Fitoplazmoze četinjača

Šafarić, Barbara

SVEUČILIŠTE U ZAGREBU

PRIRODOSLOVNO – MATEMATIČKI FAKULTET

BIOLOŠKI ODSJEK

FITOPLAZMOZE ČETINJAČA

SEMINARSKI RAD

Barbara Šafarić

Preddiplomski studij molekularne biologije

Mentor: Prof. dr. sc. Mirna Ćurković Perica

Pomoćni voditelj: dipl. ing. Marin Ježić

Zagreb, 2012.

UNIVERSITY OF ZAGREB

FACULTY OF SCIENCE

DIVISON OF BIOLOGY

PHYTOPLASMOSES OF CONIFERS

BACHELOR THESIS

Barbara Šafarić

Undergraduate Program of Molecular Biology

Supervisor: Prof. dr. sc. Mirna Ćurković Perica

dipl. ing. Marin Ježić

Zagreb, 2012

TABLE OF CONTENTS

LIST OF ABBREVATIONS

‘*Ca*. P.’ – ‘*Candidatus* Phytoplasma’

EM – electron microscope

IAA – indole-3-acetic acid

IBA – indole-3-butyric acid

PCR – Polymerase Chain Reaction

RFLP – Restriction Fragmenth Length Polymorphysm

rp gene - ribosomal protein gene

RT-PCR – real-time PCR

Taq – *Thermus aquaticus* DNA polymerase

TBE buffer – Tris - boric acid - EDTA electrophoresis buffer

TNA – Total Nucleic Acids

Tris – tris(hydroxymethyl)aminomethane

UV– ultra violet

1. GENERAL INTRODUCTION TO PHYTOPLASMAS

Phytoplasma are plant endocellular parasites spread throughout the world. They were discovered back in 1967 when a group led by Yoji Doi found pleomorphic figures in infected plants by EM that resembled mycoplasmas, animal parasites (Doi *et al.* 1967). For this reason, phytoplasmas were first called MLOs - mycoplasma like organisms. Since then, few authors have confirmed phytoplasmas' closer relation to acholeplasmas than to mycoplasmas (Lim and Sears, 1992, Gundersen *et al*. 1994, Davis *et al*. 1997).

Phytoplasmas are wall-less prokaryotes ranging in size from 200 to 800 nm with very small genome, from 530 kb to 1350 kb. The smallest phytoplasma chromosome 530 kb long, found in Bermuda grass white leaf phytoplasma isolates, is also the smallest self-replicating genome known so far (C. Marcone *et al.* 1999). Organized in nucleoid, genomes sometimes contain extrachromosomal elements, called plasmids, which are thought to play a role in integration into chromosome and can have impact on vector transmissibility (Kube *et al.* 2011). While most phytoplasmas contain circular chromosomes, '*Ca.* P. mali', '*Ca*. P. pyri' and '*Ca*. P. prunorum' which are closely related, have linear chromosomes (Kube *et al.* 2008). Complete genome sequences of four phytoplasmas have been published: '*Candidatus* Phytoplasma australiense' (Tran-Nguyen *et al.* 2008), '*Candidatus* Phytoplasma mali' strain AT (Kube *et al.* 2008), Aster yellows witches'-broom phytoplasma (Bai *et al.* 2006) and Onion yellows phytoplasma ('*Candidatus* Phytoplasma asteris' , OY strain) (Oshima *et al.* 2004). Known complete genome sequences provide a valuable insight into organization of phytoplasmas' genomes, enable better understanding of phytoplasmas' metabolism, pathogenicity, proteins involved in the interactions with hosts and, most likely, will also provide key facts for their successful cultivation *in vitro* in cell-free media.

Phytoplasmas lack a number of genes involved in: (I) essential metabolic pathways, like F_0F_1-type ATP–synthase and pentose phosphate pathway (Bai *et al.* 2008), (II) biosynthesis of amino and fatty acids, (III) metabolism of amino acids, nucleotides, sugars, glyoxylate and (IV) CO_2 fixation (Tran-Nguyen *et al.* 2008), which is the reason why they are strictly host-dependent and, so far, were not successfully cultivated on artificial medium. They reside in plant phloem, where plant's nutrients present in sap, like sugars (saccharose, raffinose, stachyose), sugar alcohols (mannitol, sorbitol), amino acids (glutamine/glutamate, asparagine/aspartate in highest concentration), nucleotides (ATP, GTP), hormones (auxins –

IAA, IBA), RNAs, enzymes, vitamins and ions (K^+, Mg^{2+}, PO_4^{3-}, Cl^-) are easily accessible to them (Pevalek-Kozlina, 2003).

