

IDENTIFICATION OF LEAFHOPPER VECTOR PROTEINS SPECIFICALLY INTERACTING WITH PHYTOPLASMA ANTIGENIC MEMBRANE PROTEIN

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Phytoplasmas are uncultivable phloem-limited phytopathogenic wall-less bacteria and are associated with severe plant diseases spread worldwide. They are transmitted in a persistent propagative manner by phloem-sucking hemipteran insects. Due to the lack of cell wall, phytoplasma membrane proteins are in direct contact with hosts and are presumably involved in determining vector specificity. Such a role has been proposed for phytoplasma plasmid-encoded transmembrane proteins and for the major phytoplasma antigenic membrane protein (Amp). The aims of our work were to discover vector proteins interacting with Amp and to investigate their role in transmission specificity. Following controlled transmission experiments, four hemipteran species were identified as vectors of the “*Candidatus Phytoplasma asteris*”, CYP strain, and three others as non vectors. Interactions between a recombinant CYP Amp and insect proteins were analysed by far Western blots and affinity chromatography. Amp specifically interacted with few membrane proteins from vector species only. Among Amp-binding vector proteins, actin and ATP synthase α and β subunits were identified by mass spectrometry and Western blots. Immunofluorescence confocal microscopy and Western blots on cell membrane and mitochondria fractions confirmed the localisation of ATP synthase, a known mitochondrial protein, in plasma membranes of midgut and salivary gland cells in the vector *Euscelidius variegatus*. Phytoplasma Amp is likely to play a crucial role in insect transmission specificity. The vector-specific interaction between phytoplasma Amp and insect ATP synthase is demonstrated for the first time. Plasma membrane ATP synthase is known as receptor for various ligands in vertebrates and arthropods. Intriguingly, phytoplasmas lack ATP synthesis genes: exploitation of host ATP synthetic machinery maybe required for phytoplasma survival or adhesion before entry in vector organs. This work also confirms the role of host actin, likely involved in internalization and intracellular motility of phytoplasmas, as shown for other intracellular pathogens.