4.1 Development and Reactivity of Polyclonal Antibodies Based on OMP Sequences of *Candidatus Liberibacter asiaticus*

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The use of serological approaches on *Candidatus Liberibacter asiaticus* (Las) research and diagnosis is limited by the absence of good antibodies as a consequence of uncultured characteristic of this bacterium. Polyclonal antibody with specific reaction against Las will be useful for both quick and low-cost diagnosis test, as well as immunomicroscopy studies. Here, we are showing results of polyclonal antibodies (PAs) produced from nine immunogenic synthetic peptides whose amino acid sequences were translated from Las - Outer Membrane Protein genomic sequences and selected based on antigenicity and hydrophilicity by Antogenicity plot. The antigenic peptides were synthesized by Bio-Synthesis, Inc. (Lewisville, TX, USA) with size ranging from 10 to 26 amino acids and were endoveneously twice inoculated (total of 500 µg) in white rabbits. Seven days after the last injection, each rabbit was bled through cardiac puncture, and the serum titer and specificity were determined by indirect enzyme-linked immunosorbent assay (ELISA) according to Clark et al. (1986). Eight of nine PAs, tested with dilutions from 1:500 to 1:32,000, resulted in a linear pattern of reactivity at O.D. of 405 nm, by ELISA, against their antigens (10 µg/ml). So, we tested by ELISA the eight preselected PAs to react against citrus asiatic-HLB symptomatic leaves. Four of eight PAs recognized Las in the infected leaves at O.D. ranging from 0.456 to 0.669 (1:2,000 dilution) with no cross-reaction observed against healthy plants as well as against some bacteria species potentially present in citrus plants like *Xanthomonas citri* pathovars, *Methylobacterium* sp. (Araujo et al., 2002), and *Xylella fastidiosa*.

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References