Experimental host plant which is most commonly used in studies of phytoplasmas is periwinkle (*Catharanthus roseus*). Not only it can be successfully infected with different 'Ca. Phytoplasma species' and strains and exhibits different symptoms of infection, periwinkle also accumulates phytoplasmas in high titers. Periwinkle, as well as the other plants used in studies, can be infected with phytoplasmas by graft inoculation, dodder transmission (*Cuscuta campestris*) or insects (Śliwa and Kamińska, 2004). In nature, phytoplasmas are transmitted from plant to plant by sap-feeding insects of the order Hemiptera, mainly leafhoppers (*Cicadellidae*), planthoppers (*Fulgoroidea*), and psyllids (*Psyllidae*) (Weintraub and Beanland, 2006). According to Hogenhout *el al.* (2008), life cycle of phytoplasmas involves replication in insect host as well as in plants.

When infected, plants exhibit a variety of symptoms which suggest phytoplasmas impact on balance of plant growth regulators. The symptoms depend on phytoplasma strains and can change with the progress of infection. In addition, some plants can be tolerant or even resistant to infection and show no symptoms at all (Lee et al. 2000). Also, there have been reports of plants' recovery from infection (Musetti et al. 2006, Osler et al. 1993). Symptoms which would be expressed as a result of a phytoplasma infection also depend on the plant species and may include shoot proliferation (like witches' broom), stunting (shortened internodes), smaller leaves and flowers, virescence (growth of green flowers instead of normal-pigmented), leaf yellowing, sterility of the flowers, phyllody (parts of flower develop incorrectly into leaves instead of petals), dwarfing and decay of tissues and whole plants (Hogenhout et al. 2008).

With the progress of research on phytoplasmas, the economic importance of some plant diseases they cause is becoming known. Plant diseases caused by phytoplasmas cause severe damages on both fruit and woody plants, decreasing quality and yield. '*Ca.* P. mali' , '*Ca.* P. pyri' and '*Ca.* P. prunorum' cause some of the most economically important plant diseases like apple proliferation, pear decline and European stone fruit yellow (Seemüller and Schneider, 2004). European Cooperation in the field of Scientific and Technical Research, COST, reported in 2008 that only in Germany, France and Italy apple proliferation ('*Candidatus* Phytoplasma mali') caused 10 - 70% loss in apple production. Based on data from 2001, COST calculated the actual losses which were stunning- in total about 25 million Euro in Germany and about 100 million Euro in Italy (COST, 2008).

2. PHYTOPLASMAS IN CONIFERS

Until 1970s phytoplasmas were considered to be habitants of angiosperms exclusively. Their presence in conifers was first reported by EM studies of 3 independent groups led by Koyama (1970), Gopo (1989) and McCoy (1989). Since phytoplasmas mostly inhabit sieve elements and move around plant through the sieve pores (Rudzinska-Langwald and Kamińska, 1999) it was thought that gymnosperms are not suitable hosts for phytoplasmas because of their small sieve pore size. Unlike angiosperms, which pore size ranges between 1 and 14 μm, sieve pores of gymnosperms are much smaller, less than 0.8 μm in diameter (http://cronodon.com/BioTech/Plant_Transport.html). Considering the fact that phytoplasmas are usually between 200 and 800 nm in diameter (Lee *et al.* 2000), it is clear why scientists have questioned gymnosperms as suitable phytoplasma hosts.

