Genetic Diversity and Geographical Distribution of Phytoplasmas Associated with Potato Purple Top Disease in Mexico

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ABSTRACT

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Potato purple top (PPT) disease has caused severe economic losses in some potato (*Solanum tuberosum*) growing areas of Mexico. Two distinct phytoplasma strains belonging to the aster yellows and peanut witches'-broom groups (16SrI and 16SrII groups) have been associated with PPT disease in several regions of Mexico. However, there has been no previous large-scale survey in the main potato growing areas in Mexico to analyze the diversity and geographical distribution of phytoplasmas. Potato samples were collected between 2003 and 2006 and were analyzed by nested polymerase chain reaction assays. On the basis of results from nucleotide sequence comparisons and virtual restriction fragment length polymorphism analysis of 16S rDNA, four different phytoplasma groups were detected in potato growing areas in Mexico. The aster yellows group (16SrI) '*Candidatus* Phytoplasma asteris' was distributed in all potato growing areas, whereas peanut witches'-broom group (16SrII) '*Candidatus* Phytoplasma aurantifolia' was detected in Guanajuato and Sinaloa, X-disease group (16SrIII) was detected in Coahuila and Guanajuato, and the Mexican periwinkle virescence (16SrXIII) was only detected in Sinaloa. Phytoplasmas from X-disease and Mexican periwinkle virescence groups were detected in potato samples for the first time in Mexico.

The potato (*Solanum tuberosum* L.) is one of the most important crops cultivated for local consumption and industry in Mexico. The national production in 2008 was 1.67 million metric tons with a value of US\$603 million (43). In recent years, phytoplasmas have been found associated with potato purple top (PPT) disease in Mexico (29). This disease is considered an important limiting factor in the potato production in this country. In 2003 and 2004, the incidence of PPT disease caused significant economic losses in the Mexican state of Coahuila. Currently, PPT disease has been spread in all major potato growing areas in Mexico. Symptoms of the disease are purple discoloration (purple

top) or yellowing of upper leaflets, apical leafroll, axillary buds, and the formation of aerial tubers. Storage tubers from affected plants do not sprout, or the sprouting is of extremely weak stems deficient in chlorophyll giving the appearance of white threads (34). However, we have observed that some infected tubers showed normal sprouting and could be an important spreading factor of phytoplasma diseases in potato growing areas. Moreover, the crops are unmarketable since the tubers show a normal appearance, but a dark color is observed when they are processed, causing a considerable economic loss in the potato industry. The symptoms of PPT disease resemble those of zebra chip, a disorder of potato recently found to be associated with '*Candidatus* Liberibacter solanacearum' in New Zealand and the United States (1,30).

Phytoplasma diseases of potato have been reported in several countries around the world including Mexico (29,34), the United States (3,22–25,40,44,48), Canada (20,21), Bolivia (18), Australia (12), Korea (19), Russia (7), and New Zealand (32). At least eight distinct phytoplasma strains

have been associated with PPT disease around the world. The most common phytoplasma groups causing the disease are aster yellows (16SrI-A and 16SrI-B), peanut witches'-broom (16SrII), clover proliferation (16SrVI-A), and stolbur (16SrXII-A). Recently, '*Candidatus* Phytoplasma americanum' (16SrXVIII-A and 16SrXVIII-B) and X-disease group (16SrIII-M) have been associated with PPT disease in the United States (23,24). Leyva-López et al. (29) characterized two phytoplasma strains belonging to the aster yellows and peanut witches'-broom groups (16SrI and 16SrII groups) associated with PPT disease in samples collected in several regions of Mexico. However, there has been no previous large-scale survey of the main potato growing areas in Mexico to analyze the genetic diversity and geographical distribution of phytoplasmas. This paper reports the results of a 4-year survey on the genetic diversity and geographical distribution of phytoplasma groups associated with PPT disease in Mexico. In addition, two phytoplasma strains associated with PPT disease not previously reported in Mexico were molecularly characterized by virtual restriction fragment length polymorphism (RFLP) profiles and 16S rDNA sequence analysis.

