‘Candidatus Phytoplasma malaysianum’, a novel taxon associated with virescence and phyllody of Madagascar periwinkle (Catharanthus roseus)

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This study addressed the taxonomic position and group classification of a phytoplasma responsible for virescence and phyllody symptoms in naturally diseased Madagascar periwinkle plants in western Malaysia. Unique regions in the 16S rRNA gene from the Malaysian periwinkle virescence (MaPV) phytoplasma distinguished the phytoplasma from all previously described ‘Candidatus Phytoplasma’ species. Pairwise sequence similarity scores, calculated through alignment of full-length 16S rRNA gene sequences, revealed that the MaPV phytoplasma 16S rRNA gene shared 96.5% or less sequence similarity with that of previously described ‘Ca. Phytoplasma’ species, justifying the recognition of the MaPV phytoplasma as a reference strain of a novel taxon, ‘Candidatus Phytoplasma malaysianum’. The 16S rRNA gene F2nR2 fragment from the MaPV phytoplasma exhibited a distinct restriction fragment length polymorphism (RFLP) profile and the pattern similarity coefficient values were lower than 0.85 with representative phytoplasmas classified in any of the 31 previously delineated 16Sr groups; therefore, the MaPV phytoplasma was designated a member of a new 16Sr group, 16SrXXXII. Phytoplasmas affiliated with this novel taxon and the new group included diverse strains infecting periwinkle, coconut palm and oil palm in Malaysia. Three phytoplasmas were characterized as representatives of three distinct subgroups, 16SrXXXII-A, 16SrXXXII-B and 16SrXXXII-C, respectively.

Possessing small, AT-rich genomes and living a transkingdom parasitic life, phytoplasmas are pleomorphic, cell wall-less bacteria that infect many plant species and are responsible for numerous agriculturally and ecologically important plant diseases worldwide (Doi et al., 1967; Marcone et al., 1999; Lee et al. 2000). Phytoplasmas inhabit phloem sieve cells of infected plants and are transmitted by phloem-feeding insects, mainly leafhoppers and psyllids (Weintraub & Beanland, 2006; Hogenhout et al., 2008). Phylogenetic studies of genes encoding 16S rRNA and a large set of concatenated core housekeeping proteins have suggested that existing phytoplasmas share a common ancestor and are descended from low G + C Gram-positive bacteria in the Bacillus–Clostridium group (Woese et al., 1980; Weisburg et al., 1989; Gundersen et al., 1994; Zhao et al., 2005). Although the evolutionary events that led to the origin of the first phytoplasma remain to be unveiled, a hypothesis has been put forward (Wei et al., 2008a) based on the unique architecture discovered in phytoplasma genomes (Jomantiene & Davis, 2006; Jomantiene et al.,

The GenBank accession numbers for the nucleotide sequences from the ‘Candidatus Phytoplasma malaysianum’ reference strain and two other related strains described in the manuscript are EU371934, EU498727 and EU498728, respectively.

A supplementary figure is available with the online version of this paper.

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resolved. In this communication, we report that the MaPV phytoplasma represents a new taxon and a novel 16Sr group, 16SrXXXII.

**Malaysian periwinkle virescence (MaPV) phytoplasma represents a novel taxon**

As all attempts to isolate and cultivate phytoplasmas in cell-free media have been unsuccessful, measurable phenotypic characters remain largely inaccessible for phytoplasma taxonomy and classification. Consequently, genes with different degrees of nucleotide sequence conservation have been used to assess genetic relatedness and phylogenetic relationships of diverse phytoplasmas. Being highly conserved across the phytoplasma clade, yet containing ample information for differentiation of a wide array of phytoplasma strains, 16S rRNA gene sequences have served as the primary character for phytoplasma molecular taxonomy under the provisional status *Candidatus* for incompletely described prokaryotes (Murray & Stackebrandt, 1995; IRPCM, 2004). To characterize molecularly the MaPV phytoplasma, a partial *rrn* operon spanning a near-full-length 16S rRNA gene, 16S–23S rRNA intergenic spacer and partial 23S rRNA gene were amplified by PCR from genomic DNA extracted from four infected periwinkle plants, cloned and sequenced as described previously (Nejat et al., 2009). A representative nucleotide sequence the partial MaPV phytoplasma *rrn* operon was deposited in GenBank under accession no. EU371934. The 16S rRNA gene portion of the *rrn* operon contains a sequence, 2665'- CAAGACTATGATGTGTAGCTGGACT-3', matching the signature sequence (5'-CAAGAYBATKATGTKTAGC-YGGDC-3') that defines the taxa in the provisional genus *Candidatus Phytoplasma* (IRPCM, 2004).

