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First report of a '*Candidatus* phytoplasma pyri' strain in Argentina

Franco Daniel Fernández[1] · **Diana Marini**[2] · **Roberto Farrando**[2] · **Luis Rogelio Conci**[1]

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Abstract During the 2015 growing season, peach plants (*Prunus persica*) showing chlorotic, curling leaves and midrib thickening were detected in production fields in Mendoza province (Midwest region, Argentina). These symptoms resemble those described for Peach Yellow Leaf Roll disease, a '*Candidatus* Phytoplasma pyri' related strain. By PCR of the 16S rDNA gene, a phytoplasma was detected in symptomatic samples. The RFLP profile, sequence identity and phylogenetic analyses showed that the Peach Yellows phytoplasma belongs to group 16SrX-C and is identical to those described as '*Candidatus* Phytoplasma pyri'. This is the first report of a phytoplasma from 16Sr group X infecting peach trees in Argentina and in South America.

Keywords Yellowing · Phytoplasma · Peach · 16SrX-group · RFLP

✉ Luis Rogelio Conci
conci.luis@inta.gob.ar

Franco Daniel Fernández
fernandez.franco@inta.gob.ar

Diana Marini
marini.diana@inta.gob.ar

Roberto Farrando
Farrando.roberto@inta.gob.ar

[1] Instituto de Patología Vegetal IPAVE-CIAP (Instituto Nacional de Tecnología Agropecuaria), Camino 60 cuadras km 5 ½ (X5020ICA), Córdoba, Argentina

[2] EEA Junín (Instituto Nacional de Tecnología Agropecuaria), Carril Isidoro Busquets La Colonia Junín, 5573, Junín, Mendoza, Argentina

Argentina is the main peach (*Prunus persica*) producer of the MERCOSUR (Mercado Común del Sur), with production reaching about 270,000 tons in 2012 (Dimeagro 2012). Mendoza, located in the Mid-West area, constitutes the main peach producing province in the country with a cultivated area of 15,000 h. Around 70% of Mendoza peach production is processed for industry while 10% is exported (IDR 2010). In most peach producing areas in the world, trees are severely affected by yellows and decline diseases of phytoplasma etiology (Marcone et al. 2014). In the NW region of Argentina (Jujuy province) phytoplasmas belonging to the X-disease group (16SrIII-B subgroup) have been previously detected affecting peach production. The symptomatology includes leaf yellowing, reddening, curling necrosis, little leaf, premature defoliation, internode shortening, die back and progressive decline (Fernández et al. 2013). The X-disease (16SrIII) is one of the most widespread phytoplasma groups; not only is it represented by a high number of subgroups, but it also has a wide host range (Galdeano et al. 2013; Conci et al. 2014).

To date, phytoplasmas from four ribosomal groups (16SrI, 16SrIII, 16SrVII and 16SrXIII) have been detected in over 20 plant species in Argentina, some of which are economically important, such as garlic (*Allium sativum*), China-tree (*Melia azedarach*), strawberry (*Fragaria* x *anannassa*) and alfalfa (*Medicago sativa*), causing variable damages and losses. During the 2015 growing season, peach plants showing chlorotic, curling leaves and midrib thickening (Fig. 1) were detected in production fields located in Tres Porteñas, San Martín and Tupungato (Mendoza province- Midwest region). The symptoms resembled those described for Peach Yellow Leaf Roll disease (PYLR) reported in California (USA) (Blomquist and Kirkpatrick 2002) and Spain (Sabaté et al. 2014), and associated with '*Candidatus* Phytoplasma pyri'. There is no report of '*Candidatus* Phytoplasma pyri' affecting peach or any other plant species in Argentina. Thus, the aim of

Fig. 1 Peach leaf showing chlorotic, curling leaves and midrib thickening (S) vs symptomless leaf (H)

this work was to identify the causal agent of this disease and to perform its molecular characterization.

