

First report of '*Candidatus Phytoplasma fraxini*' (group 16SrVII phytoplasma) associated with a peach disease in Canada

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Peach (*Prunus persica*), family Rosaceae, is native to China. In Canada, the province of Ontario produces 82% of the nation's peach crop. The Canadian Clonal Genebank, also located in Ontario, currently holds 83 accessions of peach. The collection is routinely monitored for symptoms of phytoplasma including decline, leaf reddening, yellowing, shortening of internodes, witches' broom and reduced fruit size.

Leaf samples from trees exhibiting symptoms and symptomless trees representing sixteen accessions were collected from June to August 2009. Total DNA was extracted (DNeasy plant extraction kit, QIAGEN). Phytoplasma universal primers specific for 16S rDNA (R16mF2/R1 and R16F2n/R2; Gundersen & Lee, 1996) were used in a nested PCR assay. Nested PCR products of expected size (~1250 bp) were obtained for accessions PRU0430 (cv. HW274, from Canada), PRU0380 (cv. GF 305 from France), PRU0334 (cv. RedSkin from USA), PRU0155 (cv. Harblaze from Canada) and PRU0375 (cv. Babygold #5 from USA). Symptomless plants yielded no PCR products. Amplicons were purified (Wizard PCR Clean-up, Promega), cloned (pGEM-T Easy Vector, Promega), and sequenced (Robarts Institute, London, Canada). The obtained sequences shared 100% identity with each other and a representative corresponding to PRU0430 was deposited in GenBank (Accession No. GU223903).

BLAST analysis showed that the *Prunus* phytoplasma shared 99% 16S rDNA sequence identity with those of phytoplasmas in the group 16SrVII (ash yellows) for which '*Candidatus Phytoplasma fraxini*' is the reference strain. Restriction fragment length polymorphism analysis (RFLP) of amplicons using *AluI*, *RsaI* and *MseI* yielded profiles similar to those of 16SrVII-A phytoplasma subgroup. Phytoplasmas of group 16SrVII have been reported in different hosts in Colombia, Argentina, Brazil and Chile (Fiore *et al.*, 2007). Particularly in North America, including Canada,

16SrVII phytoplasmas have been associated with ash yellows and lilac witches' broom diseases (Sinclair *et al.*, 1996; Griffiths *et al.*, 1999). To our knowledge, this is the first report of a 16SrVII-related phytoplasma strain identified in peach after an incidental report in peach in Southern Italy (Paltrinieri *et al.*, 2003). The 16SrVII phytoplasma has caused devastating effects in ornamentals. Therefore its presence in peach is of great phytosanitary significance due to its commercial interest, and represents a potential threat for disease spread to other fruit crops, particularly *Prunus* sp.

References

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Bacterial leaf spot of coffee caused by *Pseudomonas syringae* pv. *tabaci* in Brazil

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Coffee (*Coffea arabica*) is widely planted and an economically important crop in Brazil both for domestic consumption and export. During 2006 a new disease affected coffee seedlings cv. Catuai in a commercial nursery located in Arandu county, State of São Paulo. Disease incidence was estimated to be about 1–2% of seedlings. Initially small brown lesions were observed on leaves and then becoming black and angular; alternatively, there were irregular lesions surrounded by a large yellow halo, which sometimes coalesced. These symptoms were similar to those caused by *Pseudomonas syringae* pv. *garcae* or *Burkholderia andropogonis*, pathogens of coffee (Amaral *et al.*, 1956; Rodrigues-Neto *et al.*, 1981).

From diseased tissues fluorescent pseudomonad bacteria were isolated on King's B medium. Colonies were creamy white, rounded, with irregular margins. The isolates selected (IBSBF 2240, 2241 and 2249) were positive for levan production and tobacco hypersensitivity; and negative for oxidase, protopectinase and arginine dihydrolase (LOPAT group 1a) (Lelliott *et al.*, 1966). Also, the bacterial strains produced acid from D-mannitol, inositol, D-sorbitol and erythritol but not from adonitol. D(-) tartrate and L-lactate were not utilised. Ice nucleation was negative.

For comparison, the type and reference strains of *P. syringae* pv. *garcae* (IBSBF 248^b), *P. syringae* pv. *syringae* (IBSBF 281¹) and *P. syringae* pv. *tabaci* (IBSBF 1972¹) were included in the identification assays. Biochemical tests were applied according to Braun-Kiewnick & Sands (2001) and molecular tests were based on PCR-RFLP of the *hrpL* gene of *P. syringae* pv. *syringae*. Pathogenicity of the three strains was confirmed by spraying bacterial suspensions (approximately 10⁸ cfu mL⁻¹) on healthy leaves of coffee seedlings previously wounded with a sterile needle and then covered with transparent plastic bags for 3 days. Control plants were treated with sterile distilled water. After 2 weeks, lesions developed only on inoculated leaves and identical bacteria to those inoculated were re-isolated. In molecular tests, the *hrpL* gene was employed using the primers pshrp 1F (5'-CTCAGA/GGCGTTCATC/TCA-3') and pshrp 2R (5'-TCAGGCC/TAACGGGTCA/TATCT-3'). In PCR-RFLP using *AluI*, *HaeIII*, *HinfI*, *HpaII* and *TaqI* restriction endonucleases, the isolated strains showed identical profiles with *P. syringae* pv. *tabaci*. Based on biochemical and molecular tests the pathogen was identified as *P. syringae* pv. *tabaci*. This is the first report of this species causing disease on coffee.