MATERIALS AND METHODS

Plant samples. Foliage from 575 potato plants (173 asymptomatic and 402 symptomatic) and 2,419 asymptomatic potato tubers (potato seed and freshly harvested tubers) were collected between 2003 and 2006 in the main potato growing areas in Mexico including Sinaloa (SIN), Sonora (SON), Chihuahua (CHI), Guanajuato (GTO), Coahuila (COAH), Jalisco (JAL), and Baja California (BC) (Fig. 1 and Table 1). BC is not one of the main potato producers, but it is important as a seed potato producer. At least three fields from each potato growing area and five potato plants per field were sampled.

DNA extraction. Total nucleic acid was extracted as described elsewhere (49), except that 200 mg of fresh tissue was

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transferred to a 1.5-ml tube and ground in 800 µl of preheated CTAB extraction buffer (60°C) (3% [foliage] and 4% [tubers] CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0, 0.2% mercaptoethanol), followed by incubation at 60°C for 30 min. Samples were extracted with chloroform-isoamylalcohol (24:1). The aqueous DNA layer was precipitated with 600 µl of cold isopropanol. DNA pellets were washed with 70% ethanol, dried, and suspended in 20 to 80 µl of sterile twice-distilled water.

Detection of phytoplasmas in potato samples. Two “universal” phytoplasma nested primers were used for the detection of phytoplasma. R16mF2/R16mR1 and R16F2n/R16R2 primers were used to amplify a portion of the 16S rDNA (9). Polymerase chain reaction (PCR) amplifications were conducted in an automated thermocycler (model Icycler, BioRad, USA), as previously described (39). PCR products were analyzed by electrophoresis in a 1% agarose gel and visualized by staining with ethidium bromide and UV illumination. DNA samples extracted from potato tissues (foliage and tubers) were used as template for PCR. DNA extracted from asymptomatic foliage and tubers and sterile water were used as negative controls. Total genomic DNA from a previ-

ously characterized phytoplasma infecting *Opuntia* spp., belonging to the peanut witches'-broom group (16SrII, GenBank accession number AF200718), was used as positive control (28).

Cloning of PCR products and sequencing of DNA. To verify nested PCR fragments amplified by universal phytoplasma primer pairs R16mF2/R16mR1 and R16F2n/R16R2 (9), a representative num-

Table 1. Phytoplasma detections in potato tubers and foliage of different geographic areas in Mexico using nested polymerase chain reaction (PCR) with primers pairs R16mF2-R16mR1/R16F2n-R16R2

Location	Year	Plant tissue positive samples/ total samples (% ^a)		Classification 16Sr group and subgroup
		Tuber	Foliage	
Sinaloa	2003	4/200 (2)	0/68 (0)	ni
Sinaloa	2004	5/95 (5)	0/60 (0)	ni
Sinaloa	2005	50/569 (9)	5/6 (83)	16SrI-S
Sinaloa	2006	45/100 (45)	14/26 (54)	16SrI-S, 16SrII-C, 16SrXIII-D
Coahuila	2003	7/97 (7)	13/134 (10)	16SrI-S, 16SrI-T
Coahuila	2004	9/40 (23)	1/11 (9)	16SrI-S, 16SrI-T, 16SrIII-S
Coahuila	2005	16/55 (29)	24/25 (96)	16SrI-S, 16SrI-T, 16SrIII-S
Coahuila	2006	10/73 (14)	16/55 (29)	16SrI-S, 16SrI-T, 16SrIII-S
Guanajuato	2003	6/499 (1)	1/34 (3)	16SrI-S, 16SrII-M
Guanajuato	2004	2/246 (1)	9/152 (6)	16SrI-S, 16SrI-T, 16SrIII-S
Guanajuato	2005	na	4/4 (100)	16SrI-S, 16SrI-T, 16SrIII-S
Sonora	2004	13/39 (33)	na	16SrI-V
Sonora	2005	1/3 (33)	na	16SrI-V
Chihuahua	2004	8/100 (8)	na	ni
Chihuahua	2005	0/200 (0)	na	ni
Baja California	2004	8/60 (13)	na	16SrI-S
Jalisco	2004	0/15 (0)	na	ni
Jalisco	2006	4/28 (14)	na	16SrI-S, 16SrI-U
Total		188/2419	87/575	

^a Percentage of positive samples over total plants. ni: not identified, na: not available.