Surveys were performed in production fields located in Junín, San Martín, Tunuyán and Tupungato Departments (Mendoza province, Argentina) during March 2015. Yellowing and rolling peach leaves were collected (n = 31) and symptomless (n = 4) samples were used as negative controls. For molecular diagnosis total DNA extractions were conducted following the Doyle and Doyle (1990) method. The DNA quantity and quality was assessed by agarose gel electrophoresis and spectrophotometry (NanoDrop®-ND 1000). Phytoplasma detection was assayed by 16S rDNA gene PCR using the universal primer pairs P1/P7 (~1.8 kb) (Deng and Hiruki 1991; Schneider et al. 1995) and R16F2n/R16R2 (~1.2 kb) (Gundersen and Lee 1996) on direct and nested reactions, respectively. Each PCR reaction consisted of 50 ng of DNA, 0.4 mM of each primer, 200 mM of each dNTP, 1 U of GoTaq® DNA Polymerase, 1X polymerase buffer (Promega, USA) and sterile water to a final volume of 40 μl. For nested PCR, 1 μl of 1:25 dilution of the P1/P7-PCR product was used. DNA from periwinkle (*Catharanthus roseus*) infected with ACLL phytoplasma (Torres et al. 2011) was used as a positive control. Amplifications were analyzed through electrophoresis in agarose gels, stained with Gel Red® and visualized using a UV transilluminator. The 1.2 kb amplicons (16S rDNA partial gene) were subjected to digestion with the enzymes *Tru*1I (*Mse*I) (Thermo Scientific, USA), *Rsa*I, *Alu*I and α*Taq*I (NEB, USA) according to the manufacturer's instructions. The RFLP profiles were identified in agarose Metaphore (1.5%:0.5%) gels, stained with Gel Red® and visualized using a UV transilluminator. Selected amplicons (1.2 kb, 16S rDNA partial sequence) were purified with MicroSpin S-400HR Columns (Amersham Biosciences, Sweden) and cloned in the pGEM®-T Easy vector system (Promega, USA). For each isolate, 3 clones were selected and sequenced (3X coverage per base position) using an automated DNA sequencer (Unidad Genómica, Instituto de Biotecnología-INTA; Argentina). The assembly was conducted with Staden program package (Staden et al. 2000) and the consensus sequences were deposited in GenBank (NCBI). The sequences were analyzed with the BLAST program (Altschul et al. 1990) and 16Sr group/subgroup affiliations were performed using the website tool *i*Phyclassifier (Zhao et al. 2009). To infer the phylogenetic relationships, 29 phytoplasma reference sequences (1.2 kb) related to those obtained in this work were aligned using MUSCLE (Edgar 2004). The phylogenetic tree was computed by the minimal-evolution method (Rzhetsky and Nei 1994) using MEGA6 software (Tamura et al. 2013). Bootstrap (1000 replicates) was used as statistical support for the inferred clades and evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004).

In PCR reactions, four out of thirty one (~13%) symptomatic plant DNA extracts amplified to produce 1.8 kb and 1.2 kb fragments; no product was obtained from symptomless plants. The four isolates showed an identical PCR-RFLP (1.2 kb) profile when *Tru*1I, *Rsa*I, *Alu*I and α *Taq*I restriction enzymes were used (results not shown). This actual and virtual RFLP profiles were also identical to that described for '*Candidatus* Phytoplasma pyri' by Seemüller and Schneider (2004). Two DNA samples, named as PeY-Arg5 and PeY-Arg7 (PeY: Peach Yellows), were sequenced and deposited in GenBank with the accession numbers KX639783 and KX639784. Blast searches showed the highest similarity (~99%) with the 16S rDNA gene sequence of 16SrX phytoplasma group (Apple proliferation). The in silico RFLP profiles (*i*PhyClassifier) demonstrated that PeY is a variant of the subgroup 16SrX-C (similarity coefficient = 0.99). Sequences from 16SrX or Apple proliferation group (subgroups 16SrX-A, 16SrX-B and 16SrX-C) and other related '*Candidatus* Phytoplasmas species', were used to construct a phylogenetic tree. In the resulting cladogram, three clearly defined clades were observed within the 16SrX group corresponding to subgroups 16SrX-A,- B and -C ('*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma prunorum' and '*Candidatus* Phytoplasma pyri', respectively) (Fig. 2). Peach Yellows isolates were grouped within the 16SrX-C subgroup clade (bootstrap = 97), which was consistent with the RFLP profile and sequence identity.

PYRL is an important peach disease that causes chlorosis, leaf tip downward curling and leaf margin rolling in midsummer due to a cork layer deposition. The causal agent is a subtype of '*Candidatus* Phytoplasma pyri' (Seemüller and Schneider 2004) which is a member of the Apple

