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StAMP encoding the antigenic membrane protein of stolbur phytoplasma is useful for molecular epidemiology

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Abstract

The antigenic membrane protein of stolbur phytoplasma has been cloned and characterized. The expression of StAMP in *Escherichia coli* produced a 16 kDa peptide recognized by an anti-stolbur monoclonal antibody. *Stamp* is submitted to a positive diversifying selection pressure (Fabre *et al.*, 2011). The genetic diversity of *stamp* was evaluated among a collection of stolbur phytoplasma strains representative of the *tuf* and *sec*Y genetic diversity of stolbur phytoplasmas in the Euro-Mediterranean basin. Most of the French, Italian and Croatian strains clustered on the same phylogenetic branch (*tuf*-type b cluster I). A second branch of the phylogenetic tree corresponded to strains of central and Eastern Europe (*tuf-type* b cluster II), while a third branch grouped strains of the east of the Mediterranean basin (Greece, Serbia, Lebanon, and Azerbaijan). Strains of the *tuf*-type a genotype clustered together in an independent monophyletic branch of the *stamp* phylogenetic tree. In conclusion, *stamp* variability seems to be correlated to geographical origin in the case of the *tuf*-type b strains.

Key words: 'Bois noir' disease, molecular epidemiology, bacterial surface protein, positive selection.

Introduction

'Stolbur' phytoplasma (StolP) affects a wide range of crops and wild plants in the Euro-Mediterranean area including solanaceous crops, grapevine, lavender, strawberry, sugar beet, maize, stinging nettles and bindweed. It is transmitted by three *Fulguromorpha* planthoppers of the family *Cixiidae*.

Analysis of stolP *tuf*-type b variability led to the discovery that different *tuf* genotypes can be associated with infection of bindweed and nettle (Langer and Maixner, 2004). Due to its complex ecology, StolP is difficult to trace without the help of variable genetic markers. We recently described the striking genetic diversity of *vmp*1 encoding a variable membrane protein specific to StolP (Cimerman *et al.*, 2009). Variability of *vmp*1combined with that of *tuf* or *sec*Y proved to be efficient to differentiate StolP strains (Fialová *et al.*, 2009; Murolo *et al.*, 2010; Pacifico *et al.*, 2009). We report here the isolation and characterization of *stamp*, the gene encoding the antigenic membrane protein of stolbur phytoplasma. Due to the synteny of the *gro*L-*amp*-*nad*E locus between phytoplasma in the 16SrI and 16SrXII groups, the cloning of *stamp*, a StolP homolog of '*Ca.* P. asteris' *amp* was undertaken. Its usefulness as a genetic marker possibly correlated to StolP geographical origin or to association with insect vector species or ecotypes is currently evaluated.

Materials and methods

StolP-infected periwinkles were maintained by graft inoculation. Grapevines, bindweeds, nettles, lavenders, potatoes, tomatoes, peppers, eggplant, cherry, and common medlar, were collected in France, Italy, Germany, Hungary, Croatia, Serbia, Greece, Bulgaria, Lebanon, Azerbaijan and Egypt. *Hyalesthes obsoletus* (Signoret, 1865) insect vectors were collected in Germany, Italy, Slovenia and Croatia. Nucleic acids were extracted as previously described (Maixner *et al.*, 1995)

For fluorescence microscopy with 2A10 anti stolP mAB, fresh periwinkle midribs were processed as previously described (Garnier *et al.*, 1990). Methods for amplification of *gro*L-*stamp*-*nad*E and its cloning in *E. coli* as well as the detection of StAMP expression in *E. coli* was recently published (Fabre *et al.*, 2011).

Nested-PCR amplification and sequencing of *stamp* (Fabre *et al.*, 2011) produced chromatograms that were assembled and edited using GAP4. ClustalW multiple alignments and maximum of parsimony phylogenetic analyses were performed by MEGA 4. Inference of positive selection and determination of dN/dS was according to PARRIS method (Scheffler *et al.*, 2006).

