Production of Monoclonal Antibodies to Potato Virus Y Helper Component-Protease and Their Use for Strain Differentiation

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ABSTRACT

Canto, T., Ellis, P., Bowler, G., and López-Abella, D. 1995. Production of monoclonal antibodies to potato virus Y helper component-protease and their use for strain differentiation. Plant Dis. 79:234-237.

Ten monoclonal antibodies (MAbs) and a rabbit antiserum containing polyclonal antibodies (PAbs) were prepared against helper component-protease (HC-Pro) purified from plants infected with a non-aphid-transmissible strain of potato virus Y (PVY-O NAT). All MAbs reacted with PVY HC-Pro in two enzyme-linked immunosorbent assay (ELISA) protocols, and seven were useful for Western blot analysis. Six MAbs and the PAbs were compared in direct antigen-binding ELISA for reactivity with 22 isolates of PVY, potato virus A (PVA), tobacco etch virus (TEV), and pepper mottle virus (PepMoV). None of the MAbs or the PAbs reacted with PVA or TEV HC-Pro. The immunoreactivity of one MAb was similar to the PAbs, reacting with all 22 PVY isolates and with PepMoV, but failing to react with PVA and TEV HC-Pro. Another MAb reacted with all of the common strains of PVY (PVY^O) and a stipple-streak strain of PVY (PVY^C) but not with any of the tobacco veinal necrosis strains (PVY^N). The other MAbs could be used to differentiate a subgroup within the PVY^O and PVY^N strain groups. These MAbs proved useful for identifying strains of PVY.

Potato virus Y (PVY) is the type species of the proposed *Potyvirus* genus (5,7). This flexuous, rod-shaped virus is composed of a single-stranded, positive-sense RNA encapsidated by about 2,000 copies of a single coat protein (CP). The 9.7-kb genomic RNA has a genome-linked viral protein covalently linked at the 5′ end and a 3′ poly(A) tail (7). The viral genome is expressed as a single polyprotein that is proteolytically processed to generate the final gene products (27). At least three viral proteins with protease activity are involved in this process (9,10,21,33). One of them is the helper component-protease (HC-Pro), a nonstructural protein located in the amino region of the viral polyprotein (4). The HC-Pro is required for aphid transmission (16,20), in addition to the CP (3,4,30).

Most serological methods developed for the diagnosis of viral infection are based on the accumulation of viral particles in the infected plants. Although in vivo regulation of the viral polyprotein processing is still not well understood, potyvirus genetic organization should result in the production of equimolar amounts of every viral protein. In fact, potyviruses induce inclusion bodies in the plant cell related to nonstructural viral proteins. Cytoplasmic cylindrical inclusions are present in all members of the group, and these and other types of inclusions are specific and characteristic for each potyvirus (13,24). In addition, infection by PVY and some other potyviruses induces the production of cytoplasmic amorphous inclusions that are serologically related to the viral HC-Pro (6,12,24).

Several monoclonal antibodies (MAbs) to potyviral nonstructural proteins or to inclusion bodies have been described (17,24). The objective of this study was to produce MAbs against purified PVY HC-Pro and to determine their usefulness in PVY strain identification. A preliminary report has been published (8).

Accepted for publication 25 October 1994.

MATERIALS AND METHODS

Preparation of immunogens. Several potyviruses including PVY, tobacco etch (TEV), and pepper mottle (PepMoV) were used in this work. Two pepper isolates from Spain, PVY-O and PVY-O NAT, a non-aphid-transmissible isolate, were provided by F. Ponz, Centro Nacional de Investigaciones Agrarias (INIA), Madrid, and M. P. Luis Arteaga, Servicio de Investigación Agraria de Aragón, Spain, respectively. Cultures of PVY^N from potato (PVY^N-C3 and -PEI) were supplied by G. Rose, Agricultural Scientific Services, East Craigs, Edinburgh, and R. Stace-Smith, Agriculture and Agri-Food Canada, Vancouver Research Station, respectively. PVY^N-10, -18–21, -25, -33, -36, -37, -54; PVY^O-5, -17, -49, -50; and PVY^C-31 were provided by G. Adams, Biologische Bundesanstalt, Braunschweig, Germany. The cultures of PepMoV (PepMoV-FL and -M) and TEV (TEV-AF and -TN) were supplied by R. Christie, University of Florida, Gainesville.

