OBTENTION OF SPECIFIC MONOCLONAL ANTIBODIES AND ANTISERA AGAINST *XYLELLA FASTIDIOSA* AND THEIR USE FOR DETECTION AND DIAGNOSIS

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Monoclonal antibodies (MAb) specific to *Xylella fastidiosa* were obtained by fusion of a nonsecreting myeloma cell line with spleen cells from immunized BALB/c mice by intraperitoneal injections of 0.1 ml of 10^8 cfu/ml of *X. fastidiosa* subsp. *fastidiosa* (LMG17159 strain) (somatic antigens O) emulsified in Freund's incomplete adjuvant. Specific antibody-secreting hybridoma selected by indirect-ELISA was three times cloned under conditions of limiting dilution and established hybrids were grown in HT medium. Ten MAb lines producing the highest bacterial titre were selected, isotype determined and their specificity tested. Three MAbs (MAb2G1/PPD, MAb1C6/PPD and MAb9F7/PPD) were selected for their wide reaction spectrum against *X. fastidiosa* strains and good specificity. Furthermore six polyclonal antisera against *X. fastidiosa* were raised in CalifornianxNeozelander rabbits with O antigens from Conn Creek, LMG15099 and LMG17159 strains. LMG17159-O antiserum was selected for the higher titre and because it recognized all the *X. fastidiosa* strains challenged. Polyclonal immunoglobulins as trapping/coating antibodies and specific MAb2G1/PPD as intermediate-detecting antibodies (DASI-ELISA method) reached a sensitivity of 10^5 cfu/ml of *Xylella fastidiosa* in almond extracts and of 10^5-10^6 in olive extracts. A DAS-ELISA prototype was then developed, prior to commercial distribution, using MAb2G1/PPD conjugated with alkaline phosphatase. The sensitivity reached was 10^5 cfu/ml and showed excellent specificity. One hundred twelve samples of different almond tree plots from the Demarcated Zone for *X. fastidiosa* in Alicante (Spain) were analysed comparatively by the developed DAS-ELISA, the LOEWE kit and the protocols of real-time PCR by Harper et al. (2010) and Francis et al. (2006). The agreement between the techniques was almost perfect according to the estimated Cohen's kappa index, even in symptomless almond trees. The production of specific MAbs to *X. fastidiosa* will supply a continuous source of homogenous and well characterized antibodies to increase the accuracy of diagnosis and detection methods. A direct tissue-print or DTBIA kit is being also validated in order to supply an available user-friendly system to test in a low cost, fast, discreet, sensitive, an accurate manner this harmful bacterium in samples from nurseries, gardens and wide surveys, such as is available for other plant pathogens.