Etiology

Differentiation of Soybean Mosaic Virus Isolates by One-Dimensional Trypsin Peptide Maps Immunoblotted with Monoclonal Antibodies

J. H. Hill, H. I. Benner, T. A. Permar, T. B. Bailey, R. E. Andrews, Jr., D. P. Durand, and R. A. Van Deusen

First three and last authors: Department of Plant Pathology; fourth author: Department of Statistics; fifth and sixth authors: Department of Microbiology, Iowa State University, Ames 50011.

Journal Series Paper J-13243 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project No. 2700. Supported in part by the Iowa Biotechnology Grants Program and Pioneer Hi-Bred International, Inc.

Accepted for publication 22 June 1989 (submitted for electronic processing).

ABSTRACT

Hill, J. H., Benner, H. I., Permar, T. A., Bailey, T. B., Andrews, R. E., Jr., Durand, D. P., and Van Deusen, R. A. 1989. Differentiation of soybean mosaic virus isolates by one-dimensional trypsin peptide maps immunoblotted with monoclonal antibodies. Phytopathology 79:1261-1265.

Twelve monoclonal antibodies were used to differentiate 11 isolates of soybean mosaic virus by immunoblot analysis of one-dimensional trypsin peptide maps of the virion capsid protein. Although all isolates showed unique patterns that reflected epitopic specificity of the viral coat protein, the virus isolates formed three distinct groups. These results suggest potential for using this approach to study antigenic drift and plant virus epidemiology.

Additional keywords: potyviruses, serology.

Strains of the same plant virus can be differentiated on the basis of symptoms on inoculated differential host plants. Differences in vector and serologic specificity also are commonly used for this purpose (e.g., 12). Serologic specificity has generally been documented by using polyclonal antibodies. However, some strains of plant viruses, which are clearly distinguished on the basis of biological criteria, have been difficult to differentiate by using polyclonal antibodies.

For example, early studies, using polyclonal antiserum, were unsuccessful in demonstrating antigenic diversity among strains of soybean mosaic virus (SMV) (9,13). Recent studies in this laboratory, using polyclonal antiserum against SMV, suggest the presence of serologic diversity (1,11). These studies were limited, however, because of the polyspecific nature of the antiserum.

The specific recognition of unique epitopes on a virus capsid protein by monoclonal antibodies provides a useful means to obviate this difficulty. Although differential reactions in conventional immunosorbent assays using monoclonal antibodies can demonstrate antigenic diversity of virus isolates (4), we decided to test the possibility that a combination of one-dimensional peptide mapping and immunoblotting would enhance the potential for identification of strain differences. Therefore, we used a panel

of monoclonal antibodies to examine the antigenic diversity of SMV strains by immunoblot analysis and one-dimensional peptide mapping (3) of virus protein partially digested with a specific protease. The data demonstrate considerable antigenic diversity among SMV strains.

MATERIALS AND METHODS

Source of virus and monoclonal antibodies. One SMV isolate was selected from each of the seven strain groups identified by Cho and Goodman (2) from analysis of pathogenic variation from a total collection of 98 isolates. For purposes of this study, these selected isolates are referred to as isolates G1–G7. Additional isolates included the 12-18 and 0 isolates differentiated by aphid transmission (9), an SMV isolate from Brazil obtained from M. T. Lin, and the well-characterized Ia 75-16-1 isolate (6) previously identified as belonging to strain group G2 (?, *unpublished*). All SMV isolates were purified from infected *Glycine max* (L.) Merr. 'Williams' using previously described methods (6).

The monoclonal antibodies S1 and S2 have been described previously (5). The other monoclonal antibodies described herein were generated by using the Ia 75-16-1 isolate of SMV as the immunogen according to previously described procedures (4,5). The isotype class and subclass as well as light-chain components were determined by enzyme-linked immunosorbent assay (ELISA) using specific rabbit antimouse immunoglobulins (Zymed Laboratories, San Francisco, CA).