Viruses were mechanically inoculated and propagated in *Nicotiana tabacum* L. 'Xanthi nc'. At 15–25 days postinoculation, systemically infected plants were used as source material for HC-Pro purification and serological tests. The HC-Pro was purified from plants infected with PVY isolate PVY-O NAT. The HC-Pro was purified by the procedure of Thornbury et al (29) with the following minor modifications: the diethylaminoethyl cellulose column was omitted and 20 mM EDTA was present in buffers at all purification steps. The HC-Pro band, approximately 50 kDa, was excised from sodium dodecyl sulfate (SDS) polyacrylamide gels and used as immunogen (8).

Antiserum. A rabbit antiserum containing polyclonal antibodies (PAbs) against PVY HC-Pro was produced as trapping antibodies for enzyme-linked immunosorbent assay (ELISA) and for Western blot analysis. The HC-Pro immunogen was prepared as described above. A rabbit was injected intramuscularly four times at 2-wk intervals with approximately 0.5 mg of purified HC-Pro eluted from polyacrylamide gels. Immunogen was emulsified with Freund's complete adjuvant (Sigma Chemical Co., St. Louis) for the first injection. Immunogen for subsequent injections was emulsified with Freud's incomplete adjuvant (Sigma).

Hybridoma production and MAb isotyping. Six-week-old female BALB/c mice were injected intraperitoneally (IP) with 150 μg of gradient-purified, virusfree HC-Pro without adjuvant. A second IP injection of about 100 μg of HC-Pro was given 2–3 wk later. Some of the mice were administered a third IP injection of 100 μg of HC-Pro 3–4 wk after the second. Three days after the final injection, the mice were killed by CO_2 asphyxiation, and their spleens were removed aseptically. Spleen cells were fused with FOX-NY myeloma cells (Hyclone Laboratories, Inc., Logan, UT) as described by Ellis and Wieczorek (15).

Hybridoma lines that produced antibodies to PVY HC-Pro were selected by antigen-coated plate-ELISA (ACP-ELISA) (2,18) and triple antibody sandwich-ELISA (TAS-ELISA) (11). All reagents were used at 100 μl per well in 96-well, flat-bottomed Corning assay

plates (Corning Glass, Incorporated, Scientific Products Division, Corning, NY), except in blocking steps, which used 300 µl per well.

In ACP-ELISA, plates were coated overnight at 4 C with the supernatant of an extract from tobacco leaves systemically infected with PVY-O NAT. The sap was diluted 1:10 (v/v) in sodium carbonate buffer, pH 9.6, and centrifuged at 10,000 *g* for 10 min. Sap from healthy tobacco, prepared in the same way, was used as a negative control. After the coating step, wells were filled with blocking buffer, 0.3% (w/v) nonfat powdered milk in phosphate-buffered saline (PBS) (127 mM NaCl, 2.6 mM KCl, 8.5 mM Na_2HPO_4, and 1.1 mM KH_2PO_4, pH 7.4) for 30 min at room temperature. The MAbs in hybridoma cell-culture fluids were mixed with an equal volume of PBS containing 0.1% Tween 20 and 0.3% nonfat powdered milk (PBS-T-M) were added to the wells and incubated for 2 hr at 30 C. The mouse MAbs were detected with alkaline phosphatase-labeled goat anti-mouse antibodies (Jackson Immunoresearch Laboratories, Inc., Avondale, PA). Substrate, *p*-nitrophenyl phosphate at 0.5 mg/ml in 10% diethanolamine buffer, pH 9.8, was added, and absorbance was measured at 405 nm (A_{405}) after a 3 hr incubation at 30 C. The plates were washed four times with distilled water between each step.

In TAS-ELISA, the wells were coated overnight at 4 C with PAbs immunoglobulins diluted to 1 µg/ml in PBS. The same positive and negative controls were used in both ELISA protocols, except that the sap was diluted in PBS-T-M for TAS-ELISA. The steps following sample addition were the same in both assays. Positive antibody-secreting hybridoma lines were cloned twice by limiting dilution. Isotype subclasses were determined by double antibody sandwich-ELISA with a mouse hybridoma subisotyping kit (Calbiochem, Behring Diagnostics, La Jolla, CA.).

