New Disease Reports

First report of *Xanthomonas campestris* causing black rot of chard in Cuba

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In March 2016, leaves of chard (*Beta vulgaris* var. *cicla*) with blackened veins and v-shaped necrotic lesions on the leaf margins surrounded by yellow halos, were collected from plants growing in the "Organoponico" (a system of urban organic agriculture) in Havana Province, Cuba (Figs. 1-2). The symptoms were similar to those observed in black rot disease on cabbage.

To identify the pathogen involved, symptomatic leaves were rinsed with distilled water, surface disinfected using 70% ethanol for 30 seconds, rinsed again with sterile distilled water, and dried at room temperature in a biosafety cabinet. Small sections (less than 1 cm^2) from the margins of the necrotic tissue were macerated in sterile 0.85% NaCl solution. Ten-fold dilutions were made of the macerates and 70 µl aliquots were streaked onto yeast extract-dextrose-calcium carbonate (YDC) agar (Wilson *et al.,* 1967). The plates were incubated at 28°C for 48 hr. The bacterial colonies were yellow mucoid, circular and convex. The five representative isolates that were tested were all Gram-negative rods, catalase-positive and oxidase-negative. Standard microbiological tests were performed on the isolates (Schaad *et al*., 2001) and indicated that they hydrolysed starch, esculin, gelatin and Tween 80. The isolates were able to use cellobiose, trehalose, glucose, mannose, raffinose, rhamnose, indol, inositol, and sorbitol for growth. The test results indicated that the isolates from chard were *Xanthomonas campestris* (*Xc*).

Strain pathogenicity was evaluated by spraying 10^7 CFU/ml (OD600nm approximately 0.05, T60 UV PG Instruments) of the isolates (grown as pure cultures in YDC for 48 hr at 28°C) in a sterile 0.85% NaCl solution onto the leaves of 15-day old chard plants, cultivar 'White Ribbed'. The plants were grown at 25°C, with 16 hr light and 8 hr darkness in 15-cm plastic pots containing a mixture of 1:1 of sterile soil (Ferralsol eútrico, pH 5.5) and organic material (composted garden waste). A 0.85% NaCl solution was sprayed on the leaves as a negative control. The plants were maintained at a relative humidity >80%, for 48 hr. Symptom development was checked daily. V-shaped, necrotic lesions surrounded by yellow halos developed on the leaf margins 3 to 4 days after inoculation. No symptoms were observed in negative control plants.

The bacteria that were re-isolated from symptomatic plants had the same cultural, physiological and biochemical characteristics as those used for inoculation, thus fulfilling Kochs postulates. Two representative isolates, Chard1 and Chard3 were further characterised by amplifying and sequencing their 16SrDNA and DNA gyrase subunit B (*gyr*B) genes (Hauben *et al*., 1997; Parkinson *et al*., 2009). The sequences were deposited in GenBank, under Accession Nos. MF423473, MF423474, KY770953 and KY964489. BLAST analyses of the 16SrRNA and *gyr*B gene sequences showed the greatest identity with *Xanthomonas campestris* pv. *campestris* (*Xcc*) strains ATCC33913 (AE008922), ICMP4013 (CP012146), ICMP21080 (CP012145), 8004 (CP000050) and XccRC2 (KT964517) (99% homology). However, the 16SrRNA and *gyr*B gene sequence information could not be used to identify the pathovar of the *Xanthomonas* strains (Tian *et al*., 2016).

Based on these tests, the strains were identified as *Xanthomonas campestris*. To our knowledge, this is the first report of *Xanthomonas campestris* in chard.

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Figure 1

Figure 2

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