Methods to distinguish phytoplasmas and haplotypes of ‘Candidatus Liberibacter solanacearum’

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Phytoplasmas

- Plant pathogenic bacteria that lack cell wall, pleomorphic, belonging to a monophyletic group in the class *Mollicutes*

- Inhabit the sieve tube elements and the hemolymph of the insect vectors

- Small genome size (350–1350 kb) and low C-G content, diameter ranging from 0.2 to 1 μm

- Transmitted by insect, grafting and dodder

- Can be associated with devastating diseases in many crops of agricultural interest because food reserves, ornamental and forest plant species
Phytoplasma classification

41 ‘Ca. Phytoplasma’ species, 33 ribosomal groups and 130 ribosomal subgroups have been described.

To define a new ‘Candidatus’, the sequence of the 16S rRNA gene has to be at least 1,200 nucleotides and the homology less than 97.5% with the others ‘Ca. Phytoplasma’ species described.

For the high conservation of the 16S rRNA gene, other characteristics such as the geographic localization, host plant species, insect vectors, specific antibodies... must be included for ‘Candidatus Phytoplasma’ speciation.

RFLP (Restriction fragment length polymorphism) analysis is the method used for the phytoplasma ribosomal group classification. The amplified 16S rDNA sequence is analyzed with different restriction enzymes to differentiate among phytoplasmas ribosomal groups on their restriction profiles.
University of Las Palmas de Gran Canaria, Veterinary Faculty - Mycoplasmology Laboratory - Canary Islands - Spain
- *Ca. Phytoplasma* and *Ca. liberibacter* detection: Molecular testing of symptomatic carrots

Symptomatic *Daucus carota* from the North of Gran Canaria Island
Samples from carrot (variety Cordoba) collected in 2015 and 2016 from, respectively, 26 and 8 symptomatic plants were randomly selected in two fields located in the North of Gran Canaria Island.

- DNA extraction CTAB
- PCR/RFLP analysis

- primers specific for ‘Ca. L. solanacearum’ 16S rDNA and rp protein
- generic and group specific primers for phytoplasmas 16S rDNA
### Results of ‘Ca. L. solanacearum’ and ‘Ca. P. asteris’ detection in carrot with different primer combinations

<table>
<thead>
<tr>
<th>Primers and primer combinations</th>
<th>2015</th>
<th>2016</th>
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<tbody>
<tr>
<td></td>
<td>‘Ca. P. asteris’</td>
<td>‘Ca. L. solanacearum’</td>
</tr>
<tr>
<td><strong>16S rRNA gene primer combinations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ClipoF/O12c</td>
<td>-</td>
<td>61.5%</td>
</tr>
<tr>
<td>OA2/O12c</td>
<td>-</td>
<td>84.6%</td>
</tr>
<tr>
<td>System I (P1/P7 + R16F2n/R2 + M1/M2)</td>
<td>-</td>
<td>61.5%</td>
</tr>
<tr>
<td>System II (P1/P7 + R16(I)F1/R1 + M1/M2)</td>
<td>15.4%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Ribosomal protein gene rplJ/rplL primer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL514 F/R</td>
<td>-</td>
<td>84.6%</td>
</tr>
</tbody>
</table>
Primers for ‘Candidatus Phytoplasma’ also amplify ‘Candidatus Liberibacter solanacearum’
The detected **SNPs** in the **16S rRNA, 16S/23S ISR and rplJ/rplL ribosomal protein sequences** of ‘*Ca. L. solanacearum*’ agreed with those present in the **haplotype D**
Virtual RFLP analyses on OA2/O12c amplicon sequences differentiates the reported haplotypes using 6 restriction enzymes. ‘Ca. L. solanacearum’ strains from Canary island were confirmed as haplotype D.
Discussion

The primer pair M1/M2, known as universal for phytoplasmas, detected ‘Ca. L. solanacearum’ when used in nested PCR with generic primers in first nested PCR, and aster yellows phytoplasmas in nested PCR using group specific primers in first nested PCR.

Virtual RFLP analysis were applicable for ‘Ca. L. solanacearum’ as an alternative tool for haplotype discrimination.

It could be simpler than SNPs detection on two genes since only one gene and no sequencing can provide the same result.
Thank you!