Ascitic fluid was produced as described by Ellis and Wieczorek (15) for six of the 10 hybridoma lines. Immunoglobulins were purified from the PAbs by affinity chromatography with Protein G Sepharose 4 Fast Flow (Pharmacia, Uppsala, Sweden) following the manufacturer's protocol.

The MAbs and PAbs raised against HC-Pro of PVY-O NAT were compared in ACP-ELISA with PVY isolates and other potyviruses. Antigen-coated plates were incubated for 2 hr at 30 C with HC-Pro MAb ascitic fluids and purified PAbs immunoglobulin G at 1:2,000 dilution and 1 µg/ml, respectively, in PBS-T-M.

Western blot analysis. Plant extracts were made by grinding fresh leaf tissue (1:10 w/v) in 0.625 M Tris-HCl, pH 6.8, containing 2% SDS and 10% 2-mercaptoethanol. Extracts were prepared from tobacco plants infected with PVY-O, TEV-AF, and PepMoV-FL and uninoculated tobacco plants. The proteins were separated by electrophoresis in 12.5% polyacrylamide gels (19) and transferred to nitrocellulose membranes by the method of Towbin et al (31). Culture and ascitic fluids from 10 and five, respectively, of the hybridoma lines were tested for immunoreactivity with HC-Pro in extracts from PVY-O NAT-infected tobacco. The nitrocellulose membranes were blocked, washed, and incubated with the MAbs or PAbs. Goat anti-mouse peroxidase-conjugated antibody was used as the secondary antibody. The reaction was visualized with the substrate 4-chloronaphthol and hydrogen peroxide.

Serological reaction of PVY and other potyvirus antigens. Immunoreactivity of six PVY HC-Pro MAbs was tested against sap extracts from tobacco plants infected with other strains of PVY and other potyviruses by ACP-ELISA as described above. The test antigens were extracted from plants singly infected with one isolate of PVA, two of TEV, two of PepMoV, 12 of PVY^N, nine of PVY^O, and one of PVY^C. Anti-HC-Pro PAbs was included as a control. A test was considered positive when the A_{405nm} was greater or equal to three times the mean A_{405nm} of 10 control healthy plant extracts.

RESULTS

Production of MAbs to PVY HC-Pro. Spleens of three immunized mice were used in three independent fusions. Of the approximately 12,000 possible hybridoma cell lines assayed, 12 were identified that produced antibodies specific to PVY HC-Pro. Ten of these were cloned by limiting dilution, and their isotypes were determined (Table 1). In both ACP- and TAS-ELISA, all 10 MAbs reacted with extracts from PVY-infected plants but not with healthy plant extracts. In Western blot analysis, seven MAbs reacted with a band of about 50 kDa (Table 1).

Reactivity of PVY HC-Pro MAbs and PAbs with potyvirus antigens in ELISA. Neither the MAbs nor the PAbs reacted with antigens in extracts of PVA- or TEV-infected plants. One MAb, 1A11, reacted with antigens in extracts of both isolates of PepMoV-infected plants and all isolates of PVY-infected plants (Table 2). The PAbs reacted with antigens in extracts from PVY-infected plants but only with antigens from one of the two PepMoV isolates. MAb 8B9 reacted with antigen in extracts from all of the PVY-infected plants but not with antigens from plants infected with the other potyviruses. Another MAb, 8E1, reacted with an antigen in extracts from all of the PVY^O- and PVY^C-infected plants but not with antigens from plants infected with isolates of PVY^N or other potyviruses. Three MAbs, 5B10, 1B1, and 8B3, differentiated a subgroup within PVY^N. One MAb, 1B1, also distinguished a subgroup within the PVY^O strain group.

Western blot analyses. Seven of 10 MAbs and the PAbs reacted with a band of about 50 kDa in Western blot analysis of HC-Pro in extracts of plants infected with PVY-O. MAbs 7E7, 8E1, and 2C6 did not react with the HC-Pro band of PVY-O NAT or with any other band (Table 1).

In a Western blot analysis using five MAbs and the PAbs, four of five MAbs and the PAbs reacted with HC-Pro (Fig. 1). Some bands with lower molecular weights also were detected.

