Characterization of Cucumber Mosaic Virus Isolates from California

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ABSTRACT

Daniels, J., and Campbell, R. N. 1992. Characterization of cucumber mosaic virus isolates from California. Plant Dis. 76:1245-1250.

Thirty cucumber mosaic virus (CMV) isolates from California were characterized biologically (host reaction and thermosensitivity), serologically (protein A sandwich-enzyme-linked immunosorbent assay [PAS-ELISA]) and physically (viral capsid protein migration in polyacrylamide gel electrophoresis [PAGE], peptide mapping, and migration of viral double-stranded RNAs [dsRNAs] in PAGE). All methods supported the classification of CMV isolates into two main subgroups, CMV-I and CMV-II. The subgroup CMV-I was divided further into CMV-Ia and CMV-Ib on the basis of host reaction and dsRNA patterns. A low molecular weight RNA 5, presumably a satellite RNA, was detected in two isolates. Peanut stunt virus, tomato aspermy virus, and CMV were distinguished from each other by PAS-ELISA, peptide mapping, and host reaction.

Cucumber mosaic virus (CMV), peanut stunt virus (PSV), and tomato aspermy virus (TAV) are members of the cucumovirus group (20). Cucumoviruses have isometric particles of about 30-nm diameter, a capsid protein composed of a single polypeptide with a mass of about 24 kDa, and a tripartite genome composed of four RNA species (one subgenomic). Some CMV and PSV isolates contain a fifth RNA molecule, termed satellite RNA (sRNA) (20). The sRNA molecule does not share an appreciable nucleotide sequence with the CMV RNAs, but it replicates only in the presence of CMV (2). The sRNA can greatly alter the disease symptoms caused by CMV strains (19,35).

Cucumber mosaic virus exists as a myriad of strains that are difficult to characterize and distinguish (20). Methods that have been used to characterize them include symptoms induced on test plants (25), serology (8,13,21), thermosensitivity (26), electrophoretic mobilities of intact virions and RNAs in polyacrylamide gels (23), hybridization with complementary DNA (14), competition hybridizations (30), and peptide and RNA mappings (11,28). The CMV strains or isolates analyzed by any of these methods were generally separated into two distinct categories, i.e., DTL and ToRS (8), or subgroups I and II (32), or WT and S (21).

Although CMV has been recognized in many crops in California, information about the occurrence of the two subgroups is lacking, except that "legume strains" have been reported (29,33). The objective of the present work was to characterize 30 isolates of CMV from California using six methods that have not been compared previously.

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Accepted for publication 29 July 1992.

MATERIALS AND METHODS

Virus isolates. Isolates of CMV were collected during surveys of pepper and other plants done in California in 1988 and 1989 (6, and *unpublished data*). After CMV was identified in field samples using an antiserum to CMV-C by direct double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) (4), isolates were recovered from field samples by sap inoculations onto a few indicator hosts or by graft inoculations onto *Nicotiana tabacum* L. 'Xanthi' (6). Thirty CMV isolates were selected for this study to represent geographic and host diversity (Table 1). In Table 1, the isolates are grouped according to the subgroups into which they were assigned at the end of this study. Each isolate was passed through three consecutive single-lesion transfers on *Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., or *Vigna unguiculata* (L.) Walp. and then increased in *N. tabacum* 'Havana 425.' The isolates were preserved by drying leaf tissue of infected tobacco over calcium chloride and storage at −20 C.

Additional virus isolates were CMV-Cyn from artichoke and CMV-LQ from lettuce (both provided by H. Lot, Montfavet, France); CMV-C, CMV-Py, and PSV-74-23 (33); and TAV-Till (16).

Purification of virions. The cucumoviruses were inoculated onto *N. tabacum* 'Havana 425' that was kept 2–3 wk before tissues were harvested. The method for virion purification combined several techniques. The steps from tissue extraction through precipitation with polyethylene glycol (PEG-8000) were essentially those of Lot et al (24) except that 5 mM sodium ethylenediaminetetraacetic acid (EDTA) was added to the extraction buffer, and a piece of dry ice was added to the blender to maintain a CO_2 atmosphere during trituration. The virions in the PEG precipitate were resuspended in about one-fifth of the initial volume of 5 mM borate buffer, pH 9, containing 0.5 mM sodium EDTA (1) plus 2% Triton X-100 (24). The borate buffer without Triton X-100 was used for two cycles of differential centrifugation and for sucrose density-gradient centrifugation. The virus fraction recovered from

density gradients with a fractionator was sedimented and resuspended in 1 ml of PEN buffer (10 mM sodium phosphate buffer, pH 7, with 1 mM sodium EDTA and 1 mM sodium azide) (13). Virion yields, based on an extinction coefficient of $A_{260} = 5$, were good for all isolates with up to 30 mg per 100 g of leaf tissue.

