

Methods to distinguish phytoplasmas and haplotypes of '*Candidatus* *Liberibacter solanacearum*'

Eleonora Satta

Samanta Paltrinieri - Assunta Bertaccini

Department of Agricultural Sciences

Phytobacteriology laboratory

Alma Mater Studiorum – University of Bologna - Italy

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

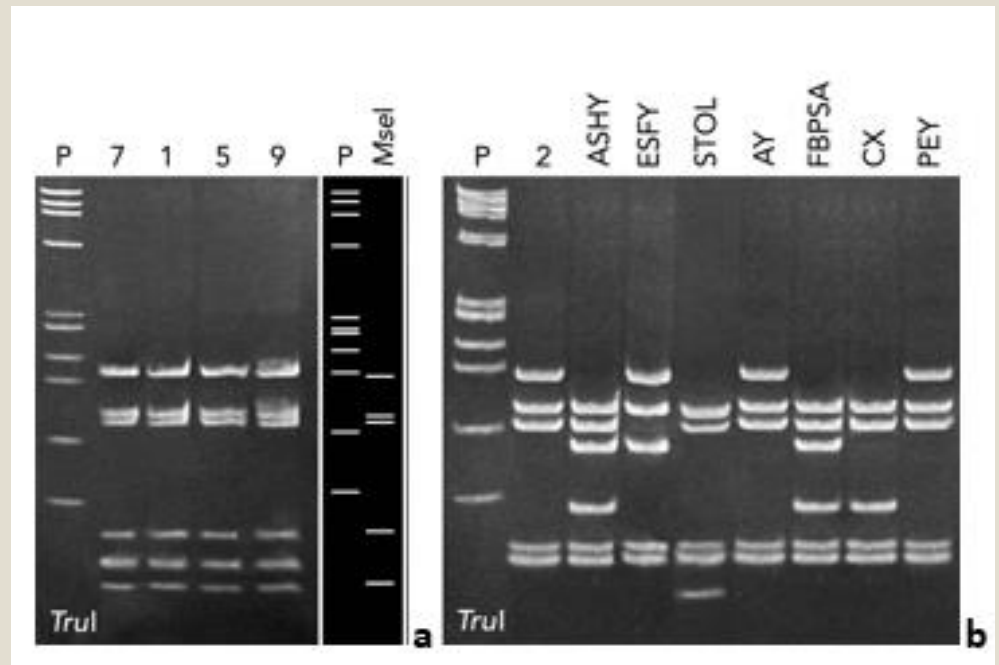
Satta E., A.S. Ramírez, S. Paltrinieri, N. Contaldo, P. Benito, J.B. Poveda, **A. Bertaccini**. 2016. **Simultaneous detection of mixed '*Candidatus Phytoplasma asteris*' and '*Ca. Liberibacter solanacearum*' infection in carrot.** *Phytopathologia mediterranea*, 55 (3): 401-409.