DISCUSSION

The usefulness of the antigenic properties of cytoplasmic inclusions for clas-

Table 1. Characteristics of monoclonal antibodies (MAbs) produced by immunization of BALB/c mice with potato virus Y (PVY) helper component-protease (HC-Pro)

		Immunoreactivity[a]				
		ACP-ELISA[c]		TAS-ELISA[d]		Western
MAbs	Subclass	PVY HC-Pro	Healthy	PVY HC-Pro	Healthy	blot[b]
5B10	IgG 1	+	−	+	−	+
8B3	IgG 1	+	−	+	−	+
8B9	IgG 1	+	−	+	−	+
7E7	IgG 1	+	−	+	−	−
8E1	IgG 1	+	−	+	−	−
7G7	IgG 1	+	−	+	−	+
1B1	IgG 3	+	−	+	−	+
1A11	IgM	+	−	+	−	+
2C6	IgM	+	−	+	−	−
3G5	IgM	+	−	+	−	+

[a] +, enzyme-linked immunosorbent assay (ELISA) reaction = A_{405nm} reading greater than or equal to three times the mean of 10 healthy extracts. Experiments were repeated four times. −, ELISA reaction = A_{405nm} reading less than three times the mean of 10 healthy extracts.
[b] + = a visible reaction with a precipitating substrate to the 50-kDa band of the PVY HC-Pro purified from tobacco infected with PVY-O NAT.
[c] Antigen-coated plate-ELISA.
[d] Triple antibody sandwich-ELISA.

sification and identification of potyviruses was demonstrated more than 20 yr ago (25). More recently, the potential use of MAbs to nonstructural virus proteins in potyvirus taxonomy has been recognized but not demonstrated (17,24). In this study we have shown that MAbs prepared with PVY HC-Pro are useful both as virus-specific probes and for distinguishing between common (PVY^O) and tobacco veinal necrosis (PVY^N) strains as well as between subgroups of these strains.

A major difficulty in potyvirus serology is presented by viruses such as PepMoV and PVY, which appear to be strains of the same virus on the basis of their CP structure (28) but which can be differentiated by cytopathology, a differential host range, and serological differences in both the CP and cytoplasmic inclusion body proteins (1,12, 23,25,26). Recent evidence based on the deduced amino acid sequence of the CP of a California isolate of PepMoV, combined with a comparison of the 3′ untranslated region nucleic acid sequence, supports serological and biological evidence that PepMoV and PVY are distinct viruses (32). The serological results obtained in this study, using ACP-ELISA and PVY HC-Pro MAbs, supports the evidence that PepMoV and PVY are related but distinct viruses. One MAb, 1A11, reacted with HC-Pro from PVY and PepMoV in both Western blot analysis and ACP-ELISA. De Mejia et al (12) also reported cross-reactivity between PepMoV and PVY in Western blot tests with an antiserum prepared against HC-Pro of PepMoV.

The primary advantage of Western blot analysis over ELISA is that antibody reactivity can be corroborated with proteins or polypeptides of a particular molecular weight, even in relatively crude plant extracts. In this study, Western blot analysis was used to confirm the ELISA results and identify the antigen as a protein with a molecular weight that corresponded to that of PVY HC-Pro. Although useful for characterizing MAbs, Western blot analysis is too complex and time-consuming for routine virus detection and identification. Further, Western blot analysis may be complicated by the presence of some bands with lower molecular weights than PVY HC-Pro (Fig. 1). These lower molecular weight bands are likely protein fragments resulting from limited proteolysis of HC-Pro when exposed to endogenous plant proteolytic enzymes.

Using a panel of MAbs raised against the CPs of several potyviruses, Jordan and Hammond (18) found that PVY and PepMoV can be differentiated by more epitopes than are conserved between them. Although based on a nonstructural protein, our work provides results similar to those based on CP serology. For example, MAb 1A11 reacted with the only conserved epitope on the HC-Pro of PepMoV and PVY identified in this study. Although this may suggest a distant serological relationship between these two viruses, the epitope was not conserved on either PVA or TEV. However, without an extensive panel of MAbs raised against the HC-Pro of several potyviruses, the degree of relatedness of these four viruses cannot be clearly defined based on the HC-Pro alone. Despite the relatively small panel of useful MAbs, our results clearly demonstrate the value of serology of nonstructural virus proteins in addition to CP-serology in taxonomic studies of the potyvirus group.

