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Use of a fragment of the *tuf* gene for phytoplasma 16Sr group/subgroup differentiation

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Abstract

The usefulness of RFLP analyses on a 435 bp fragment of the tuf gene for preliminary identification of phytoplasmas from a number of phytoplasma ribosomal groups and/or '*Candidatus* Phytoplasma' was verified. The strains employed belong to thirteen 16Sr DNA groups and 22 different subgroups and were obtained from both experimentally and naturally infected plants. The combined RFLP patterns obtained with the three restriction enzymes employed allow the distinction of a total of 18 different profiles, however no discrimination was provided for some of the ribosomal groups for which sequencing remains the main tool for phytoplasma identification.

Key words: phytoplasma identification, 16Sr RNA groups, PCR/RFLP, molecular identification, *tuf* gene.

Introduction

The increase of international trade of plant material and of phytoplasma-associated diseases worldwide demands a quick and handily system for phytoplasma identification. In the frame of a DNA barcoding project funded by EU FP7 to create a barcode database for all quarantine plant pathogens, phytoplasma barcoding is in progress and one of the selected genes is the elongation factor Tu (Tuf).

For amplification of short barcoding sequences a cocktail primer system was developed amplifying 435 bp of *tuf* gene for subsequent sequencing (M. Nicolaisen *et al*., unpublished data; Contaldo *et al*., 2010). The usefulness of this fragment for RFLP analyses for preliminary identity screening of phytoplasmas from a number of phytoplasma ribosomal groups and/or '*Candidatus* Phytoplasma' was verified in this study.

Materials and methods

Phytoplasma strains maintained in periwinkle (Bertaccini, 2010), in tomato or field collected were used for PCR amplification with newly developed primers for Tuf in a cocktail reaction (M. Nicolaisen *et al*., unpublished). The strains belong to thirteen 16S rDNA groups and to 22 different subgroups (table 1) and were both from periwinkle infected plants (Bertaccini, 2010) and from naturally infected plants such as grapevine from Italy (FD-VE 210/06), and erigeron and turnera from Brazil (Montano *et al*., 2011). Nucleic acid extraction was performed with a chloroform/phenol procedure (Prince *et al*., 1993) and 20-60 ng of DNA were used as template per reaction.

RFLP analyses were carried out on 200 ng of DNA of each amplicons with *Tru*I, *Tsp509*I, *Taq*I restriction endonucleases (Fermentas, Vilnius, Lithuania) following the instruction of the manufacturer. Restriction profiles were analyzed in both 5% polyacrilammide, and 3% agarose gels in TBE buffer.

Results

PCR products of the expected length were obtained from all strain tested. Amplicons were not observed in the negative controls devoid of DNA and in samples from healthy plants. RFLP analyses allow relevant differentiation among phytoplasmas. Both polyacrylamide and agarose gels provided discriminating profiles. The use of *Tsp509*I allowed differentiation at the 16Sr DNA group level of the majority of the strains and at subgroup level of strains in groups 16SrI, 16SrII, and 16SrIII (figure 1).

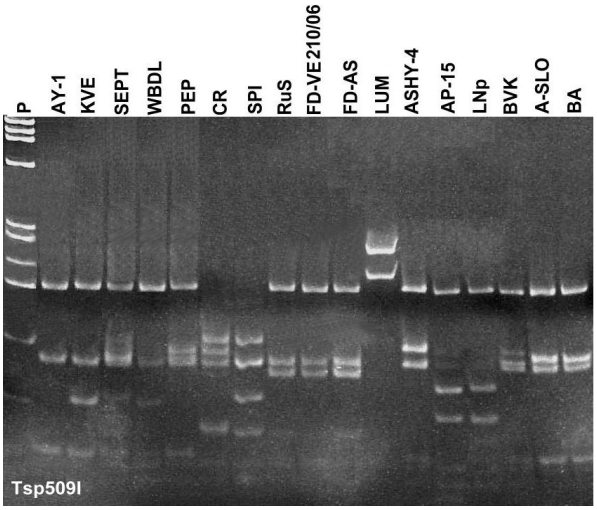

Figure 1. Polyacrylamide gel in which the different profiles distinguished by RFLP are in agreement with 16Sr DNA group/subgroup classification. Acronyms as in table 1. P, marker ΦX174 *Hae*III digested.

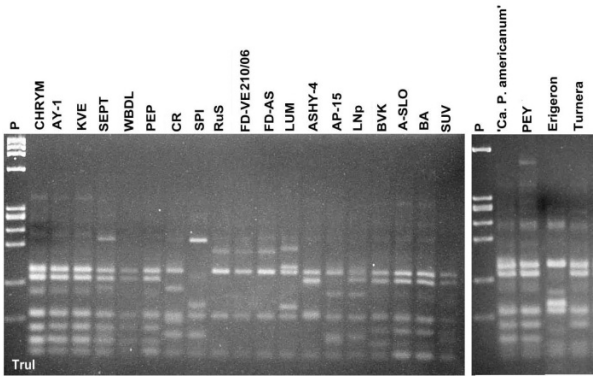

Figure 2. Agarose gel in which the different profiles distinguished by RFLP are in agreement with 16Sr DNA group/subgroup classification. Acronyms as in table 1. P, marker ΦX174 *Hae*III digested.

Table 1. Results of phytoplasma strain differentiation by RFLP analyses on *tuf* gene amplicons (identical letter indicates identical profiles).

Strain acronyms	Tuf profiles			Grouping	
	*Tru*I	*Tsp509*I	*Taq*I	16Sr	tuf
CHRYM	A	A	A	I-A	1
AY1	A	A	A	I-B	1
KVE	A	B	A	I-C	2
SEPT	A	A	B	II-A	3
WBDL	A	B	A	II-B	2
PEP	A	C	C	II-F	4
CR	B	D	B	III-B	5
SPI	C	E	B	III-E	6
RuS	D	F	D	V-E	7
FD-VE 210/06*	D	F	D	V-D	7
FD-AS	D	F	D	V-C	7
LUM	E	G	C	VI	8
ASHY-4	F	H	E	VII-A	9
Erigeron*	M	M	C	VII-B	10
PEY	A	A	C	IX-C	11
AP-15	G	I	C	X-A	12
LNp	H	I	C	X-B	13
BVK	A	L	F	XI-C	14
A-SLO	I	C	C	XII	15
BA	I	C	C	XII	15
Turnera*	I	A	C	XIII	16
SUV	L	C	C	XV	17
'*Ca*. P. americanum'**	A	A	D	XVIII-A	18

* From naturally infected species.
** Inoculated in *Solanum licopersicum*.

The use of *Tru*I differentiated less among groups, however allowed distinguishing subgroups in strains belonging to groups 16SrIII and 16SrX (figure 2). The use of *Taq*I differentiated mainly the strains at the group level. The combined RFLP patterns obtained with the three restriction enzymes allowed the distinction of a total of 18 different profiles. No RFLP discrimination was found between 16SrI-A and I–B strains, as well as 16SrV-C, V-D and V-E strains (table 1).

Discussion

RFLP analyses of tuf amplicons allowe group and subgroup discrimination that is useful for screening a large number of samples. This discrimination is in agreement with published phytoplasma groupings based on the 16S rDNA (Lee et *al.*, 1998; Montano *et al.*, 2001). The visualization of restriction profiles in agarose is a handily tool for large number of sample processing without missing identification ability. In case of phytoplasmas relevant for quarantine, sequencing may be necessary for confirmation and as the main tool for discriminating among 16SrV group strains.

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