To assess its taxonomic position, the 1523 bp near-full-length 16S rRNA gene sequence derived from the MaPV phytoplasma was queried using the iPhyClassifier software program (Zhao et al., 2009b). The results showed that the query sequence shared less than 97.5% similarity with that of any previously recognized *Ca.* Phytoplasma species reference strain. The *Ca.* Phytoplasma species most closely related to the MaPV phytoplasma was *Candidatus Phytoplasma trifolii* (GenBank accession no. AY390261), whose 16S rRNA gene sequence shared 96.5% similarity with the query sequence. According to the guidelines set forth by the International Research Program for Comparative Mycoplasmology, Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group (IRPCM), a strain can be described as a novel *Ca.* Phytoplasma species if its 16S rRNA gene sequence has <97.5% similarity to that of any previously described *Ca.* Phytoplasma species (IRPCM, 2004). Thus, the MaPV phytoplasma meets the criterion for recognition as a novel *Ca.* Phytoplasma species.

To further assess the genetic relatedness of the MaPV phytoplasma with all previously described *Ca.* Phytoplasma species, and to identify the signature sequences unique to the potentially novel taxon, a global sequence alignment was...
Table 1. Reference strains of *Candidatus Phytoplasma* species and their 16S rRNA gene RFLP group classification

<table>
<thead>
<tr>
<th>Strain name</th>
<th>GenBank accession no.</th>
<th>16S RFLP group classification*</th>
<th>Sequence identity with <em>Ca. Phytoplasma malaysianum</em></th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formally described <em>Ca. Phytoplasma</em> species:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ca. Phytoplasma malaysianum</em></td>
<td>EU371934</td>
<td>16SrXXXII-A</td>
<td>100</td>
<td>This study</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma asteris</em></td>
<td>M30790</td>
<td>16SrI-B</td>
<td>90.5</td>
<td>Lee et al. (2004a)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma aurantifolia</em></td>
<td>U15442</td>
<td>16SrII-B</td>
<td>90.4</td>
<td>Zreik et al. (1995)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma australasia</em></td>
<td>Y10097</td>
<td>16SrI-D</td>
<td>90.4</td>
<td>White et al. (1998)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma pruni</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rRNA</td>
<td>JQ044393</td>
<td>16SrI-A</td>
<td>93.7</td>
<td>Davis et al. (2013)</td>
</tr>
<tr>
<td>rRNA</td>
<td>JQ044392</td>
<td>16SrI-A</td>
<td>93.6</td>
<td>Davis et al. (2013)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma ulmi</em></td>
<td>AY197655</td>
<td>16SrV-A</td>
<td>95.7</td>
<td>Lee et al. (2004b)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma aurantifolia</em></td>
<td>AB092876</td>
<td>16SrV-B</td>
<td>95.8</td>
<td>Jung et al. (2003b)</td>
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<td><em>Ca. Phytoplasma australasia</em></td>
<td>Y10097</td>
<td>16SrV-D</td>
<td>95.7</td>
<td>Malembic-Maher et al. (2011)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma pisi</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ca. Phytoplasma pruni</em></td>
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<tr>
<td><em>Ca. Phytoplasma sudamericanum</em></td>
<td>GU292081</td>
<td>16SrVI-I</td>
<td>95.0</td>
<td>Davis et al. (2012)</td>
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<tr>
<td><em>Ca. Phytoplasma fragariae</em></td>
<td>AF092209</td>
<td>16SrVII-A</td>
<td>94.9</td>
<td>Griffiths et al. (1999)</td>
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<td><em>Ca. Phytoplasma phoenicium</em></td>
<td>AF515636</td>
<td>16SrIX-D</td>
<td>93.5</td>
<td>Verdin et al. (2003)</td>
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<tr>
<td><em>Ca. Phytoplasma pyri</em></td>
<td>AJ542543</td>
<td>16SrX-C</td>
<td>91.4</td>
<td>Seemu¨ller &amp; Schneider (2004)</td>
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<tr>
<td><em>Ca. Phytoplasma partii</em></td>
<td>X92869</td>
<td>16SrX-D</td>
<td>90.9</td>
<td>Marcone et al. (2004a)</td>
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<tr>
<td><em>Ca. Phytoplasma oryzae</em></td>
<td>AB052873</td>
<td>16SrXI-A</td>
<td>95.3</td>
<td>Jung et al. (2003a)</td>
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<tr>
<td><em>Ca. Phytoplasma australiense</em></td>
<td>L76865</td>
<td>16SrXII-B</td>
<td>90.5</td>
<td>Davis et al. (1997)</td>
</tr>
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<td><em>Ca. Phytoplasma japonicum</em></td>
<td>AB010425</td>