Fig. 1. Map of Mexico showing different geographic areas in which potato samples were collected.

ber of PPT phytoplasma isolates from different potato growing areas in Mexico and from different years of study were selected after RFLP analysis, purified with Genclean III kit (Gibco-BRL), and ligated onto the pGEM-T vector (Promega Corporation). The ligation mixture was used to transform *Escherichia coli* DH5 α (Promega). Plasmid DNA from cultures of recombinant colonies was purified using QIAprep Spin Miniprep Kit (QIAGEN GmbH). Both strands of two different clones of each phytoplasma isolate were sequenced completely twice in an ABI PRISM 377 sequencer using the Dye cycle Sequencing kit (Applied Biosystems). The sequences of 16S rDNA from PPT phytoplasmas were deposited in GenBank with the accession numbers given in Table 2 and Figure 2.

DNA sequence analysis and *in silico* enzyme digestions. The nucleotide sequence data were edited using the EditSeq program of the sequence analysis software suite Lasergene (DNASTAR Inc.). BLAST was used to compare our sequences against DNA sequences available in the National Center for Biotechnology Information (NCBI). Virtual RFLP patterns were performed from the partial sequences of the 16S rDNA gene using the virtual gel plotting program pDRAW32 (AcaClone). Each aligned DNA fragment was digested *in silico* with 17 distinct restriction enzymes (*AluI*, *Bam*HI, *BfaI*, *Bst*UI, *DraI*, *Eco*RI, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II, *Kpn*I,

Sau3AI, *MseI*, *RsaI*, *SspI*, and *TaqI*) that have been routinely used for phytoplasma 16S rRNA gene RFLP analysis (26). The virtual RFLP patterns were compared and a similarity coefficient (F) was calculated for each pair of phytoplasma strains using a Perl program developed by Wei et al. (47).

For validation of the new 16Sr (-I), -II, -III, and -XIII subgroups delineated on the basis of virtual RFLP analysis, actual gel-based PCR-RFLP analysis was performed on 16S rRNA gene F2nR2 fragments amplified from representative strains of each new -I, -II, -III, and -XIII subgroup. DNA from pepper and tomato plants infected with PeLL and ToLL phytoplasmas (16SrI-S and 16SrI-T subgroups, respectively) were used as reference strains (39) in virtual and actual RFLP analysis, and the sequences from FBP (16SrII-C), MW1 (16SrIII-F), and MPV (16SrXIII-A) were used as reference strains (28) in virtual RFLP analysis, since those phytoplasma strains had the highest identity with the new subgroups identified in this study. F2nR2 nested PCR amplicons generated with the primer pairs R16mF2/R16mR1 and R16F2n/R16R2 were digested with the respective restriction enzymes (*AluI*, *Bst*UI, *Hin*FI, *Hpa*II, *Mse*I, *Rsa*I) according to the manufacturer's instructions (New England Biolabs). The digestion products were fractionated by electrophoresis using 3.0% agarose gels and stained in ethidium bromide.

Phylogenetic analysis. Nearly complete 16S rDNA sequences from PPT phytoplasmas along with 19 ‘*Ca. Phytoplasma*’ species, other phytoplasmas representing distinct phytoplasma groups or subgroups, and *Acholeplasma palmæ* were aligned using the Clustal W program of the sequence analysis software suite Lasergene (DNASTAR). The phylogenetic tree was generated using the Clustal W program through the neighbor-joining method. *A. palmæ* was employed as the out-group to root the tree. Bootstrapping was performed 1,000 times for estimation of stability and support for the branches.

RESULTS

Detection of phytoplasmas from potato tubers and foliage samples. Phytoplasmas were detected by nested PCR with universal primer pairs R16F2n/R16R2 and R16mF2/R16mR1 in all main potato growing areas in Mexico in samples collected between 2003 and 2006. Eighty-seven out of 575 samples and 188 out of 2,419 samples from foliage and asymptomatic potato tubers, respectively, were infected with phytoplasmas (Table 1). Only two out of 173 asymptomatic potato plants gave positive PCR results. Not all the freshly harvested tubers from a phytoplasma infected potato plant were PCR positive to phytoplasmas.

