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Phytoplasmas detected in cultivated fruit plants in Lithuania

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Abstract

Because of the importance of fruits in the human nutrition and in the agricultural and food industries, we have investigated garden fruit-bearing plant species for possible phytoplasma diseases in Lithuania. Thus far strains of phytoplasmas subgroup 16SrI-B (aster yellows phytoplasma subgroup) in sourcherry, cultivated apple, and common pear; subgroup 16SrI-Q (cherry little leaf phytoplasma subgroup) in cherry; 16SrV-E (rubus stunt phytoplasma subgroup) in garden raspberry; and a new '*Candidatus* Phytoplasma' species, '*Candidatus* Phytoplasma fragariae', in cultivated strawberry were identified. This work extends knowledge of the biodiversity of phytoplasmas infecting fruit plants.

Key words: 16S rDNA, RFLP, phytoplasma, garden plants.

Introduction

Phytoplasmas are plant pathogenic, wall-less unculturable bacteria that are classified in the class *Mollicutes*. They infect plants, causing so-called yellows diseases that can result in harvest losses. Phloem-feeding insects, mainly leafhoppers, transmit phytoplasmas from plant to plant (Davis and Lee, 2000). Thus far, three 16Sr groups (16SrI, 16SrIII, and 16SrV) and eleven subgroups have been reported in Lithuania (Valiūnas, 2003).

Sourcherries (*Cerasus vulgaris* Mill., syn. *Prunus cerasus* L.), common pear (*Pyrus communis* L.), cultivated apple (*Malus domestica* Borkh.), cultivated strawberry (*Fragaria* x *ananassa* Ducheane), and garden raspberry (*Rubus idaeus* L.) plants exhibiting yellows disease symptoms were observed in Lithuania. These plants are highly valued for their nutritious fruits and are widely grown in small orchards and home gardens of mixed species. This report summarizes data on the biodiversity of phytoplasmas infecting these fruit-bearing plants in Lithuania.

Materials and methods

Samples of leaf tissue from two naturally infected sourcherry trees and from one plant each of common pear, cultivated apple tree, cultivated strawberry, and garden raspberry exhibiting yellows disease symptoms were collected in Lithuania. Nucleic acid for use as template in the polymerase chain reaction (PCR) was extracted from fresh tissue using a Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. Nested PCRs using extracted DNA and primer pairs P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) and R16F2n/R16R2 (Gundersen and Lee, 1996) were carried out and PCR products were analysed as previously described (Lee *et al*., 1998). Products from nested PCR primed by R16F2n/R16R2 were analysed by single enzyme digestion, according to the manufacturer's instructions, with *Alu*I, *Bfa*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Kpn*I, *Mse*I, *Rsa*I, *Sau*3AI, and *Taq*I (MBI Fermentas). The restriction fragment length polymorphism (RFLP) profiles of digested DNA were analyzed by electrophoresis through a 5% polyacrylamide gel. The DNA fragment size standard was ØX174 DNA/*Bsu*RI (*Hae*III) digest (MBI Fermentas). RFLP patterns were compared with those previously published (Lee *et al*., 1998).

Results

One diseased sourcherry tree exhibited symptoms of shoot proliferation and little leaf; the other exhibited shoot proliferation and decline symptoms. The diseased pear tree exhibited symptoms of reduced size of leaves, and proliferation and decline of branches. Naturally diseased apple trees exhibited symptoms of leaf yellowing, shoot proliferation and sessile leaves on the trunk. General stunting and yellowing of leaves were observed in diseased cultivated strawberry. Raspberry exhibited phyllody and yellows symptoms.

In PCRs, a phytoplasma-specific 1.2 kb 16S rDNA product was amplified from DNA of samples from diseased, but not from healthy, plants using phytoplasma universal primer pairs P1/P7 and R16F2n/R16R2 in nested PCRs, indicating that symptoms observed in diseased plants were associated with phytoplasmas. The phytoplasmas detected in the two diseased cherries, and in pear, apple, strawberry, and raspberry were termed strains cherry little leaf (ChLL), cherry proliferation (ChP), pear proliferation and decline (PPD), apple sessile leaf (ApSL), strawberry yellows (StrawY), and rubus phyllody (RuPh) phytoplasmas, respectively.