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

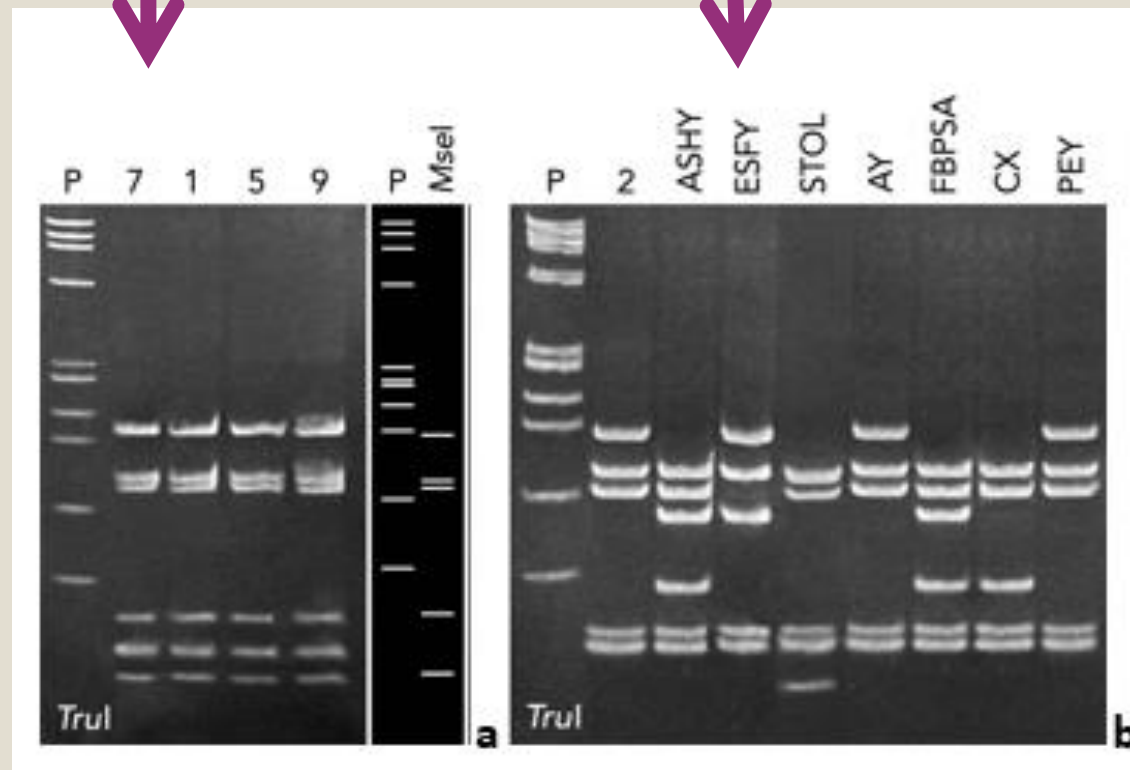
- primers specific for '*Ca. L. solanacearum*' 16S rDNA and rp protein
- generic and group specific primers for phytoplasmas 16S rDNA



Results of '*Ca. L. solanacearum*' and '*Ca. P. asteris*' detection in carrot with different primer combinations

Primers and primer combinations	2015		2016
	' <i>Ca. P. asteris</i> '	' <i>Ca. L. solanacearum</i> '	' <i>Ca. L. solanacearum</i> '
<i>16S rRNA</i> gene primer combinations			
ClipoF/O12c	-	61.5%	50.0%
OA2/O12c	-	84.6%	100%
System I (P1/P7 + R16F2n/R2 + M1/M2)	-	61.5%	75.0%
System II (P1/P7 + R16(I)F1/R1 + M1/M2)	15.4%	-	-
Ribosomal protein gene rplJ/rplL primer			
CL514 F/R	-	84.6%	75.0%

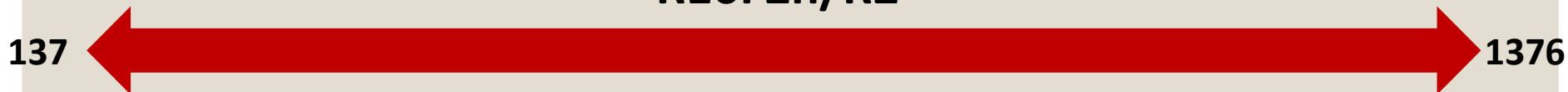
Primers
for '*Candidatus Phytoplasma*'
also amplify
'*Candidatus Liberibacter solanacearum*'



