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 secY genetic diversity of 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 Fulgoromorpha 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 nettles (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 vmp1 encoding a variable membrane protein specific to StolP (Cimerman et al., 2009). Variability of vmp1 combined with that of tuf or secY 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 groL-amp-nadE locus between phytoplasmas 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, 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.
compared to 0.64 for secY. 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 Egyptian 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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References


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