Table 2. Immunoreactivity of six anti-potato virus Y (PVY) helper component-protease (HC-Pro) monoclonal antibodies (MAbs) when tested against HC-Pro in extracts of strains of PVY- and other potyvirus-infected plants

Virus Strains	ELISA[a] reaction with MAbs[b]						
	5B10	1B1	8E1	1A11	8B3	8B9	PAb
Potato virus A							
PVA-CAN[c]	0.032	0.022	0.022	0.028	0.022	0.026	0.068
Tobacco etch virus							
TEV-AF[d]	0.026	0.020	0.022	0.029	0.020	0.023	0.030
TEV-TN[d]	0.022	0.020	0.020	0.022	0.019	0.021	0.020
Pepper mottle virus							
PepMoV-FL[d]	0.043	0.026	0.031	0.429	0.025	0.026	0.243
PepMoV-M[d]	0.046	0.029	0.034	0.537	0.028	0.031	0.624
Potato virus Y							
PVYN-C3[e]	0.057	0.026	0.030	0.206	0.032	0.221	0.549
PVYN-10[f]	0.048	0.044	0.028	0.251	0.039	0.293	0.769
PVYN-18[f]	0.069	0.095	0.033	0.276	0.042	0.715	0.947
PVYN-21[f]	0.063	0.084	0.033	0.304	0.075	0.787	1.284
PVYN-25[f]	0.051	0.042	0.029	0.272	0.052	0.487	1.062
PVYN-33[f]	0.077	0.054	0.027	0.262	0.067	0.629	0.879
PVYN-54[f]	0.072	0.057	0.036	0.243	0.063	0.408	0.860
PVYN-PEI[e]	0.094	0.076	0.037	0.334	0.078	0.727	1.709
PVYN-19[f]	0.440	0.467	0.053	0.238	0.460	2.080	2.361
PVYN-20[f]	0.266	0.254	0.060	0.258	0.752	1.657	2.085
PVYN-36[f]	0.477	0.324	0.044	0.348	0.450	1.921	2.214
PVYN-37[f]	0.296	0.260	0.035	0.226	0.254	1.132	1.290
PVYO-BC[e]	0.753	0.028	0.271	0.946	0.284	0.169	0.881
PVYO-17[f]	1.368	0.031	0.559	1.574	0.739	0.423	1.302
PVYO-49[f]	0.764	0.027	0.227	0.840	0.264	0.181	0.872
PVYO-50[f]	0.863	0.072	0.319	1.957	0.409	0.485	1.342
PVY-O[g]	0.929	1.059	0.806	2.602	0.411	1.331	1.398
PVY-O NAT[h]	1.145	1.300	1.223	2.450	0.575	1.605	1.454
PVYO-NC78[i]	0.742	0.192	0.283	0.508	0.245	0.616	2.001
PVYO-NC138[i]	1.281	0.616	2.486	2.939	1.209	2.359	2.912
PVYO-5[f]	1.035	0.889	0.764	1.906	0.437	1.193	1.182
PVYC-31[f]	1.153	1.070	0.286	2.885	0.587	2.070	1.390
Healthy controls[j]	0.037	0.035	0.029	0.061	0.032	0.024	0.133

[a] Enzyme-linked immunosorbent assay (ELISA) reaction (A_{405nm}) after 3 hr incubation of substrate at 30 C.
[b] Normal numbers = negative test; mean value of four tests <3× mean of 10 healthy control plants. Underlined numbers = positive test; mean value of four tests ≥3× mean of 10 healthy control plants.
[c] R. Stace-Smith, Agriculture Canada, Vancouver, BC.
[d] R. Christie, University of Florida, Gainesville.
[e] G. Rose, Agricultural Scientific Services, East Craigs, Edinburgh.
[f] G. Adams, Biologische Bundesanstalt, Braunschweig, Germany.
[g] F. Ponz, Centro Nacional de Investigaciones Agrarias (INIA), Madrid.
[h] M. P. Luis-Arteaga, Servicio de Investigación Agraria de Aragón, Spain.
[i] G. Gooding, North Carolina State University, Raleigh.
[j] Mean of four test results using healthy *Nicotiana tabacum* cv. Xanthi nc.