Although a substantial progress has been made with the methods of phytoplasma detection, there are still not many data available about phytoplasmas in conifers. So far, few '*Ca*. Phytoplasma species' have been detected in gymnosperms: '*Ca.* P. pini' in *Pinus halepensis* and *Pinus sylvestris* (Schneider *et at.* 2005, Śliva *et al.* 2008), and *Picea pungens* (Kamińska and Berniak, 2011); X-disease phytoplasma which belongs to16SrIII group in *Picea abies* (Kamińska and Śliva 2010), *Picea glauca* (Kamińska and Berniak, 2011) and cypress species in Italy (Paltrinieri *et al.* 1998); '*Ca.* P. trifolii' in *Araucaria heterophylla* in India (Gupta *et al.* 2009) and '*Ca.* P. phoenicium' in *Juniperus occidentalis* (Davis *et al.* 2010).

Most common symptoms in conifers are abnormal shoot proliferation, twisted needles and stunting. However, exhibited symptoms do not guarantee presence of phytoplasma since they may be caused by other stressors (Śliwa *et al*. 2007). In addition to unequal distribution throughout the plant and seasonal fluctuations, the problem with detecting phytoplasma in woody plants is in their low titers as well (Berges et al. 2000). Sometimes, false-negative results can appear due to the presence of some PCR inhibitors such as polyphenols or polysaccharides (Green and Thompson, 1999), which may inhibit the *Taq* DNA polymerase. For this reason, few methods of phytoplasma DNA extraction have been developed with the common goal of increasing phytoplasma DNA concentration and reducing presence of enzyme inhibitors (Firrao *et al*. 2007).

Impact and possible consequences of phytoplasma infection in conifers are not yet known. Scientists hypothesize that because of the phytoplasma infection, trees might be prone to fungus infections and other damaging factors which can result in premature senescence (Śliva *et al*. 2007). Furthermore, Valiunas *et al*. (2010) warns about presence of '*Ca*. P. pini' in *Pinus sylvestris* woods as a possible threat to the timber industry in Lithuania. In the past, there have been reports about phytoplasmas causing major damage in woods. Elm trees that managed to survive Dutch elm disease were often infected by phytoplasma, which almost led to extinction of elm trees in Europe and North America (Bertaccini and Duduk, 2009).

3. METHODS USED IN DETECTION AND CLASIFICATION OF PHYTOPLASMAS

Before development of molecular methods, detection of phytoplasmas was difficult. Commonly used method was electron microscopy in combination with grafting and careful monitoring of symptoms, which was very time-consuming. In the last few decades, improvements in methods used for detection and identification of phytoplasmas have been made. Two major breakthroughs, first in 1980s with the development of ELISA and second in the early 1990s with PCR and RFLP analysis, resulted in more detailed characterisation, identification and classification of phytoplasmas.

In the past, serological tools were often used for detection of phytoplasma. These include polyclonal and monoclonal antisera, immunofluorescence, dot blot, immunosorbent EM and ELISA. Today, polyclonal and monoclonal antisera are mainly used just for detection of phytoplasmas involved in apple proliferation and flavescence dorée, which are both economically important (Bertaccini and Duduk, 2009). Due to the fact that the portion of phytoplasma DNA in extracted TNA from tissue is less than 1% (Bertaccini, 2007), the need for phytoplasma DNA amplification is more than obvious. Back in 1991 Deng and Hiruki reported about the first steps in constructing PCR primers which bind to the conserved 16S rDNA region. Schneider *et al*. (1995) proposed use of P1 and P7 primers as a universal primer pair for detection of phytoplasma. In order to increase the quantity of amplified DNA, sensitivity and specificity, direct PCR is followed by a nested PCR assay. For nested PCR assays universal phytoplasma primer pairs R16mF2/R1 and R16F2n/R2 (Gundersen and Lee, 1996) are used. Universal primer pair P1/P7 is used to amplify 1.8 kb fragment, which include 16S rRNA gene, the 16S – 23S spacer region and the 5´ end of the 23S DNA gene (Schneider *et al*. 1995), while primer pair R16F2n/R2 amplifies a 1.2 kb fragment corresponding to 16S rRNA gene (Lee *et al*. 1993). Apart from PCR primers which bind to the conserved 16S rDNA region, primers specific for 16S-23S intergenic spacer region, conserved rp gene and elongation factor EF-Tu (tuf) gene were generated as well (Lee *et al.* 2000).

