Diseases Caused by Bacteria and Phytoplasmas

First Report of Bacterial Leaf Blight Caused by *Xanthomonas hortorum* pv. *carotae* on Carrots in Spain

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Plant Dis. 105:2712, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2493-PDN>. Accepted for publication 19 February 2021.

In September 2019, symptoms resembling those of bacterial leaf blight were observed on carrot plants (*Daucus carota* L. subsp. *sativus* Hoffm.) cultivar Romance cultivated in commercial plots in Chañe (Segovia), Spain. Symptoms were observed in two plots surveyed representing 3 ha, with an incidence greater than 90%, and also in some plots in other nearby municipalities sown with the same batch of seeds. The lesions observed at the ends of the leaves were initially yellow that became dark brown to black with chlorotic halos on leaflets and turned necrotic. Yellow, *Xanthomonas*-like colonies were isolated onto YPGA medium (Ridé 1969) from leaf lesions. Two bacterial isolates were selected and confirmed by real-time PCR using a specific primer set for *Xanthomonas hortorum* pv. *carotae* (Temple et al. 2013). All isolates were gram-negative, aerobic rods positive for catalase, able of hydrolyzing casein and aesculin and growing at 2% NaCl, and they were negative for oxidase and urease tests. Sequences of 16S rRNA gene showed 100% similarity with *Xanthomonas campestris*, *X. arboricola*, *X. gardneri*, and *X. cynarae* strains (GenBank accession nos. MW077507.1 and MW077508.1 for the isolates CRD19-206.3 and CRD19-206.4, respectively). However, the resulting phylogeny of multilocus sequence analysis of a concatenation of the housekeeping genes *atpD*, *dnaK*, and *efp* (Bui Thi Ngoc et al. 2010), by using neighbor-joining trees generated with 500 bootstrap replicates, grouped the

two isolates (GenBank accession nos. MW161270 and MW161271 for *atpD* for the two isolates, respectively; MW161268 and MW161269 for *dnaK*; MW161272 and MW161273 for *efp*) with the *X. hortorum* pv. *carotae* M081 strain (Kimbrel et al. 2011). A pairwise identity analysis revealed a 100% identity between all three isolates. Pathogenicity of the isolates was tested by spray inoculation (Christianson et al. 2015) with a bacterial suspension (10^8 CFU/ml) prepared in sterile distilled water at the three- to four-true-leaf stage (six plants per isolate). Sterile distilled water was used as a negative control. The inoculated plants were incubated in a growth chamber (25°C and 95% relative humidity [RH]) for 72 h and then transferred to a greenhouse at 24 to 28°C and 65% RH. Characteristic leaf blight symptoms developed on inoculated carrot plants, whereas no symptoms were observed on the negative control plants 20 days after inoculation. The bacterium was reisolated from symptomatic tissue and the identity confirmed through PCR analysis. Based on PCR, morphological and phenotypic tests, sequence analysis, and pathogenicity assays, the isolates were identified as *X. hortorum* pv. *carotae*. To our knowledge, this is the first report of bacterial leaf blight of carrot caused by *X. hortorum* pv. *carotae* in Spain, and the first molecular and pathological characterization. It is important to early detect this pathogen and take suitable measures to prevent its spread, because it could cause yield losses for a locally important crop such as carrot.

References:

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The author(s) declare no conflict of interest.

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Keywords: carrot, *Daucus carota* L. subsp. *sativus*, leaf blight, *Xanthomonas hortorum* pv. *carotae*, seed borne pathogen, diagnostics, multilocus sequence analysis, pathogenicity tests, Spain

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