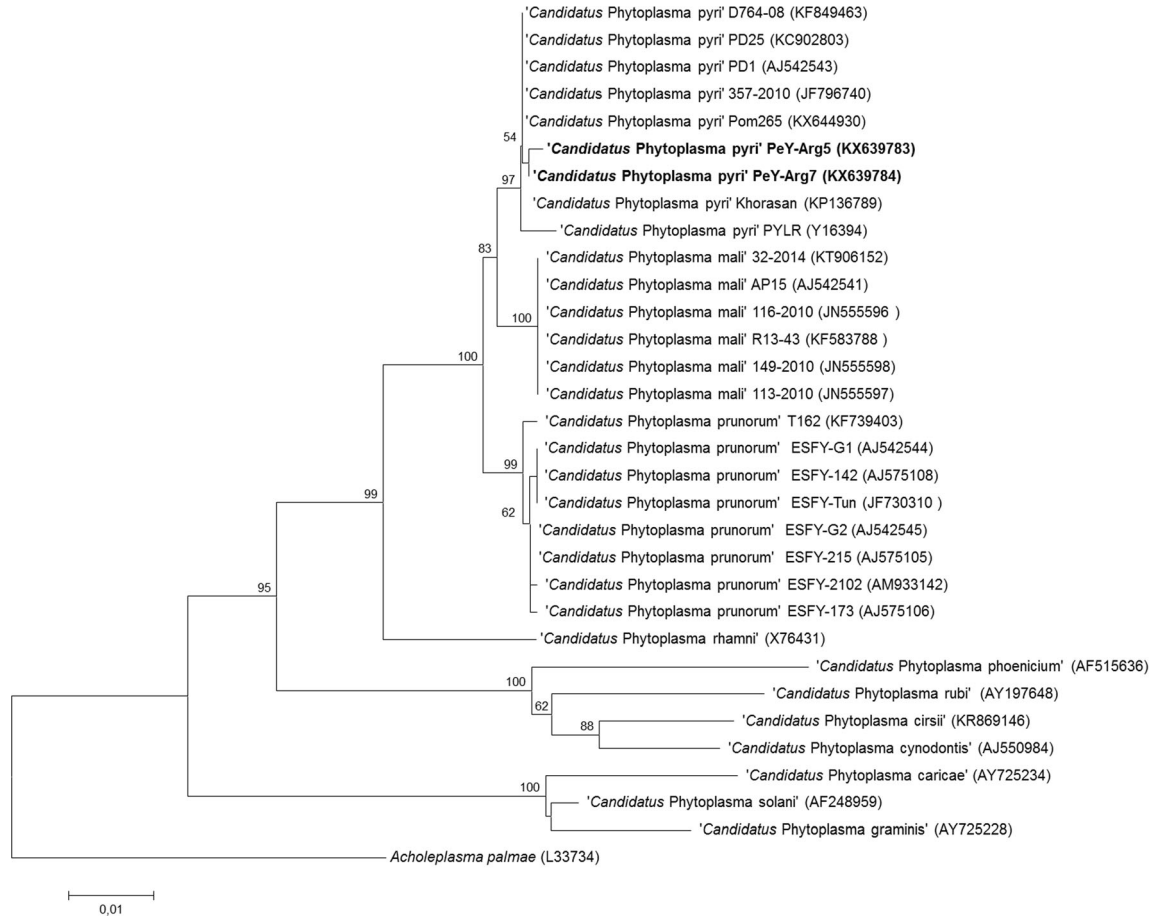

Fig. 2 Phylogenetic tree inferred from analysis of 16S rDNA sequences using the minimal-evolution method. *Acholeplasma palmae* was used as outgroup. The numbers on the branches are bootstrap (confidence) values (expressed as percentage of 1000 replicates). The GenBank accession number for each taxon is given between parentheses. The PeY strains sequenced in this paper are in *bold*

Proliferation group (16SrX). This pathogen is transmitted in the Northern Hemisphere by the psyllid *Cacopsylla pyricola*. Field transmissions to peach trees occur when psyllids migrate in late autumn from pear orchards to neighboring peach orchards to overwinter (Purcell et al. 1981; Blomquist and Kirkpatrick 2002). The Peach Yellows (PeY) phytoplasmas described in this work resembles the symptomatology associated with PYLR. RFLP-profiles, sequence identity and phylogeny confirm that PeY is a strain of the '*Candidatus* Phytoplasma pyri' (16SrX-C subgroup). Low percentage of infected symptomatic plants (~13%) found, may be due to a low phytoplasma concentration or an uneven distribution within the tissues or more likely, that the symptoms are unclear depending on the plant genotype, age or environmental conditions which makes it difficult to identify. Recently the '*Candidatus* Phytoplasma pyri' was described for the first time affecting pear in Chile (Rosany et al. 2016). Comparative analysis of the 16S rDNA sequence from this phytoplasma (KX644930) and PeY showed an identity of 99.70% and a RFLP similarity coefficient of 0.99 (data not shown). In this work we reported for the first time a '*Candidatus* Phytoplasma pyri' related strain associated with peach yellowing disease in Argentina and also in South America. Phytoplasmas of other phylogenetic groups, which are known to infect a wide range of plant hosts, have been identified in declining peach trees in the north area of the country (Fernández et al. 2013). Pears are produced in Argentina in the same region where the presence of PeY is reported; so far it has not been detected associated with this crop. The presence of this pathogen in peach trees may be due to the introduction of infected material from other regions. However, the epidemiological relevance of these 'non-peach' phytoplasmas needs to be further investigated. Furthermore, the presence of *Cacopsylla pyricola* was reported in Argentina without distribution data (SINAVIMO 2016), so the role that this insect may have in the spread of this pathogen from pear to peach trees is not known. Future research should be aimed to detect the presence of PeY phytoplasma in peach and pear orchards or weeds. These investigations should be complemented by studies of possible vector population to obtain a more comprehensive view of the whole pathosystem.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Blomquist CL, Kirkpatrick BC (2002) Identification of phytoplasma taxa and insect vectors of peach yellow leaf roll disease in California. Plant Dis 86:759–763