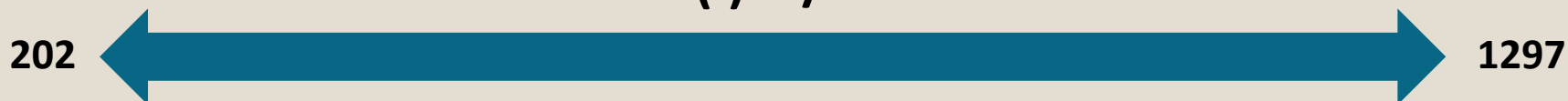
M1/M2

Primers combinations

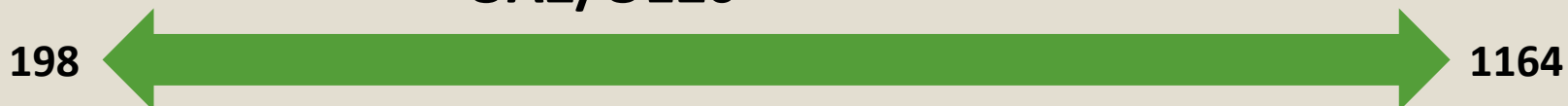
R16F2n/R2



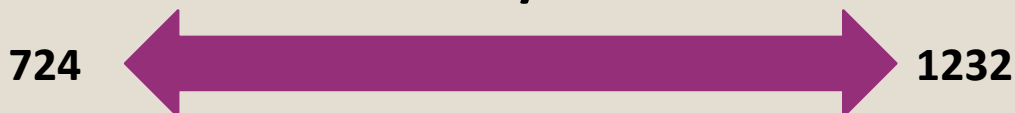
R16(I)F1/R1