For the classification of phytoplasmas, restriction fragments are generally used, which are obtained by RFLP analysis of PCR-amplified highly conserved 16S rDNA sequences after digestion with restriction enzymes (Seemüller *et al.* 1994). Some of the restriction endonucleases used in RFLP of phytoplasma 16S rDNA include *Alu*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II, *Mse*I, *Rsa*I, *Sau*3AI and *Taq*I (Salehi *et al.* 2009).

Future of phytoplasma research seems to be RT-PCR. The problem with the nested PCR is not its sensitivity but a risk of false positives. RT-PCR solves this issue because use of this method enables direct revealing of the results. For this reason there is no need for agarose gel electrophoresis in order to analyze results of PCR and the risk of contamination is much lower (Bertaccini and Duduk, 2009). And as the sequencing is becoming more and more available, new breakthroughs can be expected in the near future.

4. PHYTOPLASMA DETECTION AND CHARACTERISATION IN CONIFERS

I worked on a project which goal was to determine if conifers in Croatia are infected with phytoplasmas, and if so, which '*Ca*. Phytoplasma species' is in question.

4.1. PLANT MATERIAL

Needle samples were collected from 7 conifer species that showed symptoms, from locations across Croatia. Species included were: *Pinus halepensis*, *P. mugo*, *Juniperus oxycedrus*, *J. communis*, *P. nigra*, *Picea albies* and *Cupressus sempervirens*.

4.2. METHODS

4.2.1. TNA

Total nucleic acids extraction was performed following the protocol described by Šeruga *et al.* (2000). I checked the concentration of nucleic acids in samples using NanoDrop (Thermo Fisher Sciencetific 2000). Samples were then diluted to the final concentration of 20 ng/µl TNA.

4.2.2. PCR amplification

First, I performed direct PCR with isolated total nucleic acids (20 ng per reaction) using universal primers P1/P7. Conditions under which direct PCR was carried out: 2' 95°C, (1' 95°C, 1' 58°C, 2' 68°C) x 35 cycles + 10' 68°C. As a template for nested PCR I used 0,5 µl of reaction mixture from the direct PCR, and added universal primers R16F2n/R12R2. Conditions for nested PCR were: 1' 94°C, (1' 94°C, 2' 50°C, 3' 72°C) x 35 cycles + 7' 72°C. In PCR assays Taq polymerase and TBE buffer (Promega) were used.

4.2.3. Agarose gel electrophoresis

To make sure that PCR assays were successful and to check whether amplicons of direct and nested PCR assay are of expected size, 1.8 kb and 1.2 kb, respectively, I performed agarose gel electrophoresis on a 1% agarose gel using 1x TBE buffer. Prior to electrophoresis, SERVA DNA Stain G (SERVA Electrophoresis GmbH ('SERVA')) which emits green

fluorescence when bound to DNA, was added to the agarose gel. As a marker, *GeneRuler™ 1 kb DNA Ladder, 250-10,000 bp* (Thermo Fisher Scientific) was used. Five µl of amplicons were loaded into wells and electrophoresis was run at 6V/cm for about an hour. Gels were visualized under UV light.

4.3. RESULTS AND DISCUSSION

Presence of phytoplasma DNA was detected in six samples in total: five out of 10 samples collected from *Pinus halepensis* (commonly known as aleppo pine) and in one out of 11 samples of *Pinus mugo*, mountain pine. In samples where phytoplasma DNA was present, 1.2 kb DNA fragments were amplified (Fig 1). These results coincide with other published articles. Schneider *et al.* (2005) reported about presence of '*Candidatus* Phytoplasma pini' in *Pinus halepensis*. Kamińska *et al.* (2011) reported about phytoplasmas' presence in *Pinus mugo*, but during 2- year research they failed to detect phytoplasmas presence in *Pinus nigra*. I was unsuccessful in detecting phytoplasmas in samples collected from *Picea abies*, but there are reports of phytoplasma infection of this conifer. Kamińska and Śliwa (2010) reported about lower detectability of phytoplasmas in *P. abies*, which is approximately 70% of infected samples, while Kamińska and Berniak (2011) reported about detection of X-Disease Phytoplasma in 5 out of 24 samples collected from *P. abies.*

Further research revealed that detected phytoplasma in my samples is '*Candidatus* Phytoplasma pini' (Jezic *et al.* submitted for publication). A 16S rDNA fragments were sequenced and matched to the sequences of '*Ca.* P. pini' available in GenBank and this is the first report of '*Ca.* P. pini' in Croatia. So far, Schneider *et al*. (2005), Śliwa *et al*. (2008), Valiunas *et al*. (2010), Kaminska and Berniak (2011) and Kaminska *et al*. (2011) reported on presence of '*Ca.* P. pini' in Europe.

