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Progress towards a chromosome map of the "flavescence dorée" phytoplasma

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Abstract

A physical map of the genome of the non cultivable "flavescence dorée" phytoplasma (FDp) strain FD92 was constructed by performing restriction digests of the chromosome and resolving restriction fragments by pulsed-field gel electrophoresis (PFGE). *Sal*I, *Bss*HII, *Mlu*I and *Eag*I enzymes were used in single and double digestions to map 13 restriction sites on the FDp chromosome calculated to be 671 kbp. Eight genetic loci, including the two *rrn* operons, genes *tuf*, *uvr*B-*deg*V and *sec*Y-*map* (FD9), the FD2 marker as well as two orphan sequences (FDDH29 and FDSH05) isolated through Subtractive Suppression Hybridization (SSH) were positioned by performing Southern blot analyses.

Key words: *Mollicutes*, grapevine disease, chromosome map, pulsed-field gel electrophoresis.

Introduction

While important progress has been made in phytoplasma classification and ecology, little is known about mechanisms of phytopathogenicity and insect transmission; these research areas should benefit from the knowledge and comparative analysis of phytoplasma genomes. "Flavescence dorée" phytoplasma (FDp) is the agent of a quarantine disease of the grapevine in Europe (Boudon-Padieu, 2002). This pathogen belongs to the 16SrV group, for which the sequenced genome of '*Ca.* P. asteris' (16SrI ribosomal group) can hardly serve as a reference due to the important phylogenetic distance between these two groups (Lee *et al.,* 2004, Oshima *et al.,* 2004). This paper describes a physical map of the FDp chromosome as a first step of a genome project.

Materials and methods

FDp chromosomes were prepared according to Padovan *et al.,* (2000) from FD92 infected broad beans (Angelini *et al*., 2003). Endonuclease digestions were performed in agarose blocks with rare-cutting enzymes: *Apa*I, *Bss*HII, *Eag*I, I-*Ceu*I, *Kpn*I, *Mlu*I, *Pvu*II, *Sal*I, *Sma*I and *Xho*I. Clamped homogenous electrical field (CHEF) PFGE was performed at 6 V/cm, 14 °C, with an angle of 120° for 19 h and various ramped pulse times: 0.7 to 13 sec, 2 to 40 sec and 5 to 90 sec. FDp DNA fragments used as probes for genetic mapping were the following: FD2 (Daire *et al.,* 1992), FD9/s*ecY* gene, (Daire *et al*., 1997), *map* gene (Arnaud *et al.,* 2007), *uvrB-degV* genes (Arnaud *et al.,* 2007), FDDH 29 and FDSH05 (no homology in databases) and rDNA16S. The *tuf* gene was isolated from DNA of an aster yellows phytoplasma infected periwinkle, as described by Schneider *et al.* (1997). Southern hybridizations were performed according to standard procedures.

Results

The enzymes *Bss*HII, *Eag*I, *Mlu*I, and *Sal*I revealed a clear banding pattern with 2 or 3 fragments between 50 and 550 kbp and were consequently selected for mapping. Single and double restriction profiles are shown in figure 1A, lanes 1 to 10. Mean sizes of fragments observed were determined from at least 3 independent PFGE procedures. Such fragments were not observed with *Sal*I and *Bss*HII digested DNA prepared from healthy broad beans as control, as shown figure 1A lanes 12 and 13. Undigested DNA from infected broad beans revealed a fragment around 680 kbp (data not shown). Identification and size determinations of fragments smaller than 50 kbp were not easy to perform because of a background signal also observed for digested DNA controls and might correspond to contaminating broad bean chloroplastic or mitochondrial DNA. Therefore, fragments smaller than 50 kbp were identified by hybridization with the FD specific probes.

