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Phylogenetic position of 'bois noir' phytoplasma based on analyses of *rpsJ-rplC-rplD-rplW-rplB* gene sequences

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Abstract

In the present work, a 2,738 bp DNA segment of the operon S10, including the genes *rpsJ*, *rplC*, *rplD*, *rplW* and *rplB*, from an Italian 'bois noir' phytoplasma strain detected in grapevine was amplified. Nucleotide and amino acid sequence analyses allowed to clarify the phylogenetic position of this 'bois noir' phytoplasma strain within 16S rDNA RFLP group 16SrXII and to identify molecular markers useful for specific phytoplasma identification. The results stimulate further work aimed at obtaining the nucleotide sequence of the entire superoperon S10-*spc-alpha* for a more detailed characterization of 'bois noir' phytoplasma.

Key words: S10 operon, ribosomal proteins, single nucleotide polymorphism, RFLP.

Introduction

Phytoplasma strains associated with 'bois noir' (BN) disease of grapevine have been tentatively classified in the species '*Candidatus* Phytoplasma solani'. (IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group, 2004; according to rule 28b of the Bacteriological Code, '*Candidatus* Phytoplasma solani' is an incidental citation and does not constitute prior citation). Most '*Ca.* P. solani'-related strains belong to 16S rDNA RFLP subgroup 16SrXII-A (stolbur subgroup). Stolbur phytoplasmas infect not only grapevine, but also a wide range of wild and cultivated plants in several areas worldwide.

Stolbur phytoplasma 16S rDNA is 97.6% identical to the 16S rDNA of '*Ca*. P. australiense', its closest known relative. Because this value is above 97.5%, the designation of stolbur phytoplasma as a '*Candidatus*' species would be possible only considering its specific biological properties and comparing nucleotide sequences of multiple genes (Quaglino *et al*., 2010).

Analyses of concatenated amino acidic sequences of housekeeping proteins have previously been carried out to investigate evolutionary relationships among phytopathogenic mollicutes (Zhao *et al*., 2005). In the present study, we sequenced five ribosomal protein genes of the superoperon S10-*spc-alpha* amplified from two stolbur phytoplasma strains. Sequence analyses allowed us to clarify the phylogenetic position of BN phytoplasma within the taxonomic group 16SrXII and to identify molecular markers useful for specific phytoplasma identification.

Materials and methods

Nucleic acids were extracted from 1 g of leaf tissues of a periwinkle plant, infected by grafting with stolbur phytoplasma strain StolC, and from 1 g of leaf veins of a grapevine plant naturally infected by BN phytoplasma strain BNFc6. A segment of S10 operon, including the genes *rpsJ*, *rplC*, *rplD*, *rplW* and *rplB*, was PCR-amplified using primer pairs designed on the basis of phytoplasmal sequences deposited in the GenBank by Cimerman *et al.* (2006). Nucleotide sequences were compiled in FASTA format. Closest phytoplasmal and bacterial gene sequences were retrieved from the GenBank (table 1). Amino acid sequences were deduced from nucleotide sequences by use of the software ExPasy (http://expasy.org/).

Concatenated sequences were aligned by using the program ClustalX. Output alignment was trimmed using the program GBLOCKS to eliminate poorly aligned positions; the trimmed alignment was converted to MEGA format for phylogenetic NJ analyses.

Further, concatenated nucleotide sequences were searched for the presence of stolbur phytoplasma-specific restriction patterns by virtual RFLP assays carried out by using the program pDRAW (http://www.acaclone.com/). Digestions were performed by using both frequent cutting and rare cutting enzymes: *Alu*I, *Fau*I, *Hinf*I, *Hpa*II, *Mbo*II, *Mse*I, *Taq*I, and *Tsp*509I.

Table 1. Phytoplasmas, other prokaryotes, and GenBank accession numbers of DNAs used for phylogenetic analyses.

Species-strain	Abbreviation	Acc. No.
Stolbur - StolC	StolC	Unpublished
Stolbur - BNFc6	BNFc6	Unpublished
'*Ca*. P. australiense'	Cpaus	NC_010544
'*Ca*. P. asteris' - OYM	OYM	NC_005303
'*Ca*. P. asteris' - AYWB	AYWB	NC_007716
'*Ca*. P. mali' - AP	AP	NC_011047
Acholeplasma laidlawii	Achl	NC_010163
Bacillus cereus	Bacc	NC_011969
Clostridium botulinum	Clob	NC_010520
Mycoplasma mycoides	Mycm	NC_005364
Spiroplasma kunkelii	Spik	AY198133

Results

PCR-amplified S10 operon segments from stolbur phytoplasma-infected periwinkle and grapevine plants were sequenced. Genes (open reading frames) were identified, and their positions were recorded (table 2). The sequences from strains StolC and BNFc6 were identical.

