Disease Note

Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Canker Caused by *Pseudomonas syringae* pv. *morsprunorum* Race 1 on Sweet Cherry in Chile

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Chile is the main exporter of sweet cherries (*Prunus avium*), with a total of 228,600 t exported in the 2019 to 2020 season, and a production from the Coquimbo to the Aysén region (http://www.iqonsulting.com/yb/). In January 2019, cherry trees from a commercial orchard located near Osorno city (40°37′S, 72°54′W), Region de Los Lagos, Chile, showed symptoms such as the presence of wood cankers, necrotic spots in leaves, and premature defoliation, with a mean disease incidence near 40%. Symptomatic leaves with necrotic spots were collected for analysis, from which all the necrotic spots were extracted by incision with a sterile scalpel and macerated in 30 ml of adjusted phosphate AFT buffer (Jain et al. 2020), and subsequently 100 µl of the suspension was plated on King's B (KB) agar and incubated for 48 to 72 h at 27°C, obtaining a total of two bacterial colonies, identified as 7684.1 and 7684.2. Afterward, each colony was stroked in a new KB agar plate and incubated for 16 h at 27°C, and the obtained biomass was used in subsequent experiments. In KB agar, both colonies exhibited fluorescence under UV light and, according to the LOPAT method (Lelliott et al. 1966), they were gram negative, positive to levan and tobacco hypersensitivity tests, negative to oxidase, potato soft rot, arginine dihydrolase, and gelatin tests, and were confirmed as *Pseudomonas syringae*. Then, the 16s and *gyrB* genes of each isolate were amplified by PCR, sequenced, and compared with the NCBI GenBank database (Sarkar and Guttman 2004; Weisburg et al. 1991), finding a 99.93% genetic similarity (1,064/1,065) with a previously reported 16s sequence of a *P. syringae* pv. *morsprunorum* isolate (accession no. CP026558.1) and 99.69% (636/638) with a previously reported *gyrB* gene of *P. syringae* pv. *morsprunorum* (accession no. LC364094.1), respectively. Additionally, the closest pathovar different to *morsprunorum* aligned with our *gyrB* sequence was *P. syringae* pv. *aesculin*, with 97.8% identity (624/638). Our sequences were deposited in GenBank with the accession numbers MN528473 (16s) and MN535696 (*gyrB*) for 7684.1, and MN528474 (16s) and MN535697 (*gyrB*) for 7684.2. To identify if the isolates correspond to *P. syringae* pv. *morsprunorum* races 1 or 2, race-specific conventional PCR and qPCR assays were carried out using the specific primers described by Kałużna et al. (2016), showing that the two isolates were positive to *P. syringae* pv. *morsprunorum* race 1 in both PCR assays. Pathogenicity was tested by inoculating immature cherry fruitlets (cv. Sweetheart) with bacterial suspension at 10^8 CFU/ml. For each strain, 10 fruitlets were inoculated by pricking with a sterile needle previously immersed in the bacterial suspension (Ruinelli et al. 2019). Sterile distilled water was used as a negative control. Seven to 14 days postinoculation, necrotic and water-soaked brown lesions with yellow margins were observed on the fruits inoculated with bacterial strains. The pathogen was reisolated and confirmed as *P. syringae* pv. *morsprunorum* by 16s and *gyrB* sequencing, and as race 1 by race-specific PCRs. Our results were confirmed by the National Plant Protection Organization, (Servicio Agrícola y Ganadero de Chile [SAG]), generating the first report of *P. syringae* pv. *morsprunorum* race 1 in Chile. Thus, SAG established new protocols for quarantine of absent pests in the national territory (Resol. No. 3080, SAG, Chile), and an immediate phytosanitary program for *P. syringae* pv. *morsprunorum* (Resol. Exenta No. 8948/2019, SAG, Chile). In conclusion, our discovery contributes to the monitoring and control of the disease in Chile.

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