Peptide mapping. Purified virion preparations were dialyzed against 0.125 M Tris-HCl, pH 7.6, containing 0.1% 2-mercaptoethanol. Virion concentrations were determined by the method of Spector (14) using bovine serum albumin as a standard. After concentrations of all virus isolates were adjusted to 1.2 mg/ml, protein subunits were obtained by heating virus preparations at 100 C for 20 min with equal volumes of 1% sodium dodecyl sulfate (SDS) and 1% 2-mercapthethanol in 0.125 M Tris-HCl, pH 6.8, and then chilling in an ice bath. Protein subunit samples (300 μl) were digested with trypsin, type XIII, treated with L-1-tosylamide-2-phenylethyl chloromethyl ketone (60 μl of 0.5 mg/ml prepared in 0.125 M Tris-HCl, pH 7.6) (No. T8642, Sigma Chemical Co., St. Louis, MO) for 15 hr at 37 C. Proteolysis was terminated by heating at 100 C for 5 min, followed by immersion in an ice bath.

Peptides were separated by electrophoresis on discontinuous SDS 10–20% polyacrylamide gradient gels prepared in 0.375 M Tris-HCl, 0.1% SDS, and 0.001 M ethylenediaminetetraacetic acid (EDTA), pH 8.8. A 5% polyacrylamide stacking gel was prepared in 0.125 M Tris-HCl, 0.1% SDS, pH 6.8 (sample buffer). Immediately before electrophoresis, 15 μl of each sample was mixed with an equal volume of sample buffer containing 0.002% bromophenol blue, 2% SDS, 2% 2-mercaptoethanol, and 30% glycerol. Electrophoresis, in 0.025 M Tris-HCl, 0.192 M glycine, 0.1% SDS, 0.001 M EDTA, pH 8.3, was carried out at 25 mA (constant current) until the tracking buffer passed through the stacking gel; then the current was increased to 35 mA.

Immunoblotting of peptides. Polypeptides were transferred to nitrocellulose paper (0.45 μm, Millipore Corp., Bedford, MA) in 0.025 M Tris-HCl, 0.192 M glycine, pH 8.3, containing 20% methanol in a Trans-blot cell (Bio-Rad Laboratories, Richmond, CA) at 60 V for 3 hr. Nitrocellulose was blocked for 4 hr at 37 C with 0.05 M Tris-HCl, pH 7.4, containing 0.85% NaCl and 0.05% Tween 20 (TBS-Tween), and 3% gelatin. Blots were probed with monoclonal antibodies in either culture medium (approximately 1:10 in TBS-Tween containing 1% gelatin) or ascitic fluid (approximately 1:200 in TBS-Tween containing 1% gelatin) for 2 hr at 37 C. After being rinsed three times with TBS-Tween over a period of 30 min, the nitrocellulose blots were incubated for 1 hr at 20 C in a 1:1,000 dilution of alkaline phosphatase-conjugated anti-mouse IgG (Sigma, No. A5153) in TBS-Tween containing 1% gelatin. Reactions were detected with nitro blue tetrazolium according to Leary et al (8). After the blots were incubated in the substrate solution for 15 min, they were washed extensively in distilled water to terminate color development.

After immunoblots were photographed, the photographic negative of lanes corresponding to each SMV isolate was scanned at 700 nm using a linear transport coupled to a monochrometer. The molecular weights of all polypeptides were calculated by regression analysis with reference to molecular weight standards run in each gel.

If no immunoreactivity was observed between a polypeptide and a monoclonal antibody, a value of 0 was assigned, whereas a value of 1 was assigned to instances of positive immunoreactivity. The resulting data matrix then was analyzed by principal component analysis (10) to summarize the data in two dimensions and reveal groupings among 11 SMV isolates on the basis of immunoreactivity patterns of the various polypeptides.

