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First Report of 'Candidatus Phytoplasma asteris' in kumquat (Citrus japonica) with HLB-like Symptoms in La Paz, Baja California Sur, Mexico

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Kumquat (Family Rutacea) is a common name of citrus trees forming round or oval small edible fruit with high nutritional and pharmaceutical value, which is believed to be native to China and widely distributed in South Asia and the Asia-Pacific region. In La Paz, Baja California Sur (BCS) kumquat was introduced and cultivated as a backyard culture by Chinese descendants. In August-September of 2013 multiple symptoms of yellow-type diseases, including chlorosis, leaf dropping, malformations and blotching, and necrotic leafstalks and shoots were observed in kumquat trees in some backyard plots in La Paz. Similar

symptoms were reported earlier in relation to citrus huanglongbing (HLB) in various citrus species in different regions of BCS, and both phytoplasma and rod-shaped bacteria were detected by scanning electron microscopy (SEM) (3). Nymphs and adults of Asian citrus psyllid Diaphorina citri, known as a vector of HLB in the American continent, were detected in kumquat foliage. The attribution of HLB symptoms to walled ('Candidatus Liberibacter') and wall-less ('Ca. Phytoplasma') phloem inhabiting bacteria was reported during last decade (1). SEM and molecular techniques were used to prove the possible presence of two pathogens in kumquat trees having HLB-like symptoms. Analysis of specimens by SEM evidenced the presence of phytoplasmas in phloem tissue of all tested samples, ranged from 500 to 1,000 nm. No rod-shaped bacteria were detected. Total genomic DNA was extracted from four symptomatic and two asymptomatic leaf samples (midribs, leafstalks) using a modified CTAB extraction technique (4) and used as templates in conventional and real time PCR tests. The presence of 'Ca. Liberibacter' species was tested in real-time PCR using 16S rDNA-based TaqMan primer-probe sets specific to 'Ca. Liberibacter asiaticus' and 'Ca. Liberibacter americanus' (2). None of the symptomatic and asymptomatic samples were positive for these two 'Ca. Liberibacter' species. For phytoplasma detection nested PCR was performed with P1/P7 followed by R16F2N/R16R2 primer pairs. Amplicons of ~ 1.2 kb obtained from one symptomatic and one asymptomatic sample were cloned into pGEM-T-easy vector (Promega, Madison, WI) and sequenced (Genewiz, NJ, USA). Analysis of sequences of two amplicons in the GenBank database using BLASTn method displayed the highest sequence identity (99.4% and 99.1%) with 'Ca. Phytoplasma asteris' reference strain (Accession No.M30790). Virtual RFLP analysis (5) of sequences revealed the most similarity with reference patterns of the 16Sr group I, subgroup B (Accession No. NC005303). Two analyzed sequences were deposited in the NCBI GenBank database (Accession numbers KJ415248 and KJ415249). To our knowledge, this is the first report of 16SrI group (aster yellows) phytoplasma in BCS, and a first report of phytoplasmas in kumquat in scientific literature. The study will continue to prove whether the detected 'Ca. Phytoplasma asteris' related strain is the only pathogen associated with HLB-like symptoms in kumquat and to identify and classify the phytoplasmas detected in other citrus species in BCS. The role of D. citri as a possible vector of phytoplasmas is a subject of a special investigation. References: (1) J. Chen et al. Phytopathology 99:236, 2009. (2) W. Li et al. J. Microbiol. Methods 66:104, 2006. (3) A. Poghosyan et al. Petria 22:165, 2012. (4) R. Tapia-Tussel et al. Mol. Biotecnology 31:137, 2005. (5) W. Wey et al. IJSEM: 57:1855, 2007.

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