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# *In vitro* interactions between immunodominant membrane protein of lime witches' broom phytoplasma and leafhopper vector proteins

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## Abstract

Lime witches' broom phytoplasma (16SrII, LWB) causes major losses to lime cultivation in Iran. The role of its immunodominant membrane protein (IMP) in the specific interaction with vector proteins was addressed. The carboxy terminal moiety of LWB IMP, coding the extracellular part of the protein, was expressed (LWB fΔIMP) as a tool to study IMP interactions with insect proteins. In preliminary affinity chromatography experiments, leafhopper vector proteins with different sizes interacted with LWB fΔIMP.

**Key words:** *Hishimonus phycitis*, immunodominant membrane protein, protein interaction.

## Introduction

Immunodominant membrane proteins of phytoplasmas are in direct contact with the surrounding host cell environment, and therefore they are good candidates for interaction with host proteins. Studies on two 16SrI related phytoplasmas showed that their antigenic membrane protein (AMP) interacts specifically with certain insect vector proteins and might have a role in determining vector specificity (Suzuki *et al*., 2006; Galetto *et al*., 2010). The role of phytoplasma IMP and IdPA type membrane proteins in interactions with insect vector is still unknown. Preliminary studies showed that lime witches' broom (LWB) phytoplasma (16SrII), the causal agent of a devastating disease of lime in Iran, has the major (immunodominant) membrane protein homologous to IMP type of phytoplasma membrane proteins. Moreover, preliminary studies have indicated that some amino acids in the extracytoplasmic region of the IMPs of 16srII phytoplasmas (including LWB) are under positive selection. A more rapid evolution of this part of the gene compared to other genomic regions may further support a role for IMP in host-pathogen interactions, as already suggested for AMP (Kakizawa *et al*., 2006). The main objective of this study was to determine the interaction of LWB IMP with proteins of its leafhopper vector *Hishimonus phycitis* (Distant).

## Materials and methods

The IMP ORF of LWB excluding its transmembrane domain was amplified by PCR and cloned in pQE30 vector to express the truncated IMP protein in fusion with 6X histidine residues (LWB fΔIMP). The recombinant protein was purified and coupled to a resin affinity column. Crude extracts of leafhoppers, *H. phycitis, Macrosteles quadripunctulatus* (Kirschbaum), *Euscelidus variegatus* (Kirschbaum), *Empoasca decipiens* Paoli and *Scaphoideus titanus* Ball, were separately loaded on the IMP affinity column followed by elution of specifically bound proteins. Crude extract of the non vector aphid *Myzus persicae* (Sulzer) was separately loaded as control. All crude extracts were also loaded on affinity column coupled to a polyhistidine peptide, as control. Eluted proteins were subjected to SDS-PAGE followed by gel staining. Protein bands resolved on the gel were excised and identified by mass spectrometry. In Western blot experiment, proteins separated on SDS-PAGE were electro-blotted on a PVDF membrane. After blocking, the membranes were incubated with commercial primary antisera for 2 h at room temperature, then with HRP-conjugated secondary antibodies at room temperature for 1 h. Membranes were developed with Super Signal West Pico reagents (Pierce) and bands were visualized by VersaDoc model 4000 Imaging system (BioRad).

## Results

The LWB IMP extracellular domain (LWB ΔIMP) was successfully expressed in *E. coli* after cloning in pQE30 expression vector.

A total of 16 protein bands, detected by SDS PAGE following elution from the LWB ΔIMP-coupled affinity column, were subjected to mass spectrometry analysis for identification (table 1). P40 bands (40 kDa protein band) from different leafhopper species were identified with high confidence as actin. P50, P45 and P200 bands showed spectra matching with those of ATP synthase beta, arginine kinase and myosin heavy chain, respectively. No matches were found for P90, P150, P55 and P80 bands. No bands were detected when extracts from the aphid *M. persicae* were eluted from the LWB fΔIMP-coupled affinity column.

**Table 1.** Leafhopper proteins retained in affinity column packed with LWB ΔIMP, their observed molecular weight, results of MS/MS identification, and summary of western blot experiments to confirm MS/MS identifications.

| Insect species | Band Size (kDa) | MS/MS protein identification | Western blot (ATP synthase beta) | Western blot (actin) |
|---|---|---|---|---|
| *Hishimonus phycitis* | P90 | Unknown | + | + |
| | P50 | Unknown | | |
| | P150 | Unknown | | |
| *Macrosteles quadripunctulatus* | P90 | Unknown | + | + |
| | P80 | Unknown | | |
| | P40 | Actin | | |
| | P45 | Arginin kinase | | |
| *Euscelidius variegatus* | P90 | Unknown | + | + |
| | P40 | N/A | | |
| *Empoasca decipiens* | P90 | Unknown | + | + |
| | P50 | ATP synthase beta | | |
| | P40 | N/A | | |
| *Scaphoideus titanus* | P200 | Myosin | + | + |
| | P90 | Unknown | | |
| | P55 | ATP synthase beta | | |
| | P40 | Actin | | |

Anti actin and anti ATP synthase beta subunit polyclonal antibodies were employed in Western blot to confirm the presence of the corresponding proteins in insect eluates from LWB fΔIMP affinity column. Actin and ATP synthase beta protein bands were detected on eluates of all leafhopper species. No signal was detected in the western blots with corresponding eluates of the aphid *M. persicae*, and from leafhopper eluates of the poly-histidine control column.

## Discussion

We showed the *in vitro* interaction between lime witches' broom IMP and some proteins of leafhoppers vectors of phytoplasmas, so far not known as LWB vectors. Two of these proteins, including actin and ATP synthase beta were previously shown to participate in specific interactions with AMP of two 16SrI related phytoplasmas (Suzuki *et al*., 2006; Galetto *et al*., 2010). Sequence analysis are in progress to evaluate the existence of positive selection acting on the exposed moiety of LWB IMP, as reported for other IDPs (Kakizawa *et al*., 2009).

We showed that LWB IMP interacted *in vitro* under non denaturing conditions with several proteins from *H. phycitis*, candidate vector of the disease in Iran, as well as with other leafhoppers vectors of phytoplasmas in 16Sr groups I and V. IMP, however, did not interact with proteins of the aphid *M. persicae* which is not known as phytoplasma vector. One band (p90) was present among interacting proteins obtained from all leafhoppers, although peptides from its trypsine digestion did not match with any known sequence. Consequently, p90 could not be identified. IMP-interacting proteins p50 and p150 (*H. phycitis*), p80 (*M. quadripunctulatus*), and p40 (*E. variegatus* and *E. decipiens*) could not be identified for the same reason. These preliminary experiments showed that IMP of LWB phytoplasma interacts with several leafhopper proteins involved in cell cytoskeleton, organelle mobility, and energy production.


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