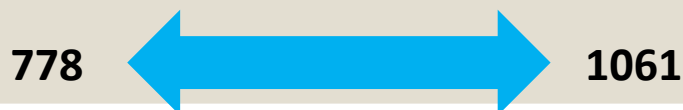
OA2/O12c



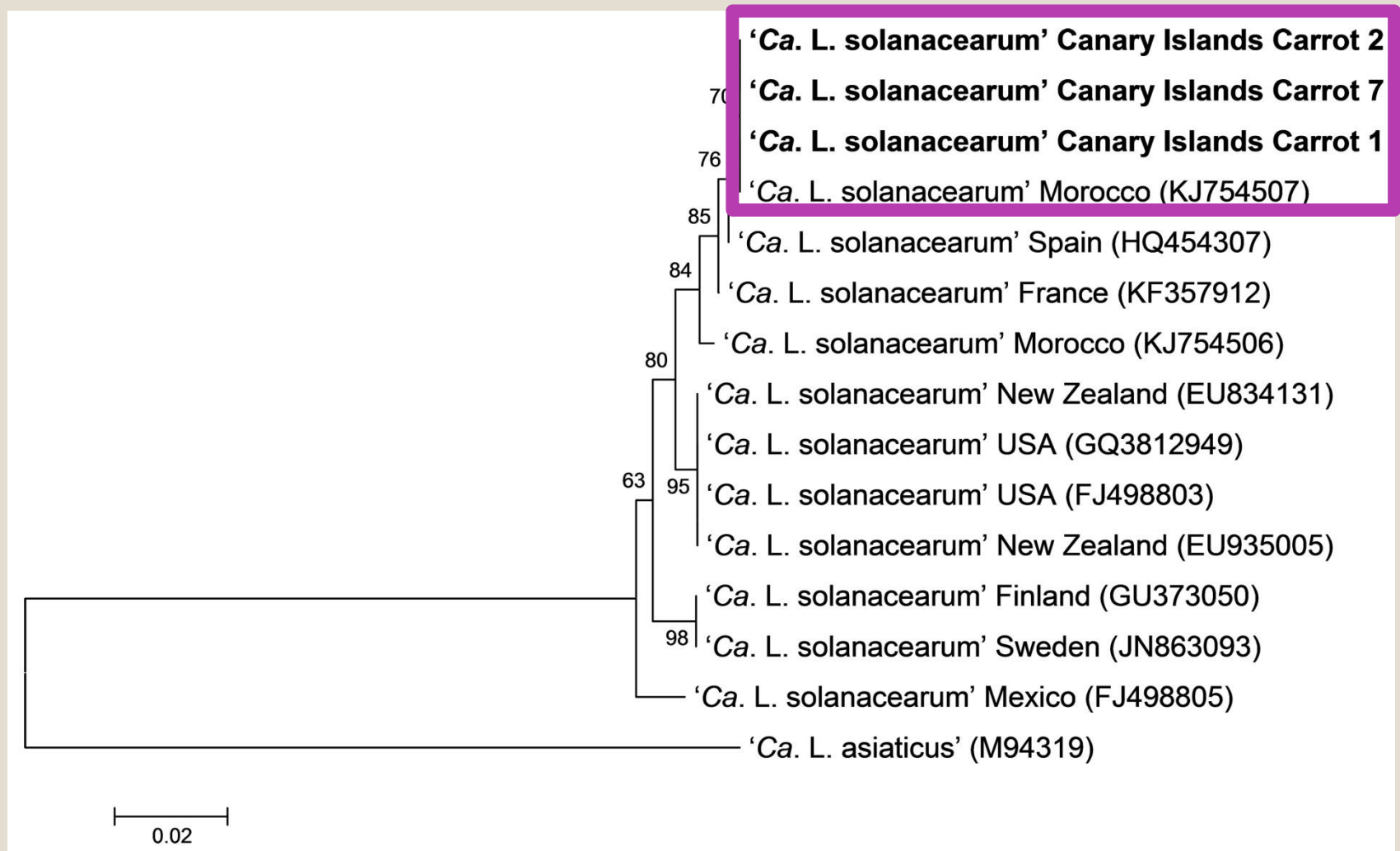
M1/M2

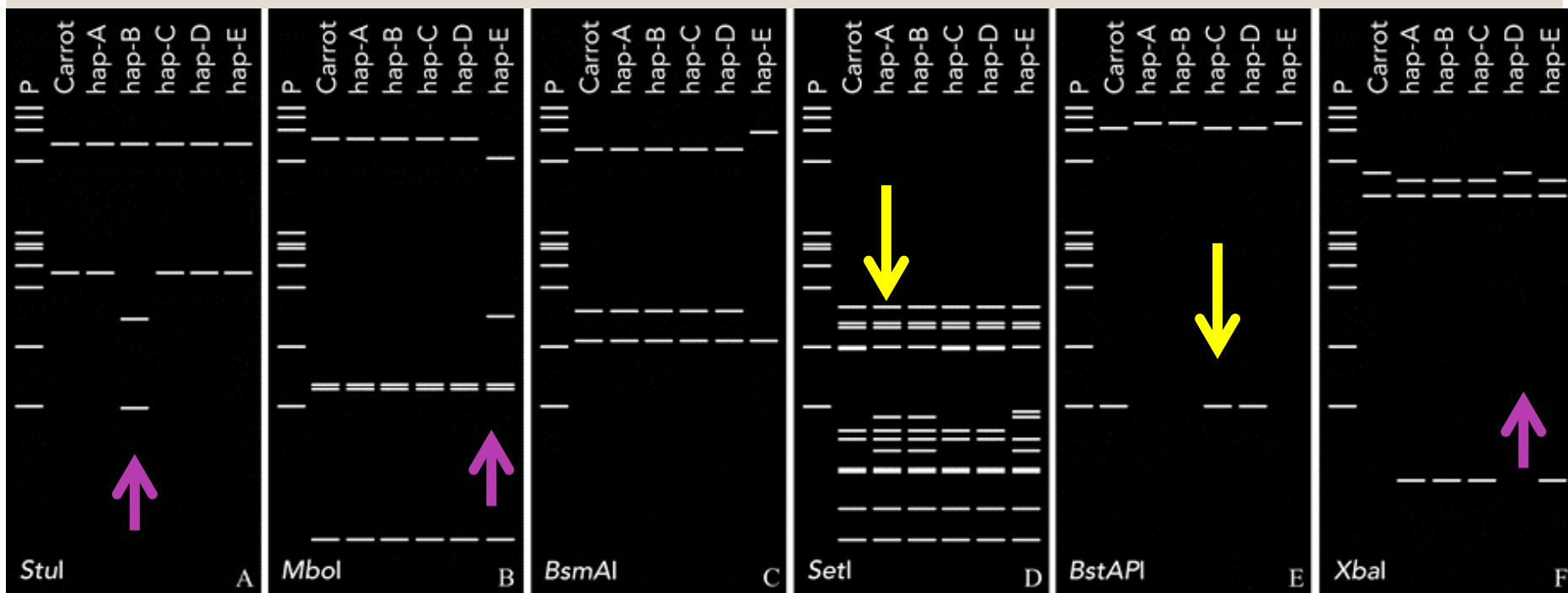


GPO3F/MGSO



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!