Although all trees, from which samples were collected, showed symptoms which pointed to possible phytoplasma infection, there was no phytoplasma DNA detected in any of the samples collected from *Juniperus oxycedrus*, *J. communis*, *Pinus nigra*, *Pica abies* or *Cupressus sempervirens*. As mentioned before, symptoms should not be taken as a sure indicator that phytoplasmas are present. Exhibited symptoms may be caused by some other biotic and/or abiotic agents. My results show that, so far, there is no widespread infection with phytoplasmas in conifer populations in Croatia. Future research should involve more sensitive methods as there is a slight possibility that phytoplasma DNA was detected in only six samples due to the very low titers, too low even for detection by nested PCR assays.

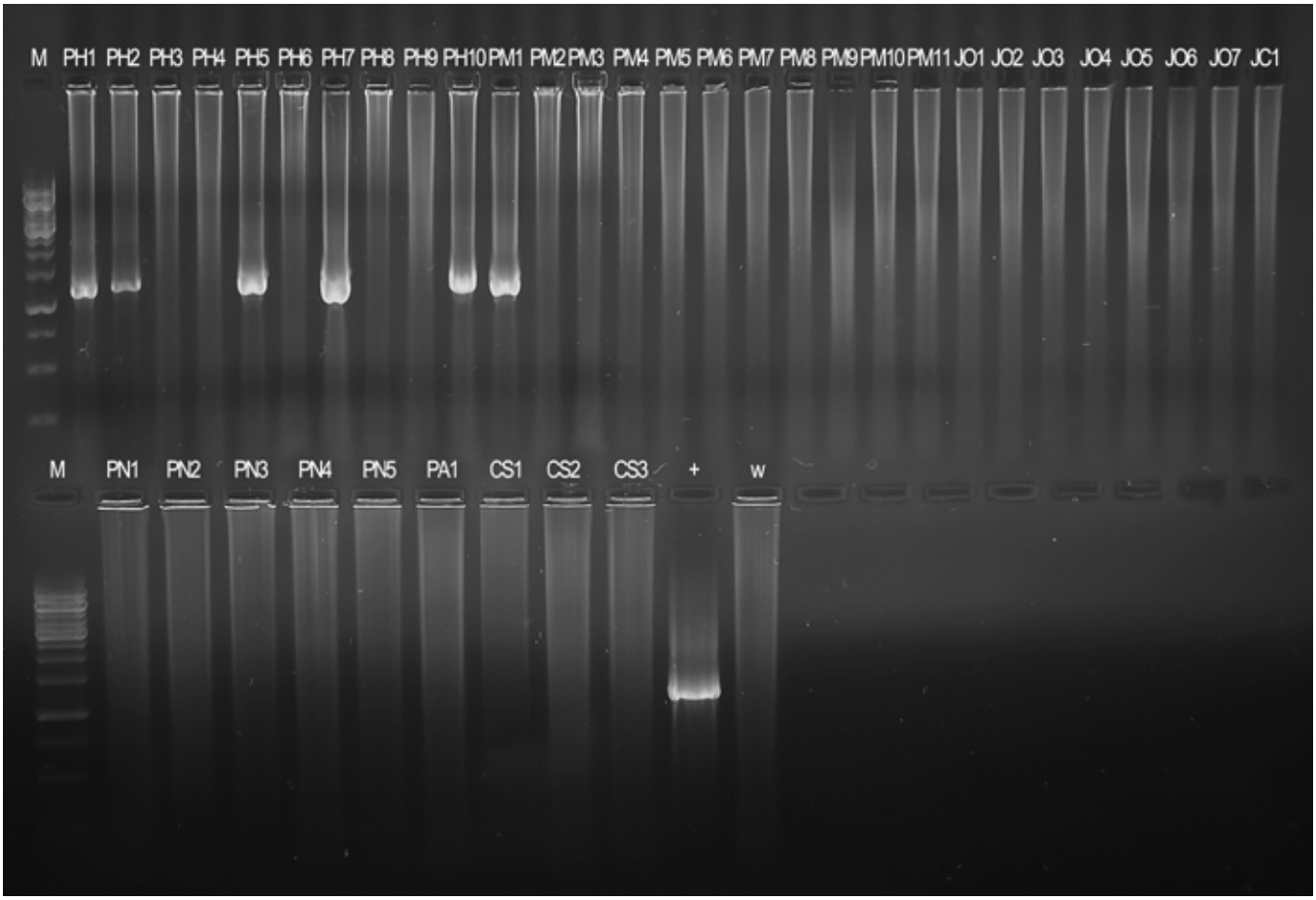

Fig 1. Electrophoresis of R16F2n/R2 PCR products on 1% agarose gel in 1X TBE buffer. Abbreviations for conifer species included in the experiment are: *Pinus halepensis* (PH), *P. mugo* (PM), *Juniperus oxycedrus* (JO), *J. communis* (JC), *Pinus nigra* (PN), *Picea. abies* (PA), *Cupressus sempervirens* (CS).

REFERENCES

Bai X, Zhang J, Ewing A, Miller SA, Radek AJ, Shevchenko DV, Tsukerman K, Walunas T, Lapidus A, Campbell JW, Hogenhout SA (2006) Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts. Journal of Bacteriology 188: 3682–3696.

Berges R, Rott M, Seemüller E (2000) range of phytoplasma concentrations in various plant hosts as determined by competitive polymerase chain reaction. Phytopathology 90: 1145-1152.

Bertaccini A, Duduk B (2009) Phytoplasma and phytoplasma dieses. Phytopathologia Mediterranea 48: 355-378.

Davis RE, Dally EL, Gundersen DE, Lee IM., Habili N (1997) '*Candidatus* Phytoplasma australiense,' a new phytoplasma taxon associated with Australian grapevine yellows. International Journal of Systematic Bacteriology 47: 262–269.