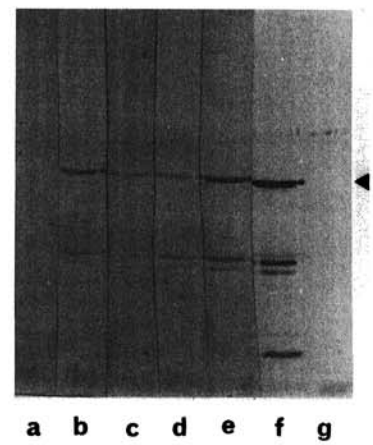

Fig. 1. Western blot analysis with monoclonal antibodies (MAbs; lanes a–e) and a polyclonal rabbit antiserum (lanes f–g) and leaf extracts from potato virus Y (PVY-O)-infected tobacco (lanes a–f) and uninoculated tobacco (lane g). MAbs: lane a, 8E1; lane b, 8B9; lane c, 8B3; lane d, 5B10; and lane e, 1B1. The arrow indicates the position of the helper component-protease.

In addition to their taxonomic value, these MAbs also have potential diagnostic uses. Although MAbs to PVY CP are valuable for detection and identification of strains of PVY (14), they have occasionally failed to correctly identify some PVY strains when used in testing for potato certification and quarantine applications (22). The PVY HC-Pro MAbs provide the basis for a second serological procedure for PVY strain identification independent of the CP. For example, MAb 8B9 reacted with antigen in extracts from all of the isolates of PVY-infected plants, which included members of all three strain groups. On the other hand, MAb 8E1 reacted only with isolates belonging to the PVY^O and PVY^C strain groups. This MAb could be used to help identify isolates of PVY^O that cross-react with PVY^N-specific CP MAbs currently used in quarantine and regulatory work (22). Although considerable emphasis has been placed on the properties of the CP, it now appears that potyvirus nonstructural proteins have a significant value in the classification of potyviruses (24). A method for PVY strain differentiation based on antibodies to both the HC-Pro and the CP used together may provide a more reliable diagnostic method than strain differentiation based on the CP alone.