<td>16SrXII-D</td>
<td>89.8</td>
<td>Sawayanagi et al. (1999)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma fragariae</em></td>
<td>DQ086423</td>
<td>16SrXII-E</td>
<td>90.2</td>
<td>Valiunas et al. (2006)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma phoenicium</em></td>
<td>AJ550984</td>
<td>16SrXIV-A</td>
<td>94.6</td>
<td>Marcone et al. (2004b)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma brasiliense</em></td>
<td>AF147708</td>
<td>16SrXV-A</td>
<td>90.3</td>
<td>Montano et al. (2001)</td>
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<td><em>Ca. Phytoplasma graminis</em></td>
<td>AY725228</td>
<td>16SrXVI-A</td>
<td>86.0</td>
<td>Arocha et al. (2005)</td>
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<tr>
<td><em>Ca. Phytoplasma caricae</em></td>
<td>AY725234</td>
<td>16SrXVII-A</td>
<td>85.7</td>
<td>Arocha et al. (2005)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma americana</em></td>
<td>DQ174122</td>
<td>16SrXVIII-A</td>
<td>89.6</td>
<td>Lee et al. (2006)</td>
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<td><em>Ca. Phytoplasma castaneae</em></td>
<td>AB054986</td>
<td>16SrXIX-A†</td>
<td>92.8</td>
<td>Jung et al. (2002)</td>
</tr>
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<td><em>Ca. Phytoplasma rhamni</em></td>
<td>X76431</td>
<td>16SrXX-A</td>
<td>91.7</td>
<td>Marcone et al. (2004a)</td>
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<td><em>Ca. Phytoplasma pini</em></td>
<td>AJ632155</td>
<td>16SrXXI-A</td>
<td>93.8</td>
<td>Schneider et al. (2005)</td>
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<tr>
<td><em>Ca. Phytoplasma omanense</em></td>
<td>EF666051</td>
<td>16SrXXIX-A‡</td>
<td>93.0</td>
<td>Al-Sady et al. (2008)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma tamaricus</em></td>
<td>FJ432664</td>
<td>16SrXXX-A</td>
<td>91.7</td>
<td>Zhao et al. (2009a)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma costaricum</em></td>
<td>HQ225630</td>
<td>16SrXXXI-A</td>
<td>88.4</td>
<td>Lee et al. (2011)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma allocasuarinae</em></td>
<td>AY135523</td>
<td>ND</td>
<td>91.4</td>
<td>Marcone et al. (2004a)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma lycopersici</em></td>
<td>EF199549</td>
<td>ND</td>
<td>71.9</td>
<td>Arocha et al. (2007)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma convolvuli</em></td>
<td>JN833705</td>
<td>ND</td>
<td>90.1</td>
<td>Martini et al. (2013)</td>
</tr>
<tr>
<td><strong>Incidentally cited or suggested novel species:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ca. Phytoplasma palmae</em></td>
<td>U18747</td>
<td>16SrXIV-A</td>
<td>95.0</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma coctostanzianae</em></td>
<td>X80117</td>
<td>ND</td>
<td>94.4</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma vitis</em></td>
<td>AF176319</td>
<td>16SrV-C</td>
<td>95.7</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma solani</em></td>
<td>AF248959</td>
<td>16SrXII-A</td>
<td>89.1</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td><em>Ca. Phytoplasma coccinegriae</em></td>
<td>Y14175</td>
<td>16SrXXII-A</td>
<td>93.6</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td>Mexican periwinkle virescence phytoplasma</td>
<td>AF248960</td>
<td>16SrXIII-A</td>
<td>90.6</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td>Chinaberry yellows phytoplasma</td>
<td>AF495882</td>
<td>ND</td>
<td>89.4</td>
<td>IRPCM (2004)</td>
</tr>
<tr>
<td>Buckland valley grapevine yellows phytoplasma</td>
<td>AY083605</td>
<td>16SrXXIII-A</td>
<td>90.2</td>
<td>Wei et al. (2007)</td>
</tr>
</tbody>
</table>
constructed using the CLUSTAL W option of the LASERGENE MEALIGN program (DNASTAR, Madison, WI). The alignment included 16S rRNA gene sequences from MaPV phytoplasma, from reference strains of all 33 formally described ‘Ca. Phytoplasma’ species, and from reference strains of 14 suggested but yet to be officially described ‘Ca. Phytoplasma’ species (Table 1). Comparisons of the aligned sequences revealed at least four regions unique to the MaPV phytoplasma 16S rRNA gene. These distinguishing regions include 1645-9-GAAATAGAAGGATAACCTTTTATTTTT-39 4075-4-GAAGAATTTAGGAT-39, 6085-6-CGGCCTTGCCTGTT-39, and 6295-5-GTCTAGCTAGTAGTGAG-39, which differ in sequence from six to 16, from two to five, from two to six, and from two to seven base positions, respectively, from the corresponding regions in the 16S rRNA genes of all previously described as well as suggested but yet to be formally described ‘Ca. Phytoplasma’ species.