Deng S, Hiruki C (1991) Amplification of 16S rRNA genes from culturable and unculturable Mollicutes. Journal of Microbiological Methods 14: 53- 61.

Doi YM, Teranaka M, Yora K, Asuyama H (1967) Mycoplasma or PLT-group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. Annals of the Phytopathological Society of Japan 33: 259–266.

European Cooperation in the field of Scientific and Technical Research, COST (2008) Action FA0807- Integrated management of phytoplasma epidemics in different crop systems. Brussels, 11th December 2008.

Firrao G, Garcia-Chapa M, Marzachì C (2007) Phytoplasmas: genetics, diagnosis and relationships with the plant and insect host. Frontiers in Bioscience 12: 1353-1375.

Gopo JM, Katerere Y, Sibindi F (1989) Isolation, purification and identification of mycoplasma-like organisms as causal agents for the yellows disease in Pinus and in *Callistris cypress* in Zimbabwe. Discovery and Innovation 1: 82–86.

Green MJ, Thompson DA, MacKenzie DJ (1999) Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. Plant Disease 83: 482-485.

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

Hogenhout SA, Oshima K, Ammar el-D, Kakizawa S, Kingdom HN, Namba S (2008) Phytoplasmas: bacteria that manipulate plants and insects. Molecular Plant Pathology 9: 403–423.

Ježić M, Poljak I, Šafarić B, Idžojtić M, Ćurković Perica M (2012) *'Candidatus* Phytoplasma pini' in pine species in Croatia. (submitted for publication)

Kamińska M, Śliwa H (2010) Detection of X-disease phytoplasma in *Picea abies* A. Dietr. tree in Poland. Acta Horticulturae 885: 183–190.

Kaminska M, Berniak H (2011) Detection and identification of three *'Candidatus* Phytoplasma' species in *Picea* spp. trees in Poland. Journal of Phytopathology 159: 796-798.

Kamińska M, Berniak H, Obdrzalek J (2011) New natural host plants of '*Candidatus* Phytoplasma pini' in Poland and the Czech Republic. Plant Pathology 60: 1023–1029.

