

Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Soft Rot Disease on Taro Caused by *Dickeya fangzhongdai* in China

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Taro (*Colocasia esculenta* [L.] Schott.) is an important root crop in the world with great economic value. In recent years, outbreaks of soft rot were observed on taro plants in several plantation areas located in Shaoguan, Guangdong Province, China (25°7'57" N, 113°19'5" E). Root tubers of taro (Paodan variety) infected by soft rot had water-soaked lesions with a dark brown to black margin including a rotten smell; they also had internal rot that was also found in root tubers with no external symptoms. In some areas, the incidence of soft rot can reach up to 30%. To isolate the causal agent, 10 pieces of taro root tubers with typical symptoms were surface sterilized with 75% ethanol and 0.1% HgCl₂ solution and then washed thrice with sterile water. The tuber slices were soaked in 50 ml of sterile water and shaken at 28°C and 200 rpm for 2 h, and 100 µl was streaked onto modified yeast extract beef (YEB) agar medium (1% peptone, 0.5% yeast extract, 0.5% sucrose, 0.5% NaCl, 1 mmol/liter MgSO₄·7H₂O, 1.5% agar, pH 7.0) plates (Zhou et al. 2011) and incubated at 28°C for 24 h. Single colonies grown on YEB were selected for preliminary inoculation onto healthy taro (Paodan variety) slices. Two of the gram-negative bacteria, named as ZXC1 and MPC2, developed symptoms consistent in rotted decay inside the root tubers after incubation for 24 h at 30°C. ZXC1 and MPC2 were biochemically profiled using a Biolog Gen III MicroPlate (Microlog 3, 5.2) (Shen et al. 2019) and were identified as *Dickeya* sp. (SIM 0.856 and 0.704). To determine the

species of the *Dickeya* isolates, 16S rRNA sequences were amplified by primers 27F and 1492R (Hauben et al. 1998). Housekeeping genes including *gyrB*, *atpD*, *rpoB*, and *infB* were also amplified using degenerate primers (Brady et al. 2008). Results from the BLASTn analysis of the 16S rRNA (GenBank accession nos. MN853405 and MN853406), *gyrB* (MN866299 and MN866303), *atpD* (MN866298 and MN866302), *rpoB* (MN866301 and MN866305), and *infB* (MN866300 and MN866304) genes in the isolates ZXC1 and MPC2 showed 99% identities to those of the previously reported *Dickeya fangzhongdai* isolates from *Phalaenopsis* (Zhang et al. 2018). Multi-locus sequence analysis by MEGA 7.0 performed with four housekeeping genes (*gyrB*, *atpD*, *rpoB*, and *infB*) showed that they clustered with *D. fangzhongdai* isolates. Analyses using scanning and transmission electron microscopy showed that ZXC1 and MPC2 bacteria were rod-shaped, 0.5 to 1.0 × 1.0 to 3.0 µm, with peritrichous flagella. Pathogenicity tests were performed thrice using surface-sterilized 2-month-old taro seedlings (Paodan variety). Six individual seedlings were inoculated using a sterile syringe with 10 µl of bacterial suspension (10⁸ CFU/ml) in Tris buffer (0.1 mol/liter Tris and 0.1 mol/liter HCl, pH 7.4). Taro seedlings injected with sterile Tris buffer were used as the negative control. These taro seedlings were grown in the greenhouse (30 ± 2°C, 90 ± 5% relative humidity). At the 25th day post-inoculation, soft rot symptoms were observed in inoculated taro, whereas all control taro plants remained symptom-free. Colonies that were small and pale yellow with irregular margins, consistent with morphological characteristics of *D. fangzhongdai*, were reisolated from symptomatic taro tubers, and the presence of the housekeeping genes was verified by sequencing as described above, fulfilling Koch's postulates. *D. fangzhongdai* is a newly emerging bacterial pathogen, which causes bleeding cankers in pear trees (Tian et al. 2016) and soft rot of *Phalaenopsis* (Zhang et al. 2018). This is the first report of *D. fangzhongdai* causing soft rot disease in taro. Considering the high incidence of soft rot, this pathogen might pose a significant threat to taro and other economically important crops. Therefore, further research is needed to investigate the host range of the pathogen and develop appropriate integrated management to contain this disease from spreading.

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