Table 2. Genes from the S10 operon of stolbur phytoplasma strains infecting periwinkle and grapevine.

Gene	Position (bp)	Encoded Protein	Amino acids
rpsJ	1-318	S10	105
rplC	319-984	L3	221
rplD	985-1616	L4	207
rplW	1617-1902	L23	96
rplB	1903-2738	L2	276

Phylogenetic analyses of both nucleotide and amino acid concatenated sequences evidenced that stolbur phytoplasma strains clustered together with '*Ca*. P. australiense', but formed a clearly distinct subclade within the cluster of taxonomic group XII (figure 1).

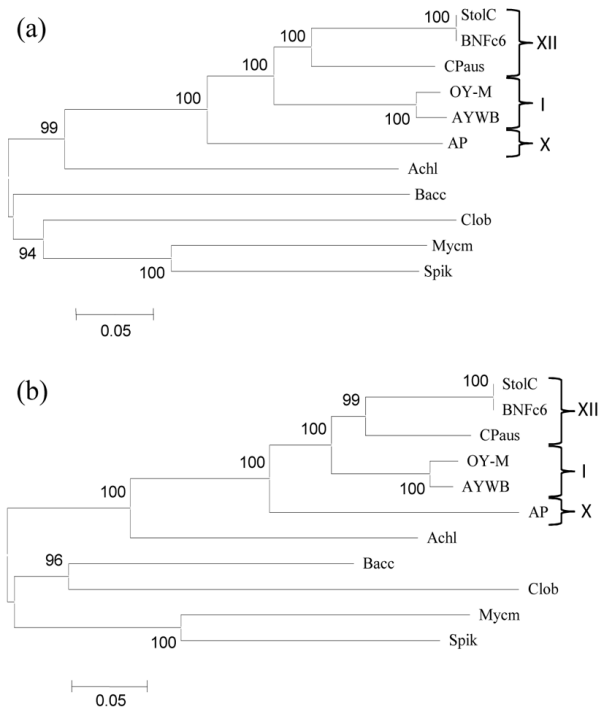

Figure 1. Phylogenetic tree constructed using NJ method of analysis of concatenated (a) rp gene nucleotide sequences and (b) amino acid sequences of rp proteins.

Further, virtual RFLP evidenced significantly different patterns distinguishing stolbur phytoplasma strains from '*Ca*. P. australiense' (figure 2).

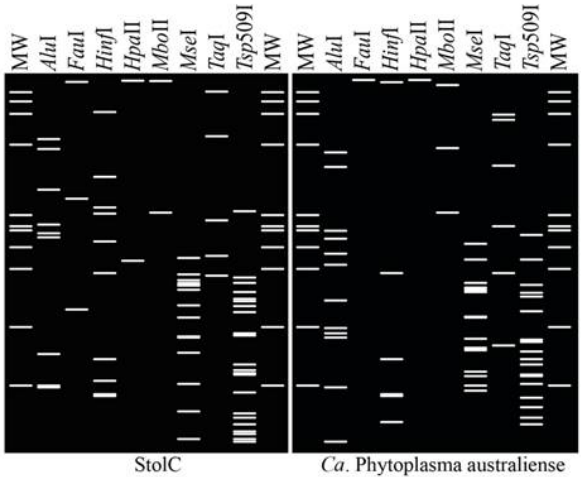

Figure 2. Virtual RFLP patterns of S10 operon segments from phytoplasmas in subgroups XII-A (strain StolC) and XII-B ('*Ca*. P. australiense').

Discussion

Preliminary results from analyses of a partial superoperon S10-*spc-alpha* and encoded proteins emphasized that stolbur phytoplasma strains cluster together in a distinct subclade within the taxonomic group 16SrXII. Further, virtual RFLP analyses confirmed reported sequence divergence between stolbur phytoplasma strains and '*Ca*. P. australiense'. These findings indicate the usefulness of ribosomal protein genes for differentiation among distinct phytoplasmas, encouraging further work aimed at defining more accurately the phylogeny of stolbur phytoplasma strains.

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