Nucleotide sequence analysis and *in silico* enzyme digestions. The F2nR2 amplified fragments (approximately 1.25

Table 2. Similarity coefficients derived from analysis of virtual restriction fragment length polymorphism (RFLP) patterns of 16S rRNA genes from phytoplasma strains in the aster yellows (16SrI), peanut witches'-broom (16SrII), X-disease (16SrIII), and Mexican periwinkle virescence (16SrXIII) groups and 17 potato-infecting phytoplasma strains^a

[illegible]

^a New subgroups are shown in bold. For each strain of a new subgroup, the similarity coefficients are equal to or lower than 0.97 with all strains in previously delineated subgroups.

kb) were analyzed by actual RFLP (data not shown) to select representative amplicons from each year and potato growing areas in Mexico. At least 20 potato phyto-

plasma isolates were selected and analyzed by actual RFLP from each amplicon cloned. The amplicons from each year and potato growing area in Mexico that yielded

the same RFLP profile and those potato phytoplasmas isolated that presented 100% identity were discarded from this study; 17 representative isolates were selected both

Fig. 2. Phylogenetic distance tree constructed by the Clustal W alignment method (DNASTAR) comparing 16S rDNA gene sequences from potato purple top phytoplasmas with sequences of 19 *Candidatus* Phytoplasma species and 14 other key phytoplasma groups and subgroups. *Acholeplasma palmae* was used as the outgroup for the phylogenetic tree reconstruction. Bootstrap (confidence) values are shown on the branches. GenBank accession numbers for sequences are given in parentheses. Phytoplasma strains from this study are in bold.

from different potato growing areas in Mexico and from different year of study. These 16S rDNA sequences were compared with those in the NCBI/GenBank database. Eleven PPT phytoplasma isolates had maximum identity (99%) with several strains of the aster yellows group (16SrI) '*Ca. P. asteris*'; three PPT phytoplasma isolates had maximum identity (99%) with several strains of the peanut witches'-broom group (16SrII) '*Ca. P. aurantifolia*'; two PPT phytoplasma isolates had maximum identity (99%) with strains of the X-disease group (16SrIII); and one PPT phytoplasma isolate had maximum identity (99%) with strains of the Mexican periwinkle virescence group (16SrXIII). These results revealed the occurrence of four different phytoplasma groups infecting potato crops in Mexico (groups 16SrI, 16SrII, 16SrIII, and 16SrXIII). '*Ca. P. asteris*' was distributed in all potato growing areas, whereas peanut witches'-broom group (16SrII) '*Ca. P. aurantifolia*' was detected in GTO and SIN, the X-disease group (16SrIII) was detected in COAH and GTO, and the Mexican periwinkle virescence group (16SrXIII) was only detected in SIN (Table 1).

Phytoplasma isolates detected in potato samples classified as strains of '*Ca. P. asteris*' were designated as PPT-BC1, PPT-BC8, PPT-BC15, PPT-COAH10, PPT-COAH19, PPT-GTO5, PPT-JAL2, PPT-JAL6, PPT-SIN6, PPT-SIN15, and PPT-SON18 (Table 2). Phytoplasma isolates detected in potato samples classified as strains of '*Ca. P. aurantifolia*' were designated as PPT-GTO29, PPT-GTO30, and PPT-SINTV (Table 2), phytoplasma isolates detected in potato samples classified as strains of the X-disease group (16SrIII) were designated as PPT-COAH and PPT-GTOP (Table 2), and one phytoplasma isolate detected in potato samples classified as a strain of the Mexican periwinkle virescence group (16SrXIII) was designated as PPT-SINPV (Table 2).

Virtual RFLP analysis of 16S rRNA sequences was used to assign PPT strains to groups and subgroups (46,47). Results from pairwise comparison of virtual 16S rRNA gene F2nR2 RFLP patterns revealed that eight of the 11 16SrI PPT phytoplasma strains identified in potato samples had a pattern type identical to PeLL phytoplasma, while one 16SrI PPT phytoplasma strain (PPT-COAH19) had a pattern type identical to ToLL phytoplasma; therefore, the eight phytoplasma strains are considered to be new members of subgroup 16SrI-S, and the PPT-COAH19 phytoplasma strain is considered to be a new member of subgroup 16SrI-T. The remaining two strains (PPT-JAL6 and PPT-SON18) showed two different virtual RFLP patterns that differed at least one band with each other and from those of the previously described subgroups, and their similarity coefficients were equal to or less

than 0.97 with each of the other known subgroups (Fig. 3 and Table 2); therefore, PPT-JAL6 and PPT-SON18 phytoplasma strains were designated as new subgroups, U and V (16SrI-U and 16SrI-V, respectively), following criteria reported by Wei et al. (47).