Host reactions. The plant species and varieties used in host reaction studies were *Capsicum annuum* L. 'Yolo Wonder B,' *C. amaranticolor, C. quinoa, Cucumis sativus* L. 'Straight 8,' *Cucurbita pepo* L. 'Small Sugar Pumpkin,' *Datura stramonium* L., *Lycopersicon esculentum* Miller 'Earlypak,' *Nicotiana glutinosa* L., *N. tabacum* 'Havana 425,' *N. tabacum* 'Xanthi,' and *V. unguiculata* 'California Blackeye.' The plants were grown in pasteurized greenhouse potting mixture and maintained in a greenhouse at about 22 C. Symptoms were observed and recorded once a week for a month after inoculation.

Aphid transmission. Colonies of nonviruliferous *Myzus persicae* (Sulzer) were used. After an acquisition access period of 1 day on peppers that had been inoculated mechanically 2 wk earlier, 10–30 aphids were placed on each of 10 healthy pepper seedlings for an inoculation access period of 1 day for each virus isolate. Plants were maintained in a greenhouse and observed 3 and 4 wk after inoculation.

Thermosensitivity. The tests, as suggested by Marchoux et al (26), were done in two growth chambers maintained at 25 or 32 C with a 16-hr photoperiod at about 25,000 lux. Two *C. quinoa* and two *V. unguiculata* plants each in separate pots were inoculated with a virus isolate, and one pot of each species was placed in each temperature regime for 3 days. The isolates that induced symptoms at both temperatures were deemed thermoresistant; those that induced symptoms only at 25 C were thermosensitive.

Serological tests. Antisera to CMV-LQ VII (=AsI) and to CMV-R (=AsII) (from H. Lot) were tested in gel double-diffusion (15) against purified virion preparations (1 mg/ml) of CMV-LQ, CMV-Cyn, PSV, and TAV. Gel plates were prepared with 0.85% agar dissolved in phosphate-buffered saline (PBS) (0.0015 M KH_2PO_4, 0.016 M Na_2HPO_4, 0.0027 M KCl, and 0.137 M NaCl).

The cucumoviruses were grouped serologically by the protein A sandwich ELISA (PAS-ELISA) method (13). The trapping and detecting antisera were AsI, AsII, AsPSV (from S. Tolin), and AsTAV-Till (from the antiserum collection at the University of California, Davis) at a dilution of 1/1,000. The antigens were purified virion preparations at 10 μg/ml or sap of infected and healthy pepper plants diluted 1/50 in PBS-T buffer. All incubations were for 2 hr at 37 C except that the protein A–alkaline phosphatase conjugate was incubated for 1 hr at 37 C. Plates were read at 405 nm in a Vmax apparatus (Molecular Devices Corp., Menlo Park, CA). The serotype was defined by the ratio AbAsII/AbAsI, in which Ab is the corrected absorbance value (average of absorbance of virus less the absorbance of the control). If the ratio was less than 0.9 (the midpoint between the highest ratio value of CMV-I serotypes and the lowest ratio value of CMV-II serotypes), the isolate was placed in the CMV-I subgroup. If the ratio was greater than 0.9, the isolate was in the CMV-II subgroup.

Molecular weight of capsid proteins. The molecular weight of capsid protein was estimated with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in discontinuous 1 × 73 × 100 mm gels. The running buffer and gels (15 mm of 4% stacking gel and 50 mm of 12% separating gel) were prepared by the method described by Laemmli (22). Purified preparations containing 0.25 mg of virions per milliliter in 62.5 mM Tris-HCl buffer, pH 6.8, with 10% glycerol, 2% SDS, 5% mercaptoethanol, and 12.5 ppm of bromophenol blue were boiled for 2 min, and then about 0.4 μg of capsid proteins was layered into the wells. Electrophoresis was done at 15 mA/gel for 3 hr.