Conci L, Saavedra Pons A, Guzman F, Fernandez F, Galdeano E, Perez Grosso T, Torres L, Meneguzzi N (2014) Advances in knowledge about phytoplasma diseases in Argentina. Chapter 2: The phytoplasmas and phytoplasma vectors in COST FA0807 international Countries. In Phytoplasmas and Phytoplasma Diseases management: how to reduce their economic impact. (ed) A. Bertaccini. Bologna, Italia. ISBN 978-88-909922-0-9 p 288

Deng S, Hiruki C (1991) Amplification of 16S rRNA genes from culturable and non-culturable mollicutes. J Microbiol Methods 14:53–61

Dirección de Mercados Agrícolas. Perfil del Mercado del Durazno (2012) Subsecretaría de Agricultura. Argentina. www.minagri.gob.ar/dimeagro

Doyle J, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797

Fernández FD, Guzmán FA, Curzel V, Bejarano N, Conci LR (2013) Detection and molecular characterization of a phytoplasma affecting *Prunus persica* L. in Jujuy, Argentina. Eur J Plant Pathol 135:627–631

Galdeano E, Guzmán F, Fernández F, Conci L (2013) Genetic diversity of 16SrIII group phytoplasmas in Argentina. Predominance of subgroups 16SrIII-J and B and two new subgroups 16SrIII-W and X. Eur J Plant Pathol 137(4):753–764

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

Instituto de Desarrollo Rural (2010) Durazno para consumo en fresco-Censo frutícola provincial (2010). Mendoza, Argentina. http://www.idr.org.ar

Marcone C, Guerra L, Uyemoto J (2014) Phytoplasmal diseases of peach and associated phytoplasma taxa. J Plant Pathol 96:15–28

Purcell AH, Nyland G, Raju BC, Heringer MR (1981) Peach yellow leaf roll epidemic in northern California: effects of peach cultivar, tree age, and proximity to pear orchards. Plant Dis 65:365–368

Rosany F, Quiroga N, Méndez P, Zamorano A, Fiore N (2016) First report of '*Candidatus* phytoplasma pyri' on pear in Chile. Plant Dis. doi:10.1094/PDIS-08-16-1144-PDN

Rzhetsky A, Nei M (1994) Unbiased estimates of the number of nucleotide substitutions when substitution rate varies among different sites. J Mol Evol 38:295–299

Sabaté J, Laviña A, Batlle A (2014) First report of '*Candidatus* phytoplasma pyri' causing peach yellow leaf roll (PYLR) in Spain. Plant Dis 98:989

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC (1995) Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas. In: Razin R, Tully JG (eds) Molecular and diagnostic procedures in mycoplasmology, vol I. Academic Press, San Diego, pp 369–380

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. Int J Syst Evol Microbiol 54:1217–1226

Sistema Nacional Argentino de Vigilancia y Monitoreo de Plagas SINAVIMO (2016) http://www.sinavimo.gov.ar/plaga/cacopsylla-pyricola

Staden R, Beal KF, Bonfield JK (2000) The Staden package, 1998. Methods Mol Biol 132:115–130

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. PNAS 101: 11030–11035

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Torres L, Galdeano E, Fernández F, Meneguzzi N, Conci L (2011) Establishment of the new subgroup 16SrI-S (rr-rp) tuf-H belonging to '*Ca*. Phytoplasma asteris' in wild and cultivated plants in Argentina. J Plant Pathol 93:311–320

Zhao Y, Wei W, Lee IM, Shao J, Suo X, Davis RE (2009) Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). Int J Syst Evol Microbiol 59:2582–2593