Comparison of RFLP patterns of 16S amplicons with patterns previously published for 16S rDNA from other phytoplasmas (Lee *et al*., 1998; Marcone *et al*., 2000) revealed that the ChLL phytoplasma belongs to group 16SrI (aster yellows phytoplasma group). The *Rsa*I RFLP pattern of 16S rDNA from ChLL phytoplasma differed from that of all other group 16SrI phytoplasmal 16S rDNAs (Lee *et al*., 1998; Marcone *et al*., 2000). On the basis of these results we classified the ChLL phytoplasma in group 16SrI, new subgroup I-Q (Valiūnas *et al*., 2005). The ChP phytoplasma was classified as a member of phytoplasma subgroup 16SrI-B (this study). RFLP analysis revealed that PPD phytoplasma belongs to phytoplasma subgroup 16SrI-B (Valiūnas *et al*., 2004), RuPh phytoplasma is related to rubus stunt (RS) phytoplasma and belongs to phytoplasma subgroup 16SrV-E (this study), ApSL phytoplasma is affiliated with subgroup 16SrI-B (Jomantiene and Davis, 2005). Results from rDNA analysis, in addition to natural host and geographical location, support recognition of StrawY phytoplasma as representative of a novel taxon, distinct '*Candidatus*' species, '*Candidatus* Phytoplasma fragariae' (Valiūnas *et al*., 2006).

Discussion

A broad biodiversity of phytoplasmas is associated with diseases of fruits. For example, in Europe, phytoplasma diseases of fruit-bearing plants are associated with strains of phytoplasma groups 16SrI, 16SrIII, 16SrV, 16SrX, and 16SrXII (Lee *et al*., 1998). We have found phytoplasmas belonging to subgroups 16SrI-B, 16SrI-Q, 16SrV-E, and new species '*Candidatus* Phytoplasma fragariae' (table 1). Thus far, in Lithuania we have not detected such severe phytoplasmas as those of apple proliferation, European stone fruit yellows, pear decline, and stolbur, all quarantine regulated pathogens already reported in several of the EU countries. The current findings add new diseases and pathogens to those known to impact important fruit crops. Future investigations are needed to discover insect vectors of these pathogens and to extend research on phytoplasma diseases to other fruit plants in Lithuania.

Table 1. Phytoplasmas detected in fruit-bearing plants in Lithuania.

Plant host	Phytoplasma strain	RFLP subgroup	Reference
Sourcherry	ChLL	16SrI-Q	Valiūnas *et al*., 2005
Sourcherry	ChP	16SrI-B	This study
Common pear	PPD	16SrI-B	Valiūnas *et al*., 2004
Cultivated apple	ApSL	16SrI-B	Jomantiene and Davis, 2005
Cultivated strawberry	StrawY, '*Candidatus* Phytoplasma fragariae'	-	Valiūnas *et al*., 2006
Garden raspberry	RuPh	16SrV-E	This study

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References

DAVIS R. E., LEE I-M., 2000.- Phytoplasma, pp. 640-646. In: *Encyclopedia of Microbiology* (LEDERBERG J., Ed.).- Academic Press Inc., 2nd edn., San Diego, USA.

DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and non-culturable mollicutes.- *Journal of Microbiological Methods,* 14: 53-61.

GUNDERSEN D. E., LEE I-M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia Mediterranea,* 35: 144-151.

JOMANTIENE R., DAVIS R. E., 2005.- Apple sessile leaf: a new disease associated with a '*Candidatus* Phytoplasma asteris' subgroup 16SrI-B phytoplasma in Lithuania.- *Plant Pathology*, 54 (2): 237.

LEE I-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology,* 48: 1153-1169.

MARCONE C., LEE I-M., DAVIS R. E., RAGOZZINO A., SEEMÜLLER E., 2000.- Classification of aster yellows-group phytoplasmas based on combined analyses of rRNA and *tuf* gene sequences.- *International Journal of Systematic and Evolutionary Microbiology,* 50: 1703-1713.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology* (RAZIN S., TULLY J. G., Eds).- Academic Press Inc., San Diego, CA, USA.

VALIŪNAS D., 2003.- Identification of phytoplasmas in Lithuania and estimation of their biodiversity and molecular evolutionary relationships.- *Summary of doctoral thesis*, Institute of Botany, Vilnius, Lithuania.

VALIŪNAS D., JOMANTIENE R., DAVIS R. E., 2004.- Identification of subgroup 16SrI-B phytoplasma from naturally infected pear in Lithuania.- *Sodininkystė ir daržininkystė, mokslo darbai*, 23 (4): 29-36.

VALIŪNAS D., JOMANTIENE R., DAVIS R. E., 2005.- A '*Candidatus* Phytoplasma asteris'-related phytoplasma associated with cherry little leaf disease represents a new subgroup, 16SrI-Q.- *Phytopathology*, 95: S106.

VALIŪNAS D., STANIULIS J., DAVIS R. E., 2006.- '*Candidatus* Phytoplasma fragariae', a novel phytoplasma taxon discovered in yellows diseased strawberry, *Fragaria x ananassa*.- *International Journal of Systematic and Evolutionary Microbiology*, 56**:** 277-281.

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