First report of bacterial leaf blight caused by *Xanthomonas hortorum* pv. *carotae* on carrots in Spain

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In September 2019, symptoms resembling those of bacterial leaf blight were observed on carrot plants (*Daucus carota* L. subsp. *sativus* Hoffm.) cv. Romance cultivated in commercial plots in Chañe (Segovia), Spain. Symptoms were observed in two plots surveyed representing three hectares, 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 develop dark brown to black with chlorotic halos on leaflets that 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, while were negative for oxidase and urease tests. Sequences of 16S rRNA gene showed 100% similarity with *Xanthomonas campestris,* X. *arboricola, X. gardneri, X. cynarae* strains (GenBank accession numbers: MW077507.1 and MW077508.1 for the isolates CRD19-206.3 and CRD19-206.4, respectively). However, the resulting phylogeny of multilocus sequence analysis (MLSA) of a concatenation of the housekeeping genes *atpD*, *dnaK*, and *efp* (Bui Thi Ngoc et al. 2010), by using neighbour-joining trees generated with 500 bootstrap replicates, grouped the two isolates with the *X. hortorum* pv. *carotae* M081 strain (Kimbrel et al. 2011) (GenBank accession numbers: MW161270 and MW161271 for *atpD* for the two isolates, respectively; MW161268 and MW161269 for *dnaK*; MW161272 and MW161273 for *efp*). 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 3 to 4 true-leaf stage (six plants per isolate). Sterile distilled water was used as 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, while no symptoms were observed on the negative control plants 20 days after inoculation. The bacterium was re-isolated 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, since it could cause yield losses for a locally important crop such as carrot.

References:

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Fig. 1S. Symptoms of bacterial leaf blight observed on carrot plants in commercial plots in Chañe (Segovia, Spain). (A) Leaves presented dark brown to black coloration with chlorotic halos on leaflets that turned necrotic; (B) detail of the lesions.


X arboricola juglandis (CFBP 7303)
X arboricola juglandis (CFBP 7304)
X arboricola juglandis (CFBP 7302)
X arboricola juglandis (CFBP 7301)
X arboricola juglandis (CFBP 7300)
X arboricola juglandis (CFBP 7299)
X arboricola juglandis (CFBP 7297)
X arboricola juglandis (CFBP 7295)
X arboricola juglandis (CFBP 7179)
X arboricola juglandis (CFBP 2564)
X arboricola juglandis (CFBP 2528)
X arboricola juglandis (CFBP 8254)
X arboricola juglandis (CFBP 7298)
X arboricola juglandis (CFBP 7294)
X arboricola juglandis (CFBP 7296)
X arboricola juglandis (CFBP 7072)
X arboricola juglandis (CFBP 176)
X arboricola juglandis (CFBP 2568)
X arboricola juglandis (CFBP 2632)
X arboricola juglandis (CFBP 6557)
X arboricola juglandis (CFBP 7071)
X arboricola juglandis (CFBP 7244)
X arboricola juglandis (CFBP 8253)
X arboricola (CFBP 7152)
X arboricola (CFBP 4023)
X arboricola celebensis (CFBP 3523)
X arboricola fragariae (CFBP 3523)
X arboricola celebensis (CFBP 7150)
X arboricola (CFBP 4021)
X arboricola zantedeschiae (CFBP 7410)
X arboricola zantedeschiae (CFBP 7411)
X arboricola arracaciae (CFBP 7403)
X arboricola arracaciae (CFBP 7404)
X arboricola arracaciae (CFBP 7405)
X arboricola arracaciae (CFBP 7406)
X arboricola arracaciae (CFBP 7407)
X arboricola (CFBP 7419)
X arboricola (CFBP 1022)
X arboricola (CFBP 7154)
X arboricola (CFBP 7278)
X arboricola corylina (CFBP 1848)
X arboricola corylina (CFBP 6600)
X arboricola corylina (CFBP 1847)
X arboricola corylina (CFBP 2565)
X arboricola corylina (CFBP 6101)
X arboricola corylina (CFBP 1159)
X arboricola corylina (CFBP 1846)
X arboricola corylina (CFBP 5956)
X arboricola pruni (CFBP 3893)
X arboricola pruni (CFBP 3898)
X arboricola pruni (CFBP 2535)
X arboricola pruni (CFBP 411)
X arboricola pruni (CFBP 3900)
X arboricola pruni (CFBP 3901)
X arboricola pruni (CFBP 3921)
X arboricola pruni (CFBP 5229)
X arboricola pruni (CFBP 5529)
X arboricola pruni (CFBP 5580)
X arboricola pruni (CFBP 5722)
X arboricola pruni (CFBP 5723)
X arboricola pruni (CFBP 5724)
X arboricola pruni (CFBP 6653)
X arboricola pruni (CFBP 7098)
X arboricola pruni (CFBP 7099)
X arboricola pruni (CFBP 7100)
X arboricola zantedeschiae (CFBP 7412)
X arboricola (CFBP 6683)
X arboricola fragariae (CFBP 6770)
X arboricola fragariae (CFBP 6771)
X arboricola (CFBP 7417)
X arboricola (CFBP 7418)
X arboricola (CFBP 7415)
X arboricola (CFBP 7416)
X arboricola fragariae (CFBP 3549)
X arboricola (CFBP 7414)
X arboricola fragariae (CFBP 6772)
X arboricola (CFBP 7413)
X arboricola fragariae (CFBP 6762)
X arboricola fragariae (CFBP 6763)
X arboricola populi (CFBP 3121)
X arboricola populi (CFBP 3122)
X arboricola populi (CFBP 3124)
X arboricola populi (CFBP 3343)
X arboricola populi (CFBP 3342)
X arboricola populi (CFBP 3344)
X arboricola populi (CFBP 2986)
X arboricola populi (CFBP 3004)
X arboricola populi (CFBP 2113)
X arboricola populi (CFBP 2669)
X arboricola populi (CFBP 3338)
X arboricola populi (CFBP 2983)
X arboricola populi (CFBP 3123)
X arboricola populi (CFBP 2985)
X arboricola populi (CFBP 2666)
X arboricola populi (CFBP 3839)
X hortorum carotae (M081)
CRD19-206.3
CRD19-206.4
X arboricola guizotiae (CFBP 7408)
X arboricola guizotiae (CFBP 7409)
0.01

Fig. 2S. Complete neighbor joining tree of concatenated, nucleotide sequences for partial *atpD*, *dnaK*, and *efp* genes from 100 *Xanthomonas* strains (Kumar et al. 2018). Numbers indicate bootstrap support (*r* = 500). Evolutionary analyses were conducted in MEGA X (Young et al 2018). The scale bars indicate the number of amino acid substitutions per site.

References:

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