The virtual RFLP pattern types revealed that one of the three 16SrII PPT phytoplasma strains identified in potato samples had a pattern type identical to faba bean phyllody phytoplasma (FBP); therefore, this phytoplasma strain (PPT-SINTV) is considered to be a new member of subgroup 16SrII-C. The remaining two strains (PPT-GTO29 and GTO30) showed the same virtual RFLP patterns, but those were different at least in one band with previously described phytoplasma subgroups, and their similarity coefficient was equal to or less than 0.97 with each of the other known subgroups (Fig. 3 and Table 2); therefore, PPT-GTO29 and PPT-GTO30 phytoplasma strains were designated as new subgroup M (16SrII-M).

Following the criteria used for the 16SrI and 16SrII phytoplasma groups, the phytoplasma strains (PPT-COAH and PPT-GTOP) belonging to the X-disease group (16Sr-III) showed the same virtual RFLP patterns, but those were different at least in one band from previously described phytoplasma subgroups, and their similarity coefficients were equal to or less than 0.97 with each of the other known subgroups (Fig. 3 and Table 2); hence, we designated PPT-COAH and PPT-GTOP phytoplasma strains as new subgroup S (16SrIII-S). The only phytoplasma strain (PPT-SINPV) belonging to the Mexican periwinkle virescence group (16SrXIII) showed a different virtual RFLP pattern from the other known subgroups (Fig. 3 and Table 2); therefore, we designated PPT-SINPV phytoplasma strains as new subgroup D (16SrXIII-D).

The actual gel-based PCR-RFLP analysis was done on likely new potato phytoplasma strains and PeLL and ToLL phytoplasma reference strains to verify the results of the virtual analysis (data not shown).

The actual gel-based RFLP analysis generated restriction profiles identical to those produced by virtual RFLP analysis (data not shown). The virtual different RFLP profiles that delineated new subgroups in the 16SrI, 16SrII, 16SrIII, and 16SrXIII phytoplasma groups are shown in Figure 4. PPT-JAL6 phytoplasma strain subgroup U (16SrI-U) can be differentiated from PeLL and/or ToLL phytoplasma strain subgroups S and T (16SrI-S and 16SrI-T, respectively) by the restriction enzyme *MseI*, while PPT-SON18 phytoplasma strain subgroup V (16SrI-V subgroup) can be differentiated from PeLL and/or ToLL phytoplasma strains by the restriction enzyme *HinfI*. The PPT-GTO29 and PPT-GTO30 phytoplasma strains sub-

group M (16SrII-M) can be differentiated from faba bean phyllody phytoplasma strain subgroup C (16SrII-C) by the restriction enzyme *BstUI*. The PPT-COAH or PPT-GTOP phytoplasma strains subgroup S (16SrIII-S) can be differentiated from milkweed yellows phytoplasma strain subgroup F (16SrIII-F) by the restriction enzymes *BstUI*, *HinfI*, and *RsaI*. The PPT-SINPV phytoplasma strain subgroup D (16SrIII-D) can be differentiated from Mexican periwinkle virescence subgroup A (16SrXIII-A) by the restriction enzymes *AluI*, *HpaII*, and *RsaI*.

Phylogenetic analysis. The 16S rDNA sequences of the above 17 phytoplasma strains associated with PPT were compared with 19 '*Ca. Phytoplasma*' species, other phytoplasmas representing distinct phytoplasma groups or subgroups, and *A. palmarum* yielding the consensus tree illustrated in Figure 2. This tree shows that 11 PPT phytoplasma strains (PPT-BC1, PPT-BC8, PPT-BC15, PPT-COAH10, PPT-COAH19, PPT-GTO5, PPT-JAL2, PPT-JAL6, PPT-SIN6, PPT-SIN15, and PPT-SON18) were clustered with '*Ca. P. asteris*', three PPT phytoplasma strains (PPT-GTO29, PPT-GTO30, and PPT-SINTV) were clustered with '*Ca. P. aurantifolia*', two PPT phytoplasma strains (PPT-COAH and PPT-GTOP) were clustered into the 16SrIII group (X-disease group), and one PPT phytoplasma strain (PPT-SINPV) was clustered into the 16SrXIII group (Mexican periwinkle virescence group).