The gels were silver stained by the method of Blum et al (3) with minor modifications. The modifications were that silver nitrate was reduced to 0.1% in the impregnation solution, that the development was done for about 1 min in 3 g of sodium carbonate per 100 ml containing 50 μl of 37% formaldehyde and 10 μl of 100 mM sodium thiosulfate, and that the reaction was stopped with 10% of 2.3 M citric acid.

Isolation of capsid protein and peptide mapping. Either 2 or 5 μl of solutions containing about 0.4 or 1 μg of capsid protein was layered into gel wells, and discontinuous PAGE was done in a mini-gel (0.75 × 73 × 100 mm) at 25 mA/gel for about 90 min. Gels were stained for 15 min in a solution of 50% methanol, 10% acetic acid, and 0.1% Coomassie blue and destained for 15 min in a mix-

Table 1. Classification in subgroups of 30 isolates of cucumber mosaic virus (CMV) from California and four reference isolates

CMV isolate	Date collected (mo/day/yr)	Origin	
		Plant species	Location[a]
CMV-Ia subgroup			
4	05/12/88	*Capsicum annuum* L.	Stanislaus Co.
7	07/07/88	*Phaseolus vulgaris* L.	Fresno Co.
13	10/17/88	*Solanum tuberosum* L.	Kern Co.
Py[b]	...	*S. tuberosum*	Kern Co.
CMV-Ib subgroup			
1	03/29/88	*Vinca minor* L.	Near pepper field 10
5	06/14/88	*Cucumis melo* L.	Stanislaus Co.
8	07/08/88	*Lycopersicon esculentum* Miller	Merced Co.
9	08/06/88	*C. annuum*	Pepper field 4
10	09/13/88	*C. annuum*	Pepper field 10
11	09/13/88	*Solanum* sp.	Pepper field 10
12	09/23/88	*L. esculentum*	Yolo Co.
14	10/21/88	*C. annuum*	Pepper field 8
17	05/02/89	*Marah fabaceus* (Naud.) Greene	Near pepper field 18
20	05/27/89	*M. fabaceus*	Near pepper field 18
21	07/06/89	*Cucurbita* sp.	Yolo Co.
22	07/11/89	*C. annuum*	Pepper field 17
23	07/18/89	*C. annuum*	Merced Co.
24	07/18/89	*Physalis* sp.	Merced Co.
25	08/22/89	*C. annuum*	Pepper field 15
26	09/19/89	*C. annuum*	Pepper field 13
27	09/19/89	*C. annuum*	Pepper field 14
28	10/10/89	*C. annuum*	Pepper field 16
29	10/11/89	*C. annuum*	Yolo Co.
30	01/24/90	*V. minor*	Yolo Co.
C[b]	...	*C. annuum*	Yolo Co.
LQ[c]	...	*Lactuca sativa* L.	France
CMV-II subgroup			
2	03/29/88	*V. major* L.	Near pepper field 10
3	03/29/88	*V. major*	Near pepper field 9
6	06/29/88	*Apium graveolens* L.	Ventura Co.
15	03/29/89	*V. major*	Near pepper field 16
16	03/29/89	*V. major*	Near pepper field 18
18	05/02/89	*V. major*	Near pepper field 18
19	05/02/89	*V. major*	Near pepper field 18
Cyn[c]	...	*Cynara scolymus* L.	France

[a]Locations listed as "Pepper field" or "Near pepper field" were located in Gilroy area and are described in Daniels (6).
[b]CMV isolates from the culture collection at the University of California, Davis.
[c]CMV isolates received from H. Lot, Montfavet, France.

ture of 40% methanol and 10% acetic acid. Gel slices with capsid protein bands were soaked in 125 mM Tris-HCl buffer, pH 6.8, and stored either in a refrigerator at 4 C for up to 2 days or in a freezer for longer periods.

