

Molecular Identification and Classification of Strawberry Phylloid Fruit Phytoplasma in Group 16SrI, New Subgroup R.

R. R. Jomantiene, USDA-ARS, Fruit Laboratory, Beltsville, MD 20705 and Institute of Botany, Vilnius, Lithuania; J. L. Maas, USDA-ARS, Fruit Laboratory, Beltsville, MD 20705; F. Takeda, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430; and R. E. Davis, USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705. Plant Dis. 86:920, 2002; published on-line as D-2002-0619-01N, 2002. Accepted for publication 10 June 2002.

Plants of commercial strawberry (*Fragaria × ananassa* Duch., cv. Camarosa) exhibiting extensive fruit phylloidy (development of leafy structures from achenes) were observed in a winter greenhouse production facility in West Virginia. In July 2001, 95 dormant, cold-stored plants were purchased from a California strawberry nursery, potted and grown in this West Virginia facility. Five of the plants developed fruits with phylloid growths. These fruits were assessed for phytoplasma infection using nested polymerase chain reactions (PCRs) in which initial ribosomal (r) DNA amplification was primed by phytoplasma-universal primer pair P1/P7 (2), and rDNA reamplification was primed by primer pair R16F2n/R16R2 (1). Amplification of phytoplasma-characteristic 1.2-kbp 16S rDNA in the nested reactions primed by R16F2n/R16R2 confirmed that the symptomatic plants were infected by a phytoplasma, termed strawberry phylloid fruit (StrawbPhF) phytoplasma. No phytoplasma DNAs were amplified from healthy plants. Restriction fragment length polymorphism (RFLP) patterns of 16S rDNA digested with *AluI*, *KpnI*, *HhaI*, *HaeIII*, *HpaII*, *MseI*, *RsaI*, and *Sau3A1* restriction endonucleases indicated that StrawbPhF phytoplasma belonged to group 16SrI (group I, aster yellows phytoplasma group) according to the phytoplasma classification system of Lee et al. (4). However, the collective patterns distinguished StrawbPhF from its closest known relative, clover phylloidy (CPh) phytoplasma, and from all other phytoplasmas classified in group 16SrI. On the basis of the RFLP patterns of 16S rDNA, the StrawbPhF was classified in group 16SrI, new subgroup R. The StrawbPhF phytoplasma 1.2-kbp 16S rDNA PCR product was cloned in *Escherichia coli* using TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA), sequenced, and the sequence deposited in GenBank under Accession No. AY102275. The StrawbPhF 16S rDNA sequence shared 99.9 and 99.8% similarity with the two sequence heterogeneous operons, *rrnA* and *rrnB*, respectively, of CPh phytoplasma, and shared 99.9% similarity with 16S rDNA of the unclassified cirsium yellows (CirY) phytoplasma (GenBank Accession No. AF200431) reported in *Cirsium arvense* L. in Lithuania (3). The restriction sites in 16S rDNA of StrawbPhF were identical to those in 16S rDNA of CPh *rrnA* and CirY. Three restriction sites (*AluI*, *HaeIII*, and *MseI*) and three base substitutions distinguished StrawbPhF 16S rDNA from *rrnB* of CPh phytoplasma. No evidence was obtained for the presence of a second (sequence heterogeneous) rRNA operon in StrawbPhF phytoplasma, as reported in CPh phytoplasma (4), which clearly distinguishes this phytoplasma from CPh phytoplasma. Future studies on StrawbPhF phytoplasma may provide important information on the evolution of phytoplasmas.

References: (1) D. E. Gundersen and I.-M. Lee. *Phytopathol. Mediterr.* 35:144, 1996. (2) R. Jomantiene et al. *Int. J. Syst. Bacteriol.* 48:269, 1998. (3) R. Jomantiene et al. *Phytopathology* 90:S39, 2000. (4) I.-M. Lee et al. *Int. J. Syst. Bacteriol.* 48:1153, 1998.