Results from the analyses of the MaPV phytoplasma 16S rRNA gene, together with geographical occurrence and identification of a natural host, justify recognition of the MaPV phytoplasma as a representative of a novel taxon. Therefore, we propose that the MaPV phytoplasma be designated the reference strain of a distinct ‘Ca. Phytoplasma’ species, ‘Candidatus Phytoplasma malaysianum’.

Description of ‘Candidatus Phytoplasma malaysianum’

‘Candidatus Phytoplasma malaysianum’ (ma.lay.si’a.num. N.L. neut. adj. malaysianum pertaining to Malaysia, referring to the country the phytoplasma was discovered).

The reference strain MaPV9R is associated with Madagascar periwinkle plants exhibiting floral virecence and phyllody symptoms.

### Table 1. cont.

<table>
<thead>
<tr>
<th>Strain name</th>
<th>GenBank accession no.</th>
<th>16Sr RFLP group classification*</th>
<th>Sequence identity with ‘Ca. Phytoplasma malaysianum’</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum bunchy shoot phytoplasma</td>
<td>AF509322</td>
<td>16SrXXIV-A</td>
<td>94.4</td>
<td>Wei et al. (2007)</td>
</tr>
<tr>
<td>Weeping tea witches’-broom phytoplasma</td>
<td>AF521672</td>
<td>16SrXXV-A</td>
<td>90.5</td>
<td>Wei et al. (2007)</td>
</tr>
<tr>
<td>Sugar cane phytoplasma D3T1</td>
<td>AJ539179</td>
<td>16SrXXXVI-A</td>
<td>93.9</td>
<td>Wei et al. (2007)</td>
</tr>
<tr>
<td>Sugar cane phytoplasma D3T2</td>
<td>AJ539180</td>
<td>16SrXXXVII-A</td>
<td>93.8</td>
<td>Wei et al. (2007)</td>
</tr>
<tr>
<td>Derbid phytoplasma</td>
<td>AY744945</td>
<td>16SrXXVIII-A</td>
<td>87.3</td>
<td>Wei et al. (2007)</td>
</tr>
</tbody>
</table>

ND, Not determined.

*Unless otherwise noted, the 16Sr RFLP group/subgroup classification status of the phytoplasma strains are based on Lee et al. (1998, 2000, 2005, 2011), Wei et al. (2007), Zhao et al. (2009a) and Davis et al. (2012).

†In the report by Jung et al. (2002), ‘Ca. Phytoplasma castaneae’ was assigned to group VI according to DNA sequence similarity rather than results from RFLP analysis. In accordance with the RFLP-based classification scheme, this phytoplasma was reassigned to group 16SrXIX by Wei et al. (2007).

‡The original reference (Al-Saady et al., 2008) reported ‘Ca. Phytoplasma omanense’ as the reference member of a new group designated group 16SrXIX. However, the group number 16SrXIX had been previously published (Wei et al., 2007) to accommodate a different phytoplasma, ‘Ca. Phytoplasma castaneae’. Therefore, ‘Ca. Phytoplasma omanense’ was reassigned to a new group, 16SrXXIX, subgroup 16SrXXIX-A (Zhao et al., 2009a).

