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Short communication

Production and characterization of a monoclonal antibody specific to the M serotype of plum pox potyvirus

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Abstract

A monoclonal antibody to an Albanian isolate of plum pox potyvirus (PPV) was obtained (MAbAL), that specifically recognized strain M of this virus. The specificity of MAbAL, assessed by comparative ELISA on 130 PPV isolates of different geographical origin, 22 of which were also tested by comparative IC-PCR, gave consistent and highly reproducible results. MAbAL seems to be elicited by a stable surface determinant that makes it particularly suitable for successful use under a wide range of conditions. MAbAL is an useful addition to the panel of PPV-specific MAbs available to date.

Plum pox potyvirus (PPV), one of the most injurious pathogens of stone fruits, is the agent of sharka disease, the cause of heavy losses to plum, apricot and peach crops in most European countries.

The European isolates of PPV fall in two main groups which can be differentiated because of the electrophoretic mobility of dissociated coat protein (CP) (Ravelonandro et al., 1988; Bousalem et al., 1994; Pasquini and Barba, 1994) and the occurence of restriction site polymorphism in the coat protein gene (Wetzel et al., 1991; Candresse et al., 1995). These PPV groups, originating from Eastern and Western Europe, respectively, proved to be related to PPV serotypes M and D identified by Kerlan and Dunez (1979). The specific detection of either serotype is of major importance, because of their different biological and epidemiological behaviour. PPV-M is readily transmitted by aphids to peach, plum and apricot, whereas PPV-D is little or not at all aphid-transmissible to and between peaches (Quiot et al., 1995). Therefore, in the areas free from PPV-M, peach may not be exposed to natural epidemics. In these areas it is of utmost importance to dispose of diagnostic tools suitable for large scale surveys for the specific identification of one or the other serotype, for the timely discovery and eradication of possible PPV-M foci. Among the few methods available for the specific identification of PPV serotypes, Western blot analysis exploits the differential electrophoretic mobility of dissociated CP, whereas PCR primers were recently designed in France (Candresse et al., 1995) for amplifying a CP sequence specific to either serotype. Both these techniques are laborious, require equipment and expertise not available in all laboratories potentially interested in PPV detection, and may not be suitable (Western blot) for large scale surveys.

For a more efficient utilization of a versatile and widely known technique like ELISA, several attempts to prepare strain-specific monoclonal antibodies (MAbs) have been made (Navratil et al., 1992; Lopez-Moya et al., 1994b; Pasquini and Barba, 1994; Asensio et al., 1995; Pasquini et al., 1995). To date, however, the only specific MAbs available are those raised to PPV-D by Cambra et al. (1994). In this

paper the production and characterization of a PPV-M-specific MAb is reported.

An Albanian isolate of PPV (PPV-AL) (Myrta et al., 1996) purified from *Nicotiana benthamiana* (Van Osten, 1972) was used for immunizing BALB/c mice (Harlan Nossan, Correzzana), then fusing immunized splenocytes and NSO/1 myeloma cells (American type Culture Collection, Rockville), as described (Boscia et al., 1992). Screening of cell culture supernatants for the selection of hybridomas secreting specific MAbs, was done by DASI-ELISA (Cambra et al., 1994). Plates were coated with IgGs from a polyclonal antiserum to PPV-AL (Myrta et al., 1996) and extracts of healthy and PPV-AL-infected *N. benthamiana* plants were used as antigen.

Comparative serological tests were done with the polyclonal antiserum to PPV-AL, and two monoclonal antibodies, i.e., MAb5B, that recognized both PPV serotypes, and MAb4DG5, specific to PPV-D (Cambra et al., 1994).

Mab to PPV-AL, obtained either from cell culture supernatant or from diluted ascitic fluid, was used for virus particle decoration (Milne, 1993) and immunogold labelling with 10 nm colloidal gold (Sigma Chemical Co., St. Louis). Virions from a purified preparation were exposed to MAbs, the grids were rinsed with PBS, incubated for 15 min with anti-mouse gold conjugate diluted 1:20 in PBS, rinsed with water applied dropwise, and stained with 2% aqueous uranyl acetate.

Western blot was as described by Hu et al. (1990). Dissociated coat protein from a purified virus preparation was transferred onto polivynildifluoride membrane (Millipore Corporation, Bedford), then incubated with MAb to PPV-AL in 2% nonfat milk powder-PBS (3μg/ml) and, after washing, was incubated first in protein-A gold conjugate solution (Bio-Rad Laboratories), then in the enhancement solution. The MW-SDS-70 kit for molecular weights 10,000–70,000 (Sigma Chemical Co., St. Louis) was used as reference marker.

For immunocapture-polymerase chain reaction (IC-PCR), 100 μl aliquots of infected plant extracts clarified by centrifuging for 5 min at 13,000 rpm, were placed in antibody-coated tubes then used for reverse transcriptase PCR (RT-PCR) as described (Wetzel et al., 1992), utilizing the primers reported by Wetzel et al. (1991). Immunocapture tubes were coated with 1 μg/ml of the anti-PPV monoclonal antibody 5B (Cambra et al., 1994). Specific amplifications of PPV-D and PPV-M sequences were obtained with primers kindly supplied by Dr. T. Candresse.

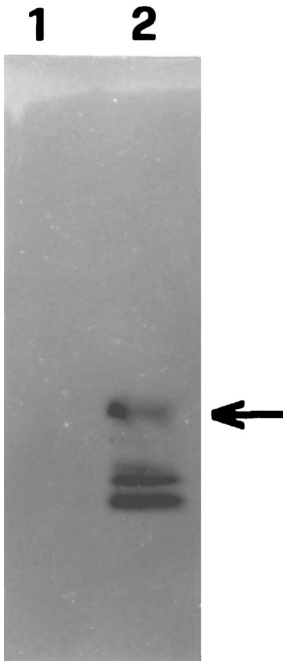

Figure 1. Western blot of dissociated coat protein (CP) preparation from purified virus, exposed to MAbAL. The antibody clearly recognized the viral CP of 38 kDa (lane 2, arrow head) and its degradation products (bands below) up to the 28 kDa component (bottom). Healthy control is in lane 1.

