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Sequencing of the ribosomal protein gene *rpl16* from '*Candidatus* Phytoplasma ulmi' infecting a historic *Ulmus minor*

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Abstract

The aim of this work is to obtain new sequences of '*Candidatus* Phytoplasma ulmi' useful for the characterization of phytoplasmas infecting a historic plant of *Ulmus minor*. At our knowledge, we described for the first time the *rpl16* gene sequence of '*Ca.* P. ulmi' strain ULW (elm witches' broom). This gene, positioned downstream the gene *rps3*, codifies the ribosomal protein L16.

Key words: Elm yellows, phytoplasma, ribosomal protein genes, *rps3*, *rpl16*.

Introduction

Elm yellows, formerly called as elm phloem necrosis, is a dangerous disease associated with phytoplasma and found in North America and Europe. The phytoplasmas associated with EY are classified as '*Candidatus* phytoplasma ulmi" (=16SrV-A) (Lee *et al*., 2004).

The pathogen is located in phloem sieve cell and cause tissue degeneration and reduced translocation from photoassimalated source organs to photoassimilated sink organs (Bertelli *et al*., 2002). The tree responds outwardly by wilting, yellowing (Sinclair, 2000) and producing witches' broom (for strain ULW). In North America EY phytoplasmas are transmitted by elm leafhopper *Scaphoideus luteolus* van Duzee and in Europe by *Macropsis mendax* (Fieber) (Carraro *et. al*., 2004). Natural infections were reported in *Ulmus minor* and *Ulmus pumila* (Carraro *et. al*., 2004).

To date, on the basis of 16S rRNA, *rpl22-rps3* and *secY* the phytoplasmas infected historic *Ulmus minor* (figure 1) are characterized (Quaglino *et. al*, 2005). The aims of this work were: (i) obtain a sequence of ribosomal protein of '*Ca*. P. ulmi', strain ULW (subgroup 16SrV-A), from symptomatic leaves (ii) identify and compare the ORFs with the GenBank sequences. In future we want compare this sequence from phytoplasmas infected asymptomatic and symptomatic leaves. At our knowledge, we described for the first time the *rpl16* sequences of '*Ca.* P. ulmi'.

Materials and methods

Amplification of ribosomal protein gene *rpl16*

DNA was extracted from 2 g of leaf veins of periwinkle (*Catharanthus roseus* L.) experimentally infected by '*Ca.* P. ulmi', strain ULW (elm witches' broom phytoplasma), by using a procedure previously described (Lee *et al.*, 1991). To obtain the sequences downstream the *rps3* gene, DNA was amplified by using the forward primer rpF1(V), positioned in the 3' end of the *rps19* gene (Lee *et al.*, 1998), differently combined with two degenerate reverse primers (L29d and L29n) designed on the basis of the alignment of the GenBank *rpl29* gene sequences of phytoplasmas, mycoplasmas and other bacteria closed to the *Mollicutes*. Direct PCR products were 1: 25 diluted in sterile water and used in nested PCR assays performed by means of the forward primer rpR1 (reverse complement, used as forward) and the reverse primer L29n. The following conditions were used for PCRs: 1 minute at 94 °C, 30 seconds at 53 °C and 7 min at 68 °C (10 min for the last cycle). DNA extracted from healthy periwinkle and sterile water devoid of DNA were used in all the PCRs as negative controls.

Sequence analysis

The obtained PCR products rp(V)F1A/L29n, covering the region between the genes *rps3* and *rps17*, was sequenced by a commercial service (Primm, Milan, Italy). ORFs searching and codified protein sequence deducing were performed by means of ExPasy Proteomic Server (http://us.expasy.org/tools/dna.html). Identity percentage of nucleotide and aminoacidic sequences were got by using the software BlastN (http://www.ncbi.nim.nih. .gov/BLAST/).

Figure 1. The historic *Ulmus minor* of Arcagna, Italy.

Table 1. BlastN analysis results and protein sequence identity.

ULW rp, size	Closest Organism	GenBank accession number	GenBank protein id	% Identity	No. of aa
L16, 140 aa	'*Ca.* P. asteris', strain OY-M	AP006628	BAD04292	77.1	140
	'*Ca.* P. asteris', strain AY-WB	NC_007716	YP_456711	76.4	140
	Mycoplasma capricolum	CP000123	ABC01382	63.5	137

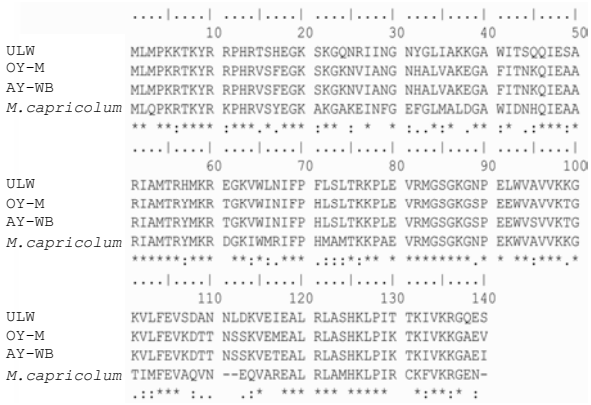

Figure 2. Alignment of L16 ribosomal protein sequences.

Results

We obtained from the ULW infected periwinkle a visible PCR product of the expected size (500 bp) after nested PCR reaction and only when undiluted DNA from direct PCR was used like template. BlastN analysis revealed the presence of one ORFs with a size of 426nt coding the ribosomal protein L16.

The protein sequence identity values are reported in table 1. ULW L16 protein showed the higher identity with L16 protein of AY-WB (77.1%) strain (subgroup 16SrI-A) and OY-M (76.4%) strain (subgroup 16SrI-B). A 32 bp long not translated region between *rps3* and *rpl16* genes was identified. Alignments of L16 sequences are showed in figure 2. Protein L16 from 16SrI (AY-WB and OY-M), 16SrV (ULW) phytoplasmas and *Mycoplasma capricolum* are characterized by a different number of amino acidic residues and some sequence blocks seem to be more conserved.

Discussion

In this work we obtained the nucleotide sequence of *rpl16* gene from '*Ca.* P. ulmi', strain ULW. Now we want to sequence *rpl16* gene from phytoplasmas infected the asymptomatic leafs and study the variability genetic of '*Ca*. P. ulmi' strain ULW to clarify the genetic variability of phytoplasmas detected in our precedent study in historic *Ulmus minor* plant. Analysis on deduced amino acidic sequences showed high identity level on L16 proteins of different phytoplasma groups.

Study of L22 and S3 ribosomal protein genes was largely used in molecular characterization of 16SrV phytoplasmas (Lee *et al.*, 2004). The information obtained in this work could be also used for the design of universal and 16SrV group specific primer pairs on the *rpl16* gene. Further experiments will employ *rpl16* gene sequence in this work in order to isolate homologous ribosomal gene of others phytoplasmas and evaluate their genetic variability.

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