‘Ca. Phytoplasma malaysianum’-related strains in other hosts

A BLAST search of 16S rRNA gene sequences deposited in GenBank, using the MaPV phytoplasma 16S rRNA gene sequence as a query, revealed that at least two other phytoplasmas are genetically closely related to the reference strain of ‘Candidatus Phytoplasma malaysianum’. These are Malayan Yellow Dwarf (MYD) phytoplasma and Malayan Oil Palm (MOP) phytoplasma; both phytoplasmas were associated with recently emerged diseases of palms in Malaysia. The MYD phytoplasma was discovered in diseased coconut palm (Cocos nucifera) trees showing yellowing symptoms in the Banting area of Selangor State, and the MOP phytoplasma was identified in oil palm (Elaeis guineensis) plants grown in the same area but exhibiting yellowing and necrosis symptoms. The 16S rRNA gene sequences from the two phytoplasmas were determined from analysis of multiple samples (six for the MYD phytoplasma and nine for the MOP phytoplasma) and a representative sequence for each phytoplasma was deposited in the GenBank (Nejat et al., 2009). Nucleotide sequence alignments revealed that the 16S rRNA gene sequences of MYD (GenBank accession no. EU498727) and MOP (GenBank accession no. EU498728) phytoplasmas shared 99.1% and 99.2% sequence identity, respectively, with that of the MaPV

[(Mollicutes) NC; NA; O, wall-less; NAS (GenBank accession number EU371934), oligonucleotide sequences of unique regions of the 16S rRNA gene are: 1645-9-GAAATAGAAGGATAACCTTTTATTTTT-39 4075-4-GAAGAATTTAGGAT-39, 6085-6-CGGCCTTGCCTGTT-39, and 6295-5-GTCTAGCTAGTAGTGAG-39, which differ in sequence from six to 16, from two to five, from two to six, and from two to seven base positions, respectively, from the corresponding regions in the 16S rRNA genes of all previously described as well as suggested but yet to be formally described ‘Ca. Phytoplasma’ species.

Results from the analyses of the MaPV phytoplasma 16S rRNA gene, together with geographical occurrence and identification of a natural host, justify recognition of the MaPV phytoplasma as a representative of a novel taxon. Therefore, we propose that the MaPV phytoplasma be designated the reference strain of a distinct ‘Ca. Phytoplasma’ species, ‘Candidatus Phytoplasma malaysianum’.

Description of ‘Candidatus Phytoplasma malaysianum’

‘Candidatus Phytoplasma malaysianum’ (ma.lay.si’a.num. N.L. neut. adj. malaysianum pertaining to Malaysia, referring to the country the phytoplasma was discovered).

The reference strain MaPV9R is associated with Madagascar periwinkle plants exhibiting floral virecence and phyllody symptoms.
Fig. 1. Phylogenetic tree inferred from analysis of 16S rRNA gene sequences. Maximum-parsimony analysis was conducted using the close-neighbour-interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). The reliability of the analysis was subjected to a bootstrap test with 1000 replicates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the nodes. Bar, 10 nt substitutions.

Furthermore, the 16S rRNA genes of MYD and MOP phytoplasmas possessed all the signature sequences that are unique to ‘Ca. Phytoplasma malaysianum’; MYD and MOP phytoplasmas are hereby termed ‘Ca. Phytoplasma malaysianum’-related strains.
Malaysian periwinkle virescence phytoplasma represents a new 16Sr RFLP group

In addition to ‘Ca. Phytoplasma’ species assignments, individual phytoplasmas are often classified into groups and subgroups according to a classification scheme established on the basis of restriction fragment length polymorphism (RFLP) patterns derived from digestions of a defined 16S rRNA gene segment (the F2nR2 fragment) by a set of 17 restriction enzymes (Lee et al., 1998b, 2000). A collection of phytoplasma 16S rRNA gene RFLP profiles established under this classification scheme have served as standard keys and ‘visible’ molecular markers for identification and classification of diverse phytoplasmas. Following recent expansions through computer-simulated RFLP analysis (Wei et al., 2007, 2008b), this 16S rRNA gene RFLP-based classification scheme has accommodated 31 groups and more than 100 subgroups (Zhao et al., 2009a; Lee et al., 2011; Davis et al., 2012). According to the guidelines of the classification scheme (Lee, et al., 1998b; Wei et al., 2008b), a new group can be proposed if the collective RFLP pattern derived from the 16S rRNA gene F2nR2 fragment of a given phytoplasma strain has lower than 0.85 similarity coefficient values with the RFLP patterns of all previously recognized groups.