Of the 420 hybridoma cultures obtained, only one reacted positively in DASI-ELISA with PPV-AL but not with healthy plant extracts. It continued to secrete antibodies after transferring, cloning and subcloning and three successive cycles of freezing and thawing in liquid nitrogen. This MAb, denoted MAbAL, belonged to the IgG2a isotype, and decorated PPV-AL particles uniformly along the whole length. In Western blot assays MAbAL recognized specifically the viral CP (38kDa) and its degradations products, including the 28kDa coat protein core (Lopez-Moya et al., 1994), but did not react with extracts from healthy *N. benthamiana* (Figure 1, markers not shown). In DASI-ELISA, MAbAL clearly reacted with virus preparations regardless of whether they had been extracted in phosphate buffer, or subjected to gentle degradation with calcium chloride (Hajimorad and Francki, 1989), or to boiling in Laemmli's (1970) degradation buffer. All this was taken as evidence that the antigenic determinant that gave rise to MAbAL is a stable surface determinant located far from the C and N termini of the CP subunit polypeptide. If this were not the case, the fast migrating electrophoretic bands, thought to be CP degradation product derived from proteolysis of the polypeptide

Table 1. Detection of different PPV isolates belonging to the D or M type, by DASI-ELISA using MAb5B (D and M specific), MAb4DG5 (D specific) and MAbAL, and by serotypes (M and D)-specific IC-RT-PCR

Isolate	Type*	ELISA MAb5B	ELISA MAb4DG5	PCR D-specif.	ELISA MAbAL	PCR M-specif.
NAT-FL/Nc	D	+	+	+	–	–
RB/Nb	D	+	+	+	–	–
R3/Nb	D	+	+	+	–	–
W-Bel/Nb	D	+	+	+	–	–
W/Nb	D	+	+	+	–	–
3.4RB/GF	D	+	+	+	–	–
Canino D/GF	D	+	+	+	–	–
Quiot/Ps	M	+	–	–	+	+
SC/Nc	D	+	+	+	–	–
SEO/GF	M	+	–	–	+	+
O6/Nb	M	+	–	–	+	+
PS/Nc	M	+	–	–	+	+
SK-68/Nc	M	+	–	–	+	+
MS-89/GF	M	+	–	–	+	+
Plovdiv/Nb	D	+	+	+	–	–
Turquia/GF	D	+	+	+	–	–
Healthy GF	–	–	–	–	–	–
Healthy Nb	–	–	–	–	–	–
PPV-AL/Nb	U	+	+	–	+	+
ISPAVE 31/GF	D	+	+	+	–	–
Grecia/GF	U	+	–	–	+	+
Turchia/GF	U	+	+	–	+	+
Marcus/GF	M	+	–	–	+	+
Peach Al/GF	U	+	–	–	+	+

* Type D (Dideron) or M (Marcus) established by previous DASI-ELISA, IC-PCR and RFLP (T. Candresse and M. Cambra, unpublished information); U = undetermined.
Nc = Nicotiana clevelandii
Nb = Nicotiana benthamiana
GF = Peach seedling GF 305
Ps = Pisum sativum

extremities (Lopez-Moya et al., 1994), would not have been recognized by MAbAL in Western blots.

The specificity of MAbAL for PPV-M was inferred from preliminary tests in which this MAb had recognized a French strain of PPV-M (kindly supplied by Dr. F. Dosba) but not an Italian strain of PPV-D (ISPAVE 31). This specificity was confirmed with a more extensive trial in which MAbAL was tested in DASI-ELISA in comparison with MAb4DG5, and MAb5B, on 130 PPV isolates, 126 of which belonging to one or the other serotype, and representing all the 21 serogroups identified by Cambra et al. (1994). The remaining four isolates (PPV-AL, Grecia, Turchia and Peach Al), from the collection of the IAM-Bari, were of undetermined serotype. The results of these tests were in complete agreement with the expectations, as MAb5B reacted with all PPV isolates, while MAbAL reacted with all isolates that were not recognized by the PPV-D specific MAb4DG5 (29), and viceversa (99), with two exceptions. These were constituted by isolates PPV-AL and Turchia which, contrary to all other sources tested that were pure strain M or D, reacted with all three MAbs.

Twentytwo PPV isolates (Table 1), including PPV-AL and Turchia, were amplified by IC-PCR using the general primers of Wetzel et al. (1991). PCR results with specific primers were in complete agreement with those of serological analysis, with the exception of PPV-AL and Turchia that were amplified only by PPV-M specific primers. We have no explanation for this behaviour which is now being further investigated. However, it does not seem to question the identification of MAbAL as a M-specific reagent.

We have shown that MAbAL is a monoclonal antibody specific to an epitope present on PPV-M but not on PPV-D particles. This MAb reacts with a stable surface determinant which is apparently not affected by the CP subunit conditions, i.e. whether the subunits are still assembled in the virion or dissociated and/or in various stages of degradation, including the 28-kDa proteolysis-resistant core (Lopez-Moya et al., 1994). This seems to make MAbAL particularly suitable for successful use under a wide range of conditions, and completes the panel of PPV-specific MAbs already obtained (Cambra et al., 1994). A new efficient tool is therefore available for the timely identification of PPV-M in the areas that it may invade, and for the accurate monitoring of its distribution in the areas where it already occurs.

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