## New Disease Reports

# First report of '*Candidatus* Phytoplasma asteris' (16Srl group) causing stunt of tomato in Cuba

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Tomato (Solanum lycopersicum) is an important vegetable in Cuba and worldwide. In 2009, a disease suspected to be associated with phytoplasmas was observed in tomato fields in Mayabeque, in the locality of San José in the western region of the country. Symptoms most frequently displayed were shortened internodes and stunting, chlorosis and reduced leaf size in approximately 40% of tomato plants but others included abnormal shoot proliferation and reduced fruit size. Leaf samples of plants with symptoms were collected and total DNA was extracted (Doyle & Doyle, 1990) and used as template in a nested PCR assay to amplify the phytoplasma 16S rDNA gene with primer pairs R16F2n/R2 (Gundersen & Lee, 1996) and fU5/rU3 (Lorenz et al., 1995). Products of the expected size (approximately 1240 and 880 bp respectively) were amplified for fifteen out of forty-five (15/45) symptom-bearing plants, but not from the symptomless plants. Restriction profiles after digestion of amplicons with HpaII, HaeIII and Sau3AI endonucleases (data not shown) were all identical to each other and to those of reference phytoplasma strains related to 'Candidatus Phytoplasma asteris' (16SrI group)(Lee et al., 1998).

A selected PCR product was cloned into the pGEM-T Easy Vector (Promega) and three clones were further sequenced. Sequence alignment with ClustalW method demonstrated that the clones from Cuban tomato phytoplasma were indistinguishable. One of the sequences was deposited in GenBank (JN383913) and compared by BLASTn with available DNA sequences. According to the results Cuban tomato phytoplasma showed the highest sequence similarity (99%) with Potato phytoplasma Islamabad (FJ178388), a strain related to '*Candidatus* Phytoplasma asteris' (Aster yellows group (16SrI)). Phylogenetic analysis revealed that Cuban tomato phytoplasma reported by Arocha *et al.* (2007) in sweet pepper (*Capsicum* sp.) in Cuba (Fig. 1).

To our knowledge, this is the first report of a phytoplasma associated with a tomato disease in Cuba. In this country, '*Candidatus* Phytoplasma asteris' has been also reported affecting other vegetables such as carrots (*Daucus carota*), cabbage (*Brassica oleracea* var. *capitata*) and beetroot (*Beta vulgaris*) (Arocha *et al.*, 2007, 2009). The results have an impact on the

tomato industry, especially since phytoplasmas of group 16SrI are known to be insect transmitted and to possess a wide host range, including weeds plants that could be a reservoir for the pathogen. Therefore further studies are required to increase the knowledge about 16SrI phytoplasmas and putative polyphagous vectors among different vegetable crops in Cuba.

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#### References

Arocha Y, Piñol B, Almeida R, Acosta K, Quiñones M, Zayas T, Varela M, Marrero Y, Boa E, Lucas JA, 2009. First report of phytoplasmas affecting organoponic crops in central and eastern Cuba. *Plant Pathology* **58**, 793. http://dx.doi.org/10.1111/j.1365-3059.2009.02027.x

Arocha Y, Piñol B, Picornell B, Almeida R, Jones P, 2007. Broad bean and sweet pepper: two new hosts associated with *'Candidatus* Phytoplasma asteris' (16SrI phytoplasma group) in Cuba. *Plant Pathology* **56**, 345. http://dx.doi.org/10.1111/j.1365-3059.2007.01518.x

Doyle JJ, Doyle JL, 1990.A rapid total DNA preparation procedure for fresh plant tissue. *Focus* **12**,13-15.

Gundersen DE, Lee IM, 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer sets. *Phytopathologia Mediterranea* **35**, 144-151.

Lee IM, Gundersen-Rindal DE, Davis RE, Bartoszyk IM, 1998. Revised classification scheme of phytoplasmas based on RFLP analysis of 16S *rRNA* and ribosomal protein gene sequences. *International Journal of Systematic and Evolutionary Microbiology* **48**, 1153-1169. http://dx.doi.org/10.1099/00207713-48-4-1153

Lorenz KH, Schneider B, Ahrens U, Seemüller E, 1995. Detection of apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology* **85**, 771-776. http://dx.doi.org/10.1094/Phyto-85-771



#### Figure 1

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