

Larch is a new host for a group 16SrI, subgroup B, phytoplasma in Ukraine

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Abstract

A phytoplasma strain belonging to group 16SrI, subgroup B ('*Candidatus* Phytoplasma asteris'), the most widely spread phytoplasma worldwide, was detected in diseased larch (*Larix* sp.) in Ukraine. Detection, identification, and classification of the larch infecting phytoplasma were accomplished through RFLP and sequence analysis of 16S rRNA and ribosomal protein (*rp*) gene sequences amplified in polymerase chain reactions. The larch infecting phytoplasma was named larch dwarfed needle proliferation (LDNP) phytoplasma. The findings revealed *Larix* as a previously unknown host of phytoplasma and indicated that '*Ca. P. asteris*' is capable of infecting a gymnosperm, while raising questions concerning the identity of possible insect vector(s) involved in transmission of '*Ca. P. asteris*' to larch and perhaps to other gymnosperms.

Key words: Gymnosperm, Pinaceae, proliferation, polymerase chain reaction, mollicute.

Introduction

Class *Gymnospermae* (gymnosperms) contains several families including *Pinaceae*, within which are classified diverse woody plants, some of which are highly valuable for their timber (Lin *et al.*, 2010). One genus in the family is *Larix* (larches), trees 15 to 50 m height that constitute a major component of various forests in cool temperate regions of the northern hemisphere. Indeed, *Larix* has been cited as one of the most widely distributed tree genera in Eurasia (Lin *et al.*, 2010). Larches are conifers, but unlike members of most genera in the *Pinaceae*, the genus *Larix* is deciduous, the leaves, or needles, turning yellow and falling in autumn. In the present study we investigated the possible presence of phytoplasma in diseased larch growing in a public park in Ukraine.

Symptoms similar to those sometimes associated with plant infection by phytoplasma were observed in diseased larch in Oleski park (L'viv region, Ukraine). The trees exhibited general yellowing of needles, dwarfed needles, and proliferation and necrosis of needles. These symptoms, and recent reports of phytoplasma infections in other Gymnosperms (Davis *et al.*, 2010; Paltrinieri *et al.* 1998; Schneider *et al.* 2005; Sliwa *et al.*, 2007; Valiunas *et al.*, 2010), drew our attention to the possibility of phytoplasma-associated disease in larch. Samples of dwarfed needles were collected and analysed for the presence of phytoplasma by widely adopted molecular detection and identification methods (Lee *et al.*, 1998). The results revealed that larch was infected by a phytoplasma of group 16SrI, subgroup B ('*Candidatus* Phytoplasma asteris'), the most widely occurring phytoplasma worldwide, infecting numerous herbaceous and woody plants.

Materials and methods

Samples of symptomatic leaves (needles) from diseased larch were collected and assayed for possible phytoplasma infection. DNA was extracted from the yellowing, dwarfed and proliferated needles using Genomic DNA Purification Kit (Fermentas, Vilnius, Lithuania). Detection of phytoplasma 16S rDNA sequences was accomplished by the use of nested polymerase chain reactions (PCRs) primed by phytoplasma-universal primer pair P1/P7 followed by nested reactions primed by R16F2n/R16R2 (F2n/R2) (Lee *et al.*, 1998). Phytoplasma ribosomal protein (*rp*) gene sequences were amplified from larch DNA templates by using PCR primed by *rp* gene primer pair rpF1/rpR1. PCR was performed as described previously (Lee *et al.*, 1998). Annealing temperature for amplification of *rp* gene sequences was 50°C. Products (1.2 kbp, R16F2n/R2 segment) of the nested PCR were subjected to enzymatic restriction fragment length polymorphism (RFLP) analysis using restriction endonucleases *AluI*, *HaeIII*, *MseI*, *RsaI*, *HhaI*, *HinfI*, *HpaII*. (Fermentas, Vilnius, Lithuania). Digested products of 16S rDNA were analyzed using electrophoresis through 5% polyacrylamide gel for 16S rDNA. PCR products of *rp* gene sequences were digested with *AluI*, *MseI*, and *Tsp9065* and analysed using electrophoresis through 10% polyacrylamide gels. DNA bands were stained with ethidium bromide and visualized using a UV transilluminator. Classification of phytoplasma was accomplished through comparisons of RFLP patterns with patterns previously published, in accordance with the classification scheme of Lee *et al.* (1998). LDNP phytoplasma 16S rDNA sequence was subjected to computer-simulated restriction digestion and virtual gel plotting using *iPhyClassifier* (Zhao *et al.*, 2009).

Amplified 16S rRNA and *rp* gene products were cloned into *Escherichia coli* by using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions and sequenced by automated sequencing of both strands to achieve a minimum of 3-fold coverage per base position.