Peptide mapping (5) was done in a discontinuous gel (15 mm of 4% stacking gel and 50 mm of 15% separating gel) measuring 1 × 73 × 100 mm. The gel slices prepared above were placed on top of the stacking gel and covered with 5 μl of chymotrypsin (Sigma C-3142) at 25 ppm, papain (Sigma P-4762) at 150 ppm, or protease V8 (Sigma P-7674) at 10 ppm. Each enzyme was in 125 mM Tris-HCl buffer, pH 6.8, containing 0.1% SDS, 10% glycerol, and 0.01% bromophenol blue. The electrophoresis was done at 15 mA/gel for 45 min, discontinued for 1 hr (during which time the assembled electrophoresis apparatus was incubated at 37 C for digestion of viral protein in the stacking gel), and then resumed for another 3.5 hr. The gels were silver stained and photographed.

Purification and analysis of dsRNAs. The dsRNAs were purified and analyzed by procedures of Valverde et al (34) from leaf tissue of *N. tabacum* 'Havana 425' harvested 10–20 days after inoculation. The electrophoresis was done in a 5% acrylamide minigel (1 × 73 × 100 mm) at 30 mA/gel for 1.25 hr. Gels were stained by silver as described for proteins and/or by soaking for 6 min in 5 μg/ml of ethidium bromide and then were photographed.

RESULTS

Host reaction. The 11 indicator plants were useful to demonstrate infectivity and to identify and maintain the virus cultures. However, host responses of three species were adequate to identify the CMV subgroups as well as PSV and TAV (Table 2). All isolates of CMV-Ia and PSV caused systemic infection of *V. unguiculata*, whereas all CMV-Ib, CMV-II, and TAV isolates induced necrotic local lesions. The CMV-II isolates and TAV caused systemic necrosis that was lethal to young plants of *N. glutinosa*; the other isolates produced systemic mosaic symptoms. All CMV isolates infected *Cucurbita pepo*; PSV and TAV did not.

Aphid transmission. All CMV isolates, as well as PSV and TAV, were transmitted by aphids.

Thermosensitivity. All CMV-Ia and CMV-Ib isolates and PSV were thermoresistant, whereas all CMV-II isolates and TAV were thermosensitive (Table 2).

Serological tests. Precipitin bands without a spur formed in gel double-diffusion tests between antiserum AsI and CMV-LQ or CMV-Cyn (Fig. 1A). In contrast, CMV-Cyn with antiserum AsII formed a large spur behind CMV-LQ (Fig. 1B). Both sera reacted weakly with TAV, but not with PSV. The weak reaction with TAV was not strong enough to be recorded in Figure 1A.

The CMV isolates were readily separated into two serogroups by testing them simultaneously against the antisera AsI and AsII in PAS-ELISA. The range in the ratio of AbAsII/AbAsI was 0.39–0.82 for 26 CMV-I isolates and 1.01–1.26 for 8 CMV-II isolates and provided a clear separation between the two serogroups (Fig. 2). The separation of CMV isolates was possible because the absorbance value (Ab) for all CMV-I isolates was smaller with antiserum AsII than with AsI, whereas the absorbance values for all CMV-II isolates were similar with both antisera, as shown for representative isolates (Table 3). These relationships were consistent for all 34 CMV isolates whether tested as purified virion preparations or as sap of infected pepper plants. The CMV isolates did not react with PSV and TAV antisera in PAS-ELISA. The PSV and TAV isolates reacted with CMV antisera, but the absorbance values were about 50% lower than with homologous virus (Table 3).

Apparent molecular weight of capsid protein. Capsid proteins of all CMV-II isolates and TAV migrated slightly faster in SDS-PAGE than those of all CMV-I isolates and PSV, as shown for representative isolates (Fig. 3). Estimated masses were 22 kDa for CMV-II, 23 kDa for TAV, and 24 kDa for CMV-I and PSV.

Isolation of capsid protein and peptide mapping. The capsid protein formed a strong, characteristic band in 12% acrylamide gel and was visualized easily by Coomassie blue staining. When gel slices containing the protein were transferred to 15% acrylamide gels and electrophoresed again, two bands were formed (Fig. 4A). The upper band resulted from loading an excess of protein on the gel and was eliminated by halving the protein loaded (0.2 μg/well), but this amount of protein was too small for peptide mapping by the procedure used.

Analyses by SDS-PAGE of partially digested CMV capsid proteins produced banding patterns that were distinct