Koyama R (1970) Mycoplasma or virus-like particles in pine trees and insects. Journal of the Japanese Forestry Society 52: 126–13.

Kube M, Mitrovic J, Duduk B, Rabus R, Seemüller E (2011) Current view on phytoplasma genomes and encoded metabolism. The Scientific World Journal, 2012: 1-25.

Kube M, Schneider B, Kuhl H, Dandekar T, Heitmann K, Migdoll AM, Reinhardt R, Seemüller R (2008) The linear chromosome of the plant-pathogenic mycoplasma '*Candidatus* Phytoplasma mali'. BMC Genomics 9: 306-320.

Lee IM., Davis RE, Gundersen-Rindal DE (2000) Phytoplasma: Phytopathogenic mollicutes. Annual Review of Microbiology 54: 221–255.

Lee IM, Hammond RW, Davis RE, Gundersen-Rindal DE (1993) Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopahtology 83: 834-842.

Marcone C, Neimark A, Ragozzino A, Lauer U, Seemller E (1999) Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups. Phytopathology 89: 805–10.

McCoy RE, Caudwell A, Chang CJ (1989) Plant diseases associated with mycoplasma-like organisms. The Mycoplasmas 5: 545–640.

Musetti R, Sanità di Toppi L, Marabottini R, Borselli S, Martini M, Badiani M, Osler R (2006) The recovery of grapevine from phytoplasmas: variation of antioxidant status in leaf tissues. Proceedings of the 15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG). Stellenbosch, South Africa, 3rd to 7th April 2006, pp. 100.

Oshima K, Kakizawa S, Nishigawa H, Jung HY, Wei W, Suzuki S, Arashida R, Nakata D, Miyata S, Ugaki M, Namba S (2004) Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. Nature Genetics 36: 27-29.

Osler R, Carraro L, Loi N, Refatti R (1993) Symptom expression and disease occurrence of a yellows disease in grapevine in northern Italy. Plant Disease 77: 496-498.

Pavelak-Kozlina B: Fiziologija Bilja. Profil International, Zagreb, 2003.

Rudzinska-Langwald A, Kamińska M (1999) Cytopathological evidence for transport of phytoplasma in infected plant. Acta Societatis Botanicorum Poloniae 68: 261-266.

Salehi M, Izadpanah K, Siampour M, Firouz R, Salehi E (2009) Molecular characterization and transmission of safflower phyllody phytoplasma in Iran. Journal of Plant Pathology 91: 453-458.

Schneider B, Cousin MT, Klinkong S, Seemüller E (1995) Taxonomic relatedness and phylogenetic positions of phytoplasmas associated with diseases of faba bean, sunnhemp, sesame, soybean, and eggplant. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 102: 225-232.

Schneider B, Torres E, Martín MP, Schröder M, Behnke HD, Seemüller E (2005) '*Candidatus* Phytoplasma pini', a novel taxon from *Pinus silvestris* and *Pinus halepensis*. International Journal of Systematic and Evolutionary Microbiology 55: 303–307.

Seemüller E, Schneider B, Mäurer R, Ahrens U, Daire X, Kison H, Lorenz K-H, Firra G, Avinent L, Sears B. B, Stackebrandt E (1994) Phylogenetic classification of phytopathogenic mollicutes by sequence analysis of 16S ribosomal DNA. International Journal of Systematic Bacteriology 44: 440-446.

Seemüller E, Schneider B (2004) '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri' and '*Candidatus* Phytoplasma prunorum', the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively. International Journal of Systematic and Evolutionary Microbiology 54: 1217–1226.

Śliwa H, Kamińska M (2005) Detection of '*Candidatus* Phytoplasma asteris' in ashleaf maple trees with shoot proliferation and decline. Journal of Fruit and Ornamental Plant Research 13: 123-134.

Śliwa H, Kamińska M, Korszun S, Adler P (2008) Detection of '*Candidatus* Phytoplasma pini' in *Pinus sylvestris* trees in Poland. Journal of Phytopathology 156: 88-92.

Šeruga M, Škorić D, Kozina B, Mitrev S, Krajačić M, Ćurković Perica M (2003) Molecular identification of a phytoplasma infecting grapevine in the republic of Macedonia. Vitis 42: 181-184.