Results

The gene *stamp* could be amplified and cloned in *E. coli*. It encodes a 157 amino acid-long protein with a predicted signal peptide and a C-terminal hydrophobic alpha helix. StAMP was 26%-40% identical to AMP of '*Ca*. P. asteris' strains and 40% identical to AMP of '*Ca*. P. japonicum'. The expression of StAMP in *E. coli* produced a 16 kDa peptide recognized by 2A10 mAB. Stamp was more variable than the house-keeping gene *sec*Y and the ratio between non synonymous over synonymous mutations (dN/dS) was 2.78 for *stamp* as

compared to 0.64 for *sec*Y. This indicates that *stamp* is submitted to a positive diversifying selection pressure.

Stamp genetic diversity was evaluated among a collection of StolP strains representative of the genetic diversity in the Euro-Mediterranean basin. Most of the French, Italian and Croatian *tuf*-type b strains as well as Egyptan potato strains clustered on the same phylogenetic branch (*tuf*B cluster I) (figure 1). Another branch corresponded to strains of Central and Eastern Europe including German, Slovenian, Hungarian, Bulgarian and Romanian strains (*tuf*-type b cluster II). A third branch grouped strains of the east of the Mediterranean basin, collected in Lebanon, Greece, Serbia and Azerbaijan (*tuf*-type b cluster III). Strains of the *tuf*-type b genotype clustered together on a monophyletic branch.

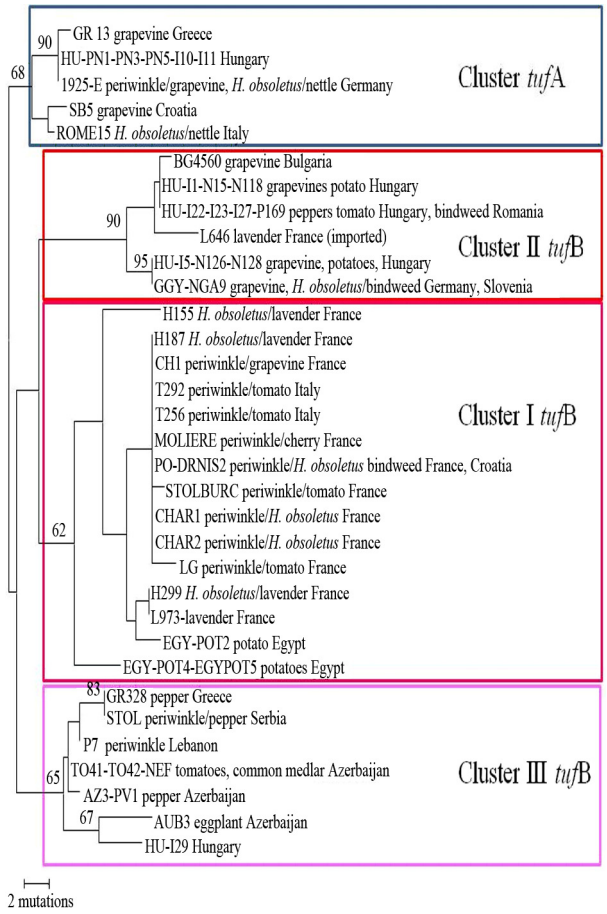

Figure 1. Phylogenetic consensus tree of StolP *stamp* sequences analysed by maximum of parsimony. Numbers above branches indicate the bootstrap values (500 replicates). Plant, insect and geographical origin are indicated on the right of StolP isolate names.

Discussion

Stamp encodes the antigenic membrane protein of StolP. Its variability is to be correlated to geographical origin of *tuf*-type b strains. Does this correlation correspond to insect vectors geographical distribution or to association with different insect species or ecotypes? To answer this question more samples will need to be analysed improving the genetic investigation by a multilocus and integrated approach with variable and house-keeping genes as well as insect population genetics.

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