DISCUSSION

PPT disease has caused severe economic losses in various Mexican states and in North America. Prior to the present study, at least eight distinct phytoplasma strains belonging to five different phytoplasma groups (16SrI, 16SrII, 16SrIII, 16SrVI, and 16SrXVIII) have been associated with PPT disease in different regions of North America and Mexico. The PPT disease was previously associated with two different phytoplasma strains belonging to the 16SrI and 16SrII groups in Mexico (29). We have found that other PPT phytoplasma strains belonging to the 16SrIII and 16SrXIII groups are also associated with PPT disease in Mexico. At least three PPT phytoplasma strains were found in potato growing areas (COAH and GTO) where high incidence of the disease has been reported (34).

The aster yellows group was the most widespread in potato growing areas in Mexico. These results are not surprising because the aster yellows phytoplasma (16SrI, '*Ca. P. asteris*') represents the most diverse and widespread phytoplasma group. Subgroups 16SrI-A, I-B, and I-C are distributed worldwide and are associated with more than 80 plant species. Subgroups 16SrI-F, I-L, I-M, and I-P appear to be restricted to the European continent.

The subgroup 16SrI-D is only found in Asia (4,27). Subgroup 16SrI-B represents the largest and most diverse strain cluster in the group (27). In Mexico, a PPT phytoplasma strain associated with PPT disease had been considered as a new member of aster yellows group, and two little leaf phytoplasma strains (PeLL and ToLL) associated with pepper and tomato diseases have also been considered as new subgroups of aster yellows group (16SrI-S and 16SrI-T, respectively) (29,39). Phytoplasma strains belonging to subgroup 16SrI-A have been associated with PPT

disease in Texas and Nebraska, USA (23) and 16SrI-B in Santa Cruz Province, Bolivia (18).

The peanut witches'-broom group (16SrII-C and 16SrII-M) was found in the GTO and SIN areas. This group is distributed worldwide, being reported in areas of Southeast Asia, Southern Europe, Africa, New Zealand, Australia, and America (4,45). Another PPT phytoplasma strain belonging to peanut witches'-broom group associated with PPT disease was found in Mexico, and it was one of the first phytoplasma strains reported on the American

continent. This phytoplasma strain was also found infecting cacti growing as weeds surrounding potato crops (29).

The X-disease group was found in the COAH and GTO areas. This group has been reported on various plant species in areas of North and South America, and constitutes one of the most ubiquitous phytoplasma groups worldwide. Subgroups 16SrIII-A and 16SrIII-F are found only in North America; 16SrIII-B in America, Asia, and Europe; 16SrIII-C, -D, -E, -G, -I, and -Q in the United States; 16SrIII-H is distributed worldwide; and 16SrIIIP

Fig. 3. Distinct virtual restriction fragment length polymorphism (RFLP) patterns derived from *in silico* digestions of 16S rRNA gene F2nR2 fragments from strains of new and previously delineated 16SrI, -II, -III, and -XIII subgroups. Recognition sites for the following 17 restriction enzymes were used in the simulated digestions: *AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *SspI*, and *TaqI*. MW = molecular weight standards from a ϕ X174 DNA-*HaeIII* digest.

and 16SrIII-R are found in Europe (4,8,17). Recently, a phytoplasma strain belonging to subgroup 16SrIII-M has been associated with PPT disease in Montana, USA (24). This phytoplasma strain is very similar or identical to phytoplasmas found in COAH and GTO.

The Mexican periwinkle virescence group was only found in the SIN area. This group appears to be restricted to the American continent. Subgroup 16SrXIII-A was detected for the first time in periwinkle samples from Mexico (10), subgroup 16SrXIII-B was detected in strawberry samples in Florida (15,16), and subgroup 16SrXIII-C was detected in chinaberry trees in Bolivia and Argentina (2,13). A phytoplasma strain belonging to Mexican periwinkle virescence group (16SrXIII-A) was previously reported in the Baja California peninsula of Mexico associated with a yellows type disease affecting tomato plants (14).