Results

To determine the possible presence of phytoplasmas in diseased larch (*Larix* sp.), we examined total DNA samples prepared from the yellowing, dwarfed and proliferated needles. Two genetic markers (16S rRNA and *rp* gene sequences) were employed for the detection, identification and classification of phytoplasmas. The product amplified in nested PCR primed by R16F2n/R16R2 from the diseased larch samples was 1.2 kbp in size (data not shown). RFLP analysis of amplified 16S rDNA revealed patterns identical to those of group 16SrI, subgroup B phytoplasmas ('*Ca. P. asteris*') (figure 1). Amplification, RFLP and nucleotide sequence analysis of *rp* gene products confirmed identification of '*Ca. P. asteris*' presence in the larch samples (data not shown). The phytoplasma detected in larch was named larch dwarfed needle proliferation (LDNP) phytoplasma. Amplicons of 16S rDNA and ribosomal protein (*rp*) genes from strain LDNP were cloned, sequenced and deposited in the GenBank database under accession numbers JF747037 and JF767009, respectively.

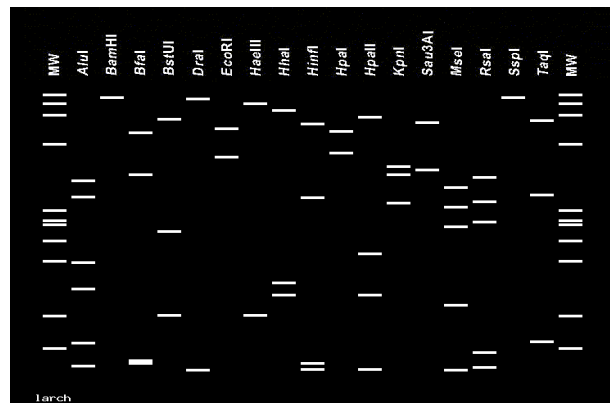


Figure 1. Virtual gel RFLP patterns from analyses of LDNP phytoplasma 16S rDNA R16F2n/R2 fragment.

Discussion

Phytoplasma infections in pine (family *Pinaceae*) have been detected in Spain, Germany, Poland, and Lithuania (Schneider *et al.*, 2005; Sliwa *et al.*, 2007; Valiunas *et al.*, 2010). Symptoms exhibited by the diseased pine trees were shoot-proliferation, dwarfed and proliferated needles, and stunting. The phytoplasma strains detected in pine belonged to group 16SrXXI, subgroup A ('*Candidatus Phytoplasma pini*'). Group 16SrIII phytoplasma was reported in cypress (Paltrinieri *et al.*, 1998). A juniper (*Juniperus occidentalis*) infecting phytoplasma in

Oregon was classified as a '*Candidatus Phytoplasma phoenicium*'-related strain and a member of group 16SrIX, subgroup E (Davis *et al.*, 2010). Our study revealed infection by a group 16SrI subgroup B phytoplasma in larch. In agreement with concepts expressed previously (Davis *et al.*, 2010), the present and other findings indicate that phytoplasma infections in gymnosperms may be more frequent than previously thought. It will be interesting to learn the identities of insect vectors transmitting the diverse phytoplasmas to gymnosperms and to learn the extent of phytoplasma infections in gymnosperms worldwide.

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References

- DAVIS R. E., DALLY E., ZHAO Y., LEE I-M., JOMANTIENE R., DETWEILER A. J., PUTNAM M. L., 2010.- First report of a new subgroup 16SrIX-E, '*Candidatus Phytoplasma phoenicium*'-related, phytoplasma associated with juniper witches' broom disease in Oregon.- *Plant Pathology*, 20: 35.
- LEE I-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic and Evolutionary Microbiology*, 48: 1153-1169.
- LIN C-P., HUANG J-P., WU C-S., HSU C-Y., CHAW S-M., 2010.- Comparative chloroplast genomics reveals the evolution of *Pinacea* genera and subfamilies.- *Genome Biology and Evolution*, 2: 504-517.
- PALTRINIERI S., PONDRELLI M., BERTACCINI A., 1998.- X-disease related phytoplasmas in ornamental trees and shrubs with witches broom and malformation symptoms.- *Journal of Plant Pathology*, 80: 261.
- SCHNEIDER B., TORRES E., MARTIN M. P., SCHRÖDER M., BEHNKE H. D., SEEMÜLLER E., 2005.- '*Candidatus Phytoplasma pini*', a novel taxon from *Pinus sylvestris* and *Pinus halepensis*.- *International Journal of Systematic and Evolutionary Microbiology*, 55: 303-307.
- SLIWA H., KAMINSKA M., KORSZUNP S., ADLER P., 2007.- Detection of '*Candidatus Phytoplasma pini*' in *Pinus sylvestris* trees in Poland.- *Journal of Phytopathology*, 156: 88-92.
- VALIUNAS D., JOMANTIENE R., IVANAUSKAS A., SNEIDERIS D., STANIULIS J., DAVIS R. E., 2010.- A possible threat to the timber industry: '*Candidatus Phytoplasma pini*' in Scots pine (*Pinus sylvestris* L.) in Lithuania, p. 38. In: *Current status and perspectives of phytoplasma disease research and management* (BERTACCINI A., LAVIÑA A., TORRES E., Eds).- Sitges, Spain, February 1-2.
- ZHAO Y., WEI W., LEE I-M., SHAO J., SUO X., DAVIS R. E., 2009.- Construction of an interactive online phytoplasma classification tool, *iPhyClassifier*, and its application in analysis of the peach X-disease phytoplasma group (16SrIII).- *Journal of Systematic and Evolutionary Microbiology*, 59: 2582-2593.

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