LITERATURE CITED

1. Abdalla, O. A., Desjardins, P. R., and Dodds, J. A. 1991. Identification, disease incidence, and distribution of viruses infecting peppers in California. Plant Dis. 75:1019-1023.
2. Al Moudallal, Z., Altschuh, D., Briand, J. P., and Van Regenmortel, M. H. V. 1984. J. Immunol. Methods 68:35-43.
3. Atreya, C. D., Raccah, B., and Pirone, T. P. 1990. A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. Virology 178:161-165.
4. Atreya, C. D., Thornbury, P. L., and Pirone, T. P. 1992. Site-directed mutations in the potyvirus HC-Pro gene affect helper component activity, virus accumulation and symptom expression in infected tobacco plants. Virology 191:106-111.
5. Barnett, O. W. 1992. A summary of potyvirus taxonomy and definitions. Arch. Virol. Suppl. 5:435-444.
6. Baunoch, D. A., Das, P., and Hari, V. 1990. Potato virus Y helper component is associated with amorphous inclusions. J. Gen. Virol. 71:2479-2482.
7. Brunt, A. A. 1992. The general properties of potyviruses. Arch. Virol. Suppl. 5:3-16.
8. Canto, T., López-Moya, J. J., Ellis, P. J., Serra, M. T., López-Abella, D., and Diáz-Ruiz, J. R. 1993. Immuno-electrophoretic analysis of potato virus Y (PVY) helper component (HC) protein. Page 349 in: Abstr. 9th Int. Congr. Virol. Glasgow, Scotland.
9. Carrington, J. C., Cary, S. M., Parks, T. D., and Dougherty, W. G. 1989. A second proteinase encoded by a plant potyvirus genome. EMBO J. 8:365-370.
10. Carrington, J. C., and Dougherty, W. G. 1987. Small nuclear inclusion protein encoded by a plant potyvirus genome is a protease. J. Gen. Virol. 61:2540-2548.
11. Converse, R. H., and Martin, R. R. 1990. ELISA methods for plant viruses. Pages 179-196 in: Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens: A Laboratory Manual. R. Hampton, E. Ball, and S. De Boer, eds. American Phytopathological Society, St. Paul, MN.
12. De Mejia, M. V. G., Hiebert, E., Purcifull, D. E., Thornbury, D. W., and Pirone, T. P. 1985. Identification of potyviral amorphous inclusion protein as a nonstructural virus-specific protein related to helper component. Virology 142:34-43.
13. Edwardson, J. R. 1992. Inclusion bodies. Arch. Virol. Suppl. 5:25-30.
14. Ellis, P. J., Bowler, G., MacKenzie, D. J., and Stace-Smith, R. 1992. Monoclonal antibodies for detection and identification of strains of potato virus Y in Canada and the United States. (Abstr.) Can. J. Plant Pathol. 14:241.
15. Ellis, P. J., and Wieczorek, A. 1992. Production of monoclonal antibodies to beet Western yellows virus and potato leafroll virus and their use in luteovirus detection. Plant Dis. 76:75-78.
16. Govier, D. A., Kassanis, B., and Pirone, T. P. 1974. A virus induced component of plant sap needed when aphids acquire potato virus Y from purified preparations. Virology 61:410-426.
17. Jordan, R. 1992. Potyvirus, monoclonal antibodies and antigenic sites. Arch. Virol. Suppl. 5:81-95.
18. Jordan, R., and Hammond, J. 1991. Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific, and potyvirus group common epitopes using monoclonal antibodies. J. Gen. Virol. 72:25-36.
19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
20. Lecoq, H., Bourdin, D., Raccah, B., Hiebert, E., and Purcifull, D. E. 1991. Characterization of a zucchini yellow mosaic virus isolate with a deficient helper component. Phytopathology 81:1087-1091.
21. Mavankal, G., and Rhoads, R. E. 1992. In vitro cleavage at or near the amino terminus of the helper component protein of the tobacco vein mottling virus polyprotein. Virology 185:721-731.
22. McDonald, J. G., Kristjansson, G. T., Singh, R. P., Ellis., P. J., and McNab, W. B. 1993. Consecutive ELISA screening with monoclonal antibodies to detect potato virus Y^N. Am. Potato J. 71:175-183.
23. Nelson, M. R., and Wheeler, R. E. 1978. Biological and serological characterization and separation of potyviruses that infect peppers. Phytopathology 68:979-984.
24. Purcifull, D. E., and Hiebert, E. 1992. Serological relationships involving potyviral nonstructural proteins. Arch. Virol. Suppl. 5:97-122.
25. Purcifull, D. E., Hiebert, E., and McDonald, J. G. 1973. Immunochemical specificity of cytoplasmic inclusions induced by viruses in the potato Y group. Virology 55:275-279.
26. Purcifull, D. E., Zitter, T. A., and Hiebert, E. 1975. Morphology, host range, and serological relationships of pepper mottle virus. Phytopathology 65:559-562.
27. Riechmann, J. L., Lain, S., and Garcia, J. A. 1992. Highlights and prospects of potyvirus molecular biology. J. Gen. Virol. 73:1-16.
28. Shukla, D. D., and Ward, C. W. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69:2703-2710.
29. Thornbury, D. W., Hellmann, G. M., Rhoads, R. E., and Pirone, T. P. 1985. Purification and characterization of potyvirus helper component. Virology 144:260-267.
30. Thornbury, D. W., Patterson, C. A., Dessens, J. T., and Pirone, T. P. 1990. Comparative sequence of the helper component (HC) region of potato virus Y and a HC-defective strain, potato virus C. Virology 178:573-578.
31. Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. (USA) 76:4350-4354.
32. Vance, V. B., Jordan, R., Edwardson, J. R., Christie, R., Purcifull, D. E., Turpen, T., and Falk, B. 1992. Evidence that pepper mottle virus and potato virus Y are distinct viruses: analyses of the coat protein and 3′ untranslated sequence of a California isolate of pepper mottle virus. Arch. Virol. Suppl. 5:337-345.
33. Verchot, J., Koonin, E. V., and Carrington, J. C. 1992. The 35 kDa protein from the amino terminus of the potyviral polyprotein processing functions as a third virus encoded proteinase. Virology 185:527-535.