An in silico analysis of the MaPV phytoplasma 16S rRNA gene F2nR2 fragment, using the RFLP pattern recognition and pattern similarity coefficient calculation program (Wei et al., 2008b; Zhao et al., 2009b), revealed a new collective RFLP pattern (Fig. 2a). The similarity coefficients of the MaPV collective pattern with pattern types of phytoplasmas in each of the 31 previously delineated groups were lower than 0.85, the threshold value for recognition of new 16Sr groups (Lee, et al., 1998b; Wei et al., 2008b). Consequently, we propose a new 16Sr group, designated

MaPV EU371934 16SrXXXII-A
MYD EU498727 16SrXXXII-B
MOP EU498728 16SrXXXII-C

Fig. 2. RFLP profiles derived from in silico digestion of 16S rRNA gene F2nR2 fragments from ‘Ca. Phytoplasma malaysianum’ reference and related strains. Sequences from strains MaPV (a), MYD (b) and MOP (c) were subjected to in silico digestions with 17 restriction enzymes: AluI, BamHI, BflI, BstUI (Thal), Dral, EcoRI, HaeIII, Hhal, HinfI, Hpal, Hpall, KpnI, Sau3AI (MboI), MseI, RsaI, SspI and TaqI. The restriction fragments were resolved on a 3% virtual agarose gel. MW, φX174DNA-HaeIII digests.

Fig. 3. Restriction enzymes that distinguish three 16SrXXXII subgroup lineages. BstUI or Hhal distinguishes subgroup16SrXXXII-A (MaPV) pattern from that of subgroup16SrXXXII-B (MYD) and subgroup16SrXXXII-C (MOP) (a and b); Sau3AI distinguishes subgroup16SrXXXII-B pattern from that of subgroup16SrXXXII-A and subgroup16SrXXXII-C (c); BflI distinguishes subgroup16SrXXXII-C pattern from that of 16SrXXXII-A and 16SrXXXII-B (d). The restriction fragments were resolved on a 3% virtual agarose gel. MW, φX174DNA-HaeIII digests.
16SrXXXII, the Malaysian periwinkle virescence phytoplasma group, with MaPV phytoplasma being the first recognized member. The RFLP profile resulting from 16S rRNA gene F2nR2 fragment digestion by restriction enzyme AflIII digestion alone was sufficient to distinguish MaPV phytoplasma from strains in all other 16Sr groups (Fig. S1, available in IJSEM Online).

The two ‘Ca. Phytoplasma malaysianum’-related strains, MYD and MOP, also exhibited new and mutually distinct 16S rRNA gene F2nR2 RFLP patterns (Fig. 2b and c). The RFLP pattern similarity coefficients were 0.87 between MYD and MaPV, and 0.90 between MOP and MaPV, indicating that both MYD and MOP phytoplasmas are members of group 16SrXXXII. Since the RFLP pattern similarity coefficient values are significantly lower than the threshold value (0.97) for recognition of new subgroups (Lee et al., 1998b; Wei et al., 2008b), we assign MaPV, MYD and MOP phytoplasmas to three different subgroups, hereby designated 16SrXXXII-A (with MaPV phytoplasma as the representative strain), 16SrXXXII-B (with MYD phytoplasma as the representative strain) and 16SrXXXII-C (with MOP phytoplasma as the representative strain). RFLP patterns from F2nR2 fragment digestions with restriction enzymes BstUI or HhaI clearly distinguished MaPV (subgroup 16SrXXXII-A) phytoplasma from MYD (16SrXXXII-B) and MOP (16SrXXXII-C) phytoplasmas (Fig. 3a and b). Similarly, restriction enzyme Sau3A can be used to distinguish 16SrXXXII-B phytoplasma from 16SrXXXII-A and 16SrXXXII-C phytoplasmas (Fig. 3c), and restriction enzyme BfaI can be used to distinguish subgroup 16SrXXXII-C phytoplasma from 16SrXXXII-A and 16SrXXXII-B phytoplasmas (Fig. 3d). Presence of three distinct subgroup lineages highlights the breadth of the genetic diversity within the Malaysian periwinkle virescence phytoplasma group and draws attention to the possibility of additional related but distinct 16SrXXXII strains in Asia.

References


