Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Leaf Spot of Coriander Caused by *Pseudomonas syringae* pv. *coriandricola* in China

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Coriander (*Coriandrum sativum* L.) or Chinese parsley is a culinary herb with multiple medicinal effects that is widely used in cooking and traditional medicine. From September to November 2019, symptoms were observed in 2-month-old coriander plants from fields in Lanzhou and Wenzhou, China. The disease developed rapidly under cold and wet climatic conditions, and the infection rate was almost 80% in open coriander fields. Typical symptoms on leaves included small, water-soaked blotches and irregular brown spots surrounding haloes; as the disease progressed, the spots coalesced into necrotic areas. Symptomatic leaf tissue was surface sterilized, macerated in sterile distilled water, and cultured on nutrient agar (NA) plates at 28°C for 48 h (Koike and Bull 2006). After incubation, six bacterial colonies, which were individually isolated from samples from two different areas, were selected for further study. Colonies on NA plates were small, round, raised, white to cream-colored, and had smooth margins. All bacterial isolates were gram-negative, rod-shaped, and nonfluorescent on King's B medium. The bacteria were positive for levan production, Tween 80 hydrolysis, and tobacco hypersensitivity but negative for oxidase, potato slice rot test, arginine dihydrolase, ice nucleation activity, indole production, and H₂S production. A suspension of a representative isolate for inoculating plants was obtained from a single colony on King's B medium for 2 to 3 days at 28°C. DNA was extracted from bacterial suspensions of YS2003200102 cultured in 20 ml of King's B medium broth at 28°C for 1 day. Extraction was

performed with a TIANamp Bacterial DNA Kit (TIANGEN, China) according to the manufacturer's recommendations. The pathogen was confirmed by amplification and sequencing of the glyceraldehyde-3-phosphate dehydrogenase A (*gapA*) gene, the citrate synthase (*gltA*) gene, the DNA gyrase B (*gyrB*) gene, and the RNA polymerase sigma factor 70 (*rpoD*) gene using *gapA*-For/*gapA*-Rev, *gltA*-For/*gltA*-Rev, *gyrB*-For/*gyrB*-Rev, and *rpoD*-For/*rpoD*-Rev primers, respectively (Popović et al. 2019). The sequences of the PCR products were deposited in GenBank with accession numbers MZ681931 (*gapA*), MZ681932 (*gltA*), MZ681933 (*gyrB*), and MZ681934 (*rpoD*). Phylogenetic analysis of multiple genes (Xu and Miller 2013) was conducted with the maximum likelihood method using MEGA7. The sequences of our isolates and 10 published sequences of *P. syringae* pv. *coriandricola* were clustered into one clade with a 100% confidence level. To confirm the pathogenicity of isolate YS2003200102, 2-month-old healthy coriander plants were inoculated by spraying the leaves with a bacterial suspension (10⁸ CFU ml⁻¹) at 28°C incubation temperature and 70% relative humidity, and sterile distilled water was applied as a negative control treatment (Cazorla et al. 2005). Three replicates were conducted for every isolate, and each replicate included six coriander plants. After 12 days, only the inoculated leaves with bacterial suspension showed bacterial leaf spot resembling that observed on naturally infected coriander leaves. Cultures reisolated from symptomatic leaves showed the same morphological characteristics and molecular traits as those initially isolated from infected leaves in the field. This bacterium was previously reported causing leaf spot of coriander in India and Spain (Cazorla et al. 2005; Gupta et al. 2013). To our knowledge, this is the first report of *P. syringae* pv. *coriandricola* causing leaf spot disease on coriander in China. Studies are needed on strategies to manage *P. syringae* pv. *coriandricola* in crops, because its prevalence may cause coriander yield loss in China.

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