Tran-Nguyen LTT, Kube M, Schneider B, Reinhardt R, Gibb KS (2008) Comparative genome analysis of '*Candidatus* Phytoplasma australiense' (subgroup tuf-Australia I; rp -A) and '*Ca*. Phytoplasma asteris' strains OY-M and AY-WB. Journal of Bacteriology 190: 3979–3991.

Valiunas D, Jomantiene1 R, Ivanauskas A, Sneideris D, Staniulis J, Davis RE (2010) A possible threat to the timber industry: '*Candidatus* Phytoplasma pini' in Scots pine (*Pinus sylvestris* L.) in Lithuania. COST Action FA0807, Current status and perspectives of phytoplasma disease research and management. Sitges, Spain, 1st and 2nd February 2010, pp 38.

Weintraub PG, Beanland L (2006) Insect vectors of phytoplasmas. Annual Review of Entomology 51: 91-111.

http://cronodon.com/BioTech/Plant_Transport.html

SUMMARY

Phytoplasmas are plant endocellular parasites spread through the world. They range in size from 200 to 800 nm. Their genome is very small, from 530 kb to 1350 kb. Phytoplasmas lack a number of genes involved in: (I) essential metabolic pathways, like F_0F_1-type ATP–synthase and pentose phosphate pathway, (II) biosynthesis of amino and fatty acids, (III) metabolism of amino acids, nucleotides, sugars, glyoxylate and (IV) CO_2 fixation. In plants they reside in sieve elements and move around through the sieve pores, where plant's sap provides nutrients. To this date, few '*Ca*. Phytoplasma species' have been detected in gymnosperms - '*Ca*. P. pini', X disease phytoplasma from 16S rIII group, '*Ca*. P. trifolii' and '*Ca*. P. phoenicum'. In Croatia, '*Ca*. P. pini' is detected in two pine species *Pinus mugo* and *P. halepensis*. Infected plants exhibit number of symptoms which include shoot proliferation (whiches' broom), stunting, leaf yellowing, phyllody and dwarfing. Due to phytoplasma infection economic losses in the production of many plant species are substantial. So far, impact and possible consequences of phytoplasma infection in conifers are not known. However, scientists do warn that phytoplasma presence in conifers might present a threat to timber industry. It is thought that phytoplasma infection causes trees to be more vulnerable to infection with fungi and/or other damaging abiotic factors.

SAŽETAK

Fitoplazme su prokarioti bez stanične stijenke te kao biljni patogeni uzrokuju bolesti biljaka diljem svijeta. S vrlo malenim genomom, od 530 do 1350 kb, prosječne su veličine od 200 do 800 nm. Nedostaju im važni geni koji sudjeluju u važnim metaboličkim putevima, kao što su (I) F_0F_1-ATP–sintaza, geni uključeni u put pentoza fosfata, (II) biosintezu aminokiselina i masnih kiselina, (III) metabolizam aminokiselina, nukleotida, šećera te (IV) geni zaduženi za fiksaciju CO_2. Fitoplazme biljku putuju floemom kroz sitaste elementne, gdje sve potrebne nutrijente crpe iz floemskog soka. Do danas je dokazano prisustvo nekoliko različitih sojeva fitoplazmi kod golosjemenjača - '*Ca*. P. pini', X-disease fitoplazma iz 16S rIII grupe, '*Ca*. P. trifolii' i '*Ca*. P. phoenicum'. U Hrvatskoj, '*Ca*. P. pini' prisutna je u dvije vrste bora, *Pinus mugo* i *P. halepensis*. Jednom inficirane, biljke pokazuju različite simptome, kao što su virescencija, filodija, vještičja metla te žućenje listova. Fitoplazme uzrokuju velike ekonomske gubitke na različitim biljnim vrstama. Posljedice infekcije golosjemenjača fitoplazmama za sada nisu poznate. Unatoč tome, znanstvenici upozoravaju kako bi fitoplazme mogle predstavljati prijetnju drvnoj industriji. Pretpostavlja se da nakon infekcije fitoplazmama, drveće postaje podložnije gljivičnim infekcijama i/ili različitim nepovoljnim abiotičkim čimbenicima