Arneodo et al. (2) determined the diversity and geographical distribution of phytoplasmas infecting china-tree in Argentina. They found that two different phytoplasmas belonging to groups 16SrIII and 16SrXIII were infecting china-tree. In addition, the RFLP analysis of these phytoplasma groups of different geographic origin did not reveal any intra-group variation. On the other hand, another study based on virtual RFLP analysis and calculated coefficients of RFLP pattern similarities revealed a remarkable genetic diversity among cactus witches'-broom (CaWB)

phytoplasma strains infecting *Opuntia* species in China (5). Our results from 16S rDNA sequence and virtual RFLP analyses of PPT phytoplasma strains from different geographic origins revealed inter- and intra-group variation. These results showed the occurrence of four different phytoplasma groups infecting potato crops in Mexico (groups 16SrI, 16SrII, 16SrIII, and 16SrXIII). Eight PPT phytoplasma strains (PPT-BC1, PPT-BC8, PPT-BC15, PPT-COAH10, PPT-GTO5, PPT-JAL2, PPT-SIN6, and PPT-SIN15) were considered to be new members of subgroup 16SrI-S; one strain (PPT-COAH19) was considered to be a new member of subgroup 16SrI-T; two strains (PPT-JAL6 and PPT-SON18) were designated as new subgroups U and V (16SrI-U and 16SrI-V, respectively) of aster yellows group; one strain (PPT-SINTV) was considered to be a new member of subgroup 16SrII-C; two strains (PPT-GTO29 and PPT-GTO30) were designated as new subgroup M (16SrII-M) of the peanut witches'-broom group; two strains (PPT-COAH and PPT-GTOP) were designated as new subgroup S (16SrIII-S) of X-disease group; one strain (PPT-SINPV) was designated as new subgroup D (16SrXIII-D) of the Mexican periwinkle virescence group.

Symptoms of plants infected by PPT resemble those of psyllid yellows (PY) and zebra chip (ZC); ZC is a new potato disease that was first observed in Texas, USA and Saltillo, Mexico in 1994 (35,41). Our results show that only 15.1% of foliage

samples from symptomatic and nonsymptomatic plants collected in representative fields of all potato growing areas in Mexico were PCR-positive for phytoplasmas. Hence, our results may suggest that one or more other pathogens may be involved in PPT disease in Mexico. In January 2008, a *Liberibacter* species designated as '*Candidatus Liberibacter solanacearum*' (33) was associated with diseases of tomato and pepper plants and other members of the family Solanaceae in New Zealand (30,31). In Mexico and the United States, the new *Liberibacter* species was associated, on September and June 2008, with potato ZC in Saltillo and Texas, respectively (1,36). In March 2009, '*Ca. L. solanacearum*' was also detected in tomato and bell pepper plants in Sinaloa, Mexico (37,38). In January 2009, '*Candidatus Phytoplasma australiense*' was found in mixed infection with '*Ca. L. solanacearum*' in New Zealand (32). Phytoplasma strains of different groups (16SrI, 16SrII, and 16SrXIII) have been detected in the psyllid *Bactericera cockerelli* collected from potato and pepper plants in Mexico, and these phytoplasmas were transmitted by this psyllid to potato, pepper, and tomato plants (6). '*Ca. L. solanacearum*' was also detected in *B. cockerelli* collected from tomato plants in Texas (11), and this psyllid was able to transmit ZC and PY to tomato and potato plants (11,42). Therefore, *B. cockerelli* seems to be involved in a new psyllid disease complex caused by different phytoplasma strains and possibly '*Ca. L. solanacearum*'. More studies are needed to establish the incidence and impact of phytoplasmas and '*Ca. L. solanacearum*' in Mexico.

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Fig. 4. Key restriction enzymes that distinguish the new phytoplasma subgroups (*AluI*, *BstUI*, *HinfI*, *HpaII*, *MseI*, and *RsaI*). Restriction patterns were obtained by virtual restriction fragment length polymorphism (RFLP) analysis of 16S rDNA sequences of strains from the new 16SrI, 16SrII, 16SrII, and 16SrXIII subgroups and sequence from a known member of each subgroup was used as a reference (GenBank accession numbers: PeLL-IS, DQ092321; ToLL-IT, DQ375238; PPT-JAL6-IU, FJ914650; PPT-SON18-IV, FJ914642; FBP-IIC, X83432; PPT-SINTV-IIC, FJ914651; PPT-GTO29-IIM, FJ914643; MW1-IIIF, AF510724; PPT-COAH-IIS, FJ914645; PPT-GTOP-IIS, FJ914646; MPV-XIIIA, AF248960; PPT-SINPV-XIID, FJ914647). MW, ϕ X174 DNA-*HaeIII* digest. New phytoplasma subgroups are in bold.

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