Hybridizations performed with either FDSH05, *uvrB-deg*V, FDDH29 (figure 1B), *map* (figure 1C), *sec*Y, FD2 or *tuf* probes revealed a single fragment. This indicated that these markers are present in a single copy on the FD92 phytoplasma chromosome. No fragments were revealed with hybridization of healthy control DNA. Hybridizations performed with *sec*Y presented the same profile as those performed with *map* and FD2, showing that these two markers are located on the same restriction fragment. *Tuf* and FDDH29 also presented the same hybridization profile. Hybridizations performed with 16S rDNA revealed 2 fragments for each digestion, suggesting the presence of 2 operons on the chromosome.

The size of the phytoplasma chromosome was estimated at 671 ± 14 kbp which represents the mean of the sums of the sizes of fragments generated by single and double digestions. A provisional physical and genetic map is presented figure 2.

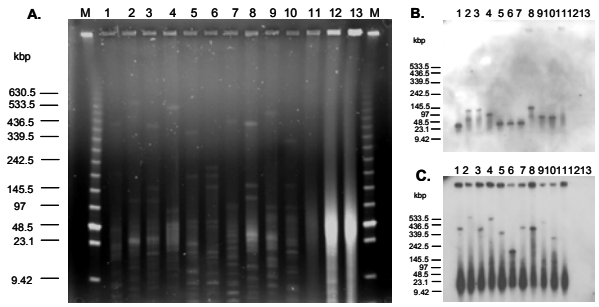

Figure 1. PFGE of DNA prepared from FDp-infected (lanes 1 to 11) and healthy (lanes 12 and 13) broad beans single or double digested with various rare-cutting enzymes. A: lanes 1, *Sal*I; 2, *Bss*HII; 3, *Eag*I; 4, *Mlu*I; 5, *Bss*HII and *Sal*I; 6, *Eag*I and *Sal*I; 7, *Mlu*I and *Sal*I; 8, *Eag*I and *Bss*HII; 9, *Mlu*I and *Bss*HII; 10, *Mlu*I and *Eag*I; 11, undigested; 12, *Sal*I; 13, *Bss*HII; M, Low range PFG marker (kbp). B and C: Southern-blot hybridizations of PFGE **p**robed with FDDH29 (B), or with *map* (C).

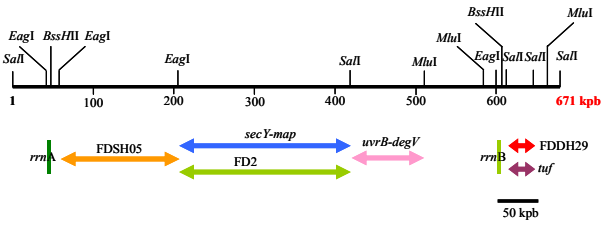

Figure 2. Provisional physical and genetic map (linear) of the FDp chromosome**.** Restriction sites are indicated above the linear scale. Restriction fragments hybridizing with rDNA16S, *tuf,* FDDH29, *uvr*B-*deg*V, *sec*Y, *map*, FD2 and FDSH05 sequences are delimited by arrows or by bars.

Discussion

The two copies of *rrn* operon were shown to be distant of about 100 kbp on the FDp chromosome, *tuf* gene lying somewhere 30 kbp upstream *rrn*B. A similar disposition was also found for onion yellows and aster yellows witches' broom phytoplasmas (Bai *et al.,* 2006; Oshima *et al.*, 2004). The estimated chromosome size of FD92 phytoplasma (671 kbp) is in accordance with that of 16SrV group phytoplasmas according to Marcone *et al.,* (1999), ranging from 680 kbp ('*Candidatus* Phytoplasma ulmi' strain ULW) to 820 kbp (rubus stunt phytoplasma). However, chromosome size is not related to phylogenetic relationships in the group since ULW is closer to elm yellows strain EY (730 kbp) than to FDp and alder yellows (750 kbp) (Angelini *et al*., 2003; Lee *et al.,* 2004; Arnaud *et al.,* 2007).

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