. Ten days postinoculation, symptoms similar to the ones in natural conditions were observed in the bacteria-inoculated plants. Based on the disease index (Li et al. 2020), we found that among the three strains, SM 2-13 displayed the highest virulence, whereas no symptoms were observed in the control plants. The same bacterial strains were reisolated from these inoculated roots and identified by the methods described above. Previous studies showed that some *Stenotrophomonas* species cause plant diseases such as rice white stripe (Singh et al. 2001), strawberry leaf black spot (Wang 2017), *Cyclobalanopsis patelliformis* leaf spot (Bian et al. 2020), and *Jatropha curcas* L. seed borne and stem necrosis (Wang et al. 2018). To our knowledge, this is the first report confirming *S. maltophilia* causing root soft rot of *P. notoginseng* in China.

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The author(s) declare no conflict of interest.

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Keywords: *Panax notoginseng*, *Stenotrophomonas maltophilia*, pathogens, soft rot

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