TABLE 1. Reaction of monoclonal antibodies with polypeptides from tryptic digests of capsid protein of soybean mosaic virus isolate G1, G2, and G3 as determined in immunoblotting experiments

Mol. wt.	G1												G2												G3											
($\times 10^3$)	1[a]	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
27.1			+			+	+		+		+	+		+	+		+	+	+		+		+	+		+	+		+	+	+		+		+	
25.2	+				+		+						+				+											+								+
23.8			+		+		+				+				+		+		+		+		+				+		+		+		+		
22.5			+		+	+					+						+	+									+		+	+						
22.1																					+												+			
21.5	+		+	+		+	+	+					+		+			+	+	+					+			+		+	+	+				
20.8															+												+									
19.5		+	+	+		+			+	+						+		+		+		+				+						+	+		+
18.6			+		+		+		+		+	+			+	+	+		+		+		+	+			+	+	+		+		+	+	+
17.5			+	+	+	+	+	+	+						+		+	+		+	+		+				+		+	+	+	+	+	+	+	
16.6				+		+		+			+	+				+				+			+	+				+		+		+			+	+
15.4		+	+		+	+	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+
15.0																																				
14.4					+		+	+	+	+							+	+	+	+	+								+		+	+		+		
13.5			+	+	+				+		+	+			+	+	+				+						+	+	+				+		+	+
12.6			+	+		+	+	+		+		+			+	+		+	+	+				+			+	+		+	+	+		+		+
11.6			+	+	+		+		+		+				+	+	+		+		+						+	+	+		+		+			
11.0																						+												+		
10.8				+												+															+					
9.4				+																																
8.2																+																				
7.5				+												+																				

[a] Arabic numerals designate monoclonal antibodies S1, S2, etc.

RESULTS AND DISCUSSION

Characterization of monoclonal antibodies. Antibodies used in these studies were designated S1–S12, and all were of the IgG class. Monoclonal antibodies S3 and S7 were IgG1; the remainder were IgG2a.

Timing of digestion of virion capsid protein with protease. In preliminary experiments, virion capsid proteins were digested for different periods with varying concentrations of thermolysin, papain, V-8 protease of *Staphylococcus aureus*, or trypsin and analyzed on SDS-gradient gels followed by Western blots stained with amido black. Results of preliminary experiments demonstrated that maximum differentiation of polypeptides was obtained by digestion with trypsin for 15 hr (data not shown).

Interaction between virion capsid proteins and monoclonal antibodies. Twenty-two polypeptides were identified by reactions with the panel of monoclonal antibodies used in this study (Tables 1–4). For a specific polypeptide, apparent molecular weights varied from 1 to 2% across all experiments. No reactions were observed between trypsin and any of the antibodies or between any polypeptides and anti-mouse IgG conjugated with alkaline phosphatase (data not shown). The immunoreactive pattern produced by monoclonal antibody S6 is shown in Figure 1 as an example.

Almost all the polypeptides obtained from each virus isolate were detected by the panel of monoclonal antibodies. However, the 15,020-MW polypeptide from isolate G7 was detected only by monoclonal antibody S9. Additionally, several polypeptides

TABLE 2. Reaction of monoclonal antibodies with polypeptides from tryptic digests of capsid protein of soybean mosaic virus isolates G4, G5, and G6 as determined in immunoblotting experiments

Mol. wt.	G4												G5												G6											
($\times 10^3$)	1[a]	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
27.1			+		+	+	+		+		+	+			+		+	+	+				+	+			+		+	+	+		+		+	
25.2				+								+				+		+										+		+						
23.8			+		+		+		+						+				+								+		+		+					
22.5					+	+											+			+								+		+	+					
22.1									+																											
21.5	+		+	+		+	+	+					+		+	+		+	+	+					+			+				+	+			
20.8			+												+		+																			
19.5			+	+								+			+		+			+	+						+					+		+		
18.6			+		+				+			+			+	+	+		+		+						+	+	+		+		+			+
17.5			+		+	+	+	+	+		+				+		+	+	+	+	+						+		+	+	+	+	+		+	
16.6				+		+		+			+					+		+		+							+		+		+			+	+
15.4	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+				+		+	+	+	+	+		+	+	+	+
15.0																																				
14.4					+		+	+					+				+	+	+	+	+	+						+		+	+	+				
13.5			+	+	+				+			+			+	+	+			+	+						+	+	+				+			
12.6			+	+		+	+	+		+		+			+	+		+	+	+							+			+	+	+		+		+
11.6			+	+	+		+		+	+					+	+	+		+		+	+					+	+	+		+		+			
11.0										+												+												+		
10.8				+				+								+				+		+						+				+				
9.4																+												+				+				
8.2				+																								+								
7.5				+												+												+								

[a] Arabic numerals designate monoclonal antibodies S1, S2, etc.

TABLE 3. Reaction of monoclonal antibodies with polypeptides from tryptic digests of capsid protein of soybean mosaic virus isolates G7, Brazil, and 75-16-1 as determined in immunoblotting experiments

Mol. wt.	G7												Brazil												75-16-1											
($\times 10^3$)	1[a]	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
27.1		+	+		+	+	+		+		+	+			+		+	+	+		+		+				+		+	+	+					
25.2				+		+						+				+		+										+								
23.8			+		+		+				+				+		+		+				+				+		+		+		+			
22.5					+	+					+						+	+												+		+				
22.1									+																								+			
21.5	+		+	+			+	+					+			+				+					+		+	+		+	+					
20.8			+								+				+												+									
19.5		+	+	+				+		+		+				+		+		+	+							+		+			+			
18.6			+		+		+		+		+	+			+		+		+		+						+	+	+	+	+		+			
17.5			+		+	+	+	+	+		+				+	+		+	+	+	+		+				+		+	+	+	+	+			
16.6				+		+		+			+	+				+													+		+		+			
15.4	+	+	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+			+		+	+	+	+	+	+	+			
15.0										+																										
14.4					+		+	+	+	+							+	+	+			+			+				+	+	+	+	+	+		
13.5			+	+	+						+				+	+	+						+				+	+	+							
12.6			+	+		+	+	+							+	+		+	+								+	+		+	+	+				
11.6			+	+	+		+		+						+	+						+					+	+	+		+					
11.0																						+												+		
10.8				+				+								+												+	+			+				
9.4																												+								
8.2				+																								+								
7.5																+																				

[a] Arabic numerals designate monoclonal antibodies S1, S2, etc.

were detected in digests from most, but not all, virus isolates, as illustrated by the 22,100-MW polypeptide, which was detected by at least one monoclonal antibody in digests from isolates G2, G3, G4, G7, Ia 75-16-1, 12-18, and 0 but not in digests from isolates G1, G5, G6, and Brazil. The inability of a monoclonal antibody to react with a specific polypeptide may be caused either by the absence of a specific epitope in that peptide or by the absence of the polypeptide in the tryptic digest.

The data reflect both diversity and conservation of epitopes on polypeptides resulting from digestion. All SMV isolates reacted similarly with monoclonal antibodies S3, S4, S5, and S7. A direct comparison of monoclonal antibodies S3, S5, and S7 suggests that these monoclonal antibodies react with the same or closely related epitopes on all SMV isolates. Monoclonal antibody S4, although similar to monoclonal antibodies S3, S5, and S7 in its reaction pattern to midrange-molecular-weight polypeptides, is primarily distinguished by its reaction with low-molecular-weight polypeptides. Other monoclonal antibodies such as S6 (Fig. 1) and S11 illustrate a unique reaction pattern with many of the virus isolates. Although each SMV isolate could be uniquely differentiated by its immunoreactive pattern of capsid protein polypeptides with the various antibodies, principal component analysis of the data suggested that the 11 SMV isolates could be placed into three groups (Fig. 2).

These data show that it is possible to uniquely identify SMV isolates on the basis of immunoreactive patterns of proteolyzed capsid proteins. Investigations of virus epidemiology might be enhanced if a virus isolate could be monitored on the basis of a strain-specific epitope (without epitope mutation) through the progression of a pandemic. Monoclonal antibodies S1–S12 used in conventional ELISA, without immunological analysis of one-dimensional peptide maps of SMV capsid proteins, were not sufficiently discriminating to discern the antigentic differences found in this study (?, *unpublished*). Although the methods employed in this study are not currently adaptable to rapid analysis

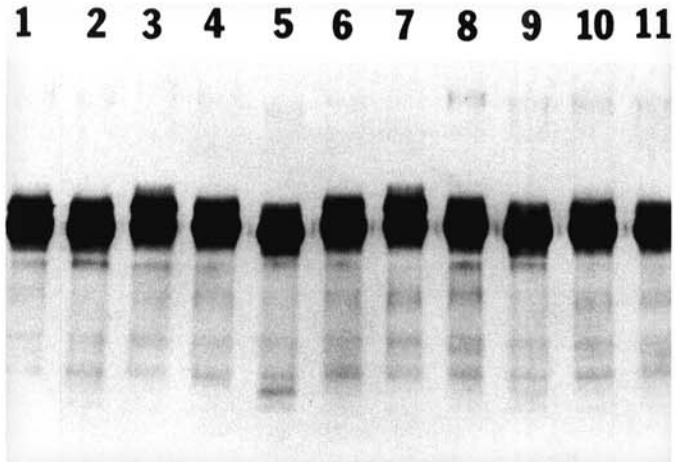

Fig. 1. Pattern produced by monoclonal antibody S6 from immunoblot analysis of one-dimensional trypsin peptide mapping of capsid proteins from soybean mosaic virus strains G1 (lane 1), G2 (lane 2), G3 (lane 3), G4 (lane 4), G5 (lane 5), G6 (lane 6), G7 (lane 7), Brazil (lane 8), 75-16-1 (lane 9), 12-18 (lane 10), and 0 (lane 11).

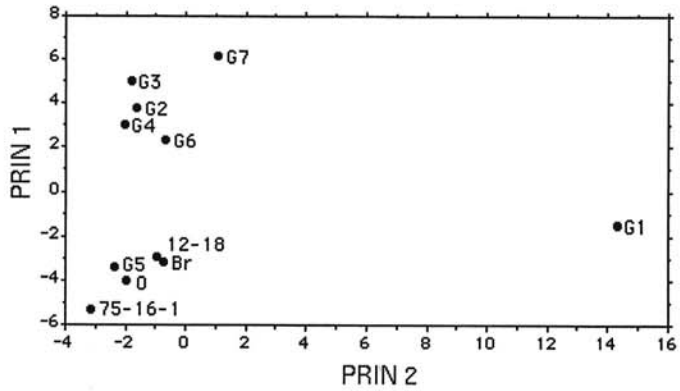

Fig. 2. Plot of the first (PRIN 1) and second (PRIN 2) principal components revealing grouping of 11 soybean mosaic virus isolates on the basis of immunoreactivity patterns of capsid protein polypeptides with 12 monoclonal antibodies. Isolates are designated G1–G7, Br (Brazil), 75-16-1, 0, and 12-18. The first two components accounted for 23.5 and 16.7% of the total variation observed, respectively.

TABLE 4. Reaction of monoclonal antibodies with polypeptides from tryptic digests of capsid protein of soybean mosaic virus isolates 12-18 and 0 as determined in immunoblotting experiments

Mol. wt. ($\times 10^3$)	12-18												0											
	1[a]	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
27.1			+		+	+	+		+		+				+		+	+	+				+	
25.2				+												+		+						
23.8			+				+								+		+		+		+			
22.5			+		+	+		+							+			+		+				
22.1									+												+			
21.5	+			+		+	+	+					+			+			+					
20.8					+										+		+							
19.5			+	+					+	+		+				+								+
18.6			+	+	+		+		+						+	+	+		+		+		+	
17.5	+		+	+	+	+	+	+	+		+		+		+		+		+	+	+			
16.6				+		+		+								+		+		+				
15.4	+		+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+
15.0																								
14.4					+	+	+	+									+	+	+		+	+		
13.5			+	+	+			+				+			+	+	+			+				+
12.6			+	+		+	+			+					+	+		+	+					
11.6			+	+	+		+			+					+	+	+		+			+		
11.0										+														
10.8				+				+								+				+				
9.4				+																				
8.2																								
7.5				+																				

[a] Arabic numerals designate monoclonal antibodies S1, S2, etc.

of virus samples from the field, the data clearly demonstrate variation among virus isolates. Attempts are now being made in our laboratory to develop methods that will be useful in the field and have sufficient discrimination to reflect the differences reported here.

Additionally, the occurrence of antigenic drift has not been demonstrated unequivocally with plant viruses, but recent work with maize dwarf mosaic virus has suggested that it may occur (7). The ability to differentiate single virus isolates by unique antigenic patterns makes it possible to detect changes in amino acid sequence that alter epitope binding patterns by specific antibody. These changes may have biological significance if alterations in specific antibody recognition are coincident with changes in exposed regions (N- and C-termini) of the capsid protein that affect aphid-transmission specificity.

LITERATURE CITED

1. Chen, L-C., Durand, D. P., and Hill, J. H. 1982. Detection of pathogenic strains of soybean mosaic virus by enzyme-linked immunosorbent assay with polystyrene plates and beads as the solid phase. Phytopathology 72:1177-1181.
2. Cho, E. K., and Goodman, R. M. 1979. Strains of soybean mosaic virus: Classification based on virulence in resistant soybean lines. Phytopathology 69:467-470.
3. Cleveland, D. W., Fischer, S. G., Kitschner, M. W., and Laemmli, U. K. 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. 252:1102-1106.
4. Diaco R., Lister, R. M., Hill, J. H., and Durand, D. P. 1986. Demonstration of serological relationships among isolates of barley yellow dwarf virus by using polyclonal and monoclonal antibodies. J. Gen. Virol. 67:353-362.
5. Hill, E. K., Hill, J. H., and Durand, D. P. 1984. Production of monoclonal antibodies to viruses in the potyvirus group: Use in radioimmunoassay. J. Gen. Virol. 65:525-532.
6. Hill, J. H., and Benner, H. I. 1980. Properties of soybean mosaic virus and its isolated protein. Phytopathol. Z. 97:272-281.
7. Jones, F. E., Hill, J. H., and Durand, D. P. 1988. Detection and differentiation of maize dwarf mosaic virus, strains A and B, by use of different class immunoglobulins in a double antibody sandwich ELISA. Phytopathology 78:1118-1124.
8. Leary, J. J., Brigati, D. J., and Ward, D. C. 1983. Rapid and sensitive colorimetric method for visualizing biotin-labelled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose. Proc. Natl. Acad. Sci. USA 80:4045-4049.
9. Lucas, B. S., and Hill, J. H. 1980. Characteristics of the transmission of three soybean mosaic virus isolates by *Myzus persicae* and *Rhopalosiphum maidis*. Phytopathol. Z. 99:47-53.
10. Morrison, D. F. 1976. Multivariate Statistical Methods. 2nd ed. McGraw Hill, New York. 415 p.
11. O'Connell-Ziegler, P., Benner, H. I., and Hill, J. H. 1986. Soybean mosaic virus: Vector specificity and coat protein properties. J. Phytopathol. 115:29-36.
12. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
13. Soong, M. M., and Milbrath, G. M. 1980. Purification, partial characterization and serological comparison of soybean mosaic virus and its coat protein. Phytopathology 70:388-391.
14. Spector, T. 1978. Refinement of the coomassie blue method of protein quantitation. Anal. Biochem. 86:142-146.