

AAA+ ATPase AP460 – A virulence factor of apple proliferation disease?

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‘*Candidatus* Phytoplasma mali’, a wall-less phytopathogenic bacterium of the class *Mollicutes* and the agent of apple proliferation (AP) disease, causes specific symptoms (undersized fruits, witches’-brooms, rosetting, enlarged stipules) and nonspecific symptoms (foliar reddening, yellowing, stunting, decline) on apple plants. ‘*Ca. P. mali*’ colonizes phloem sieve elements inducing impairment of phloem function (e.g. callose deposition on sieve plates and phloem necrosis).

However, symptom expression of AP disease is highly variable, ranging from non-symptomatic to mildly, moderately and severely affected trees. Additionally, differences in disease development of infected trees (no symptoms at all, temporary or permanent recovery) were monitored in long-term observations. These variations in severity and variability of symptom expression are due to differences in the virulence of AP phytoplasma accessions (phytoplasma population in a tree). They are phenotypically classified into avirulent, mildly, moderately or severely virulent. Molecular analyses of AAA+ (ATPases Associated with various cellular Activities) protein genes from infected trees of different symptom expression revealed the existence of single and multiple strain accessions. Multiple infections are usually composed of differently virulent strains and shifts in the population may alter

symptomatology. This research focuses on the membrane-associated AAA+ ATPase AP460. Since virulence-specific, as well as suppression-related clustering, was previously identified in the AP460 gene, there is indication that AP460 plays a significant role in phytoplasma pathogenicity. The current study examined 147 full-length deduced AP460 protein sequences of single- and multiple-strain accessions of different virulence. In sequence alignments two relevant regions were detected: region 1 in the N-terminal part possessed conserved substitutions associated with suppression of virulence. The more diverse region 2 in the C-terminus contained conserved substitutions, not only associated with two different groups of virulence but also with suppression of virulence. Three of these suppression-related substitutions, occurring next to key residues of ATPase motifs, seem to affect ATPase function. Membrane topology prediction programs (Phobius, PolyPhobius) indicate an extracellular C-tail orientation of AP460, essential for being a virulence factor. An immunohistochemical localization procedure will be employed for confirming the predicted orientation. A purified recombinant C-terminally 6xHis-tagged AP460- Δ TM protein (transmembrane domain deleted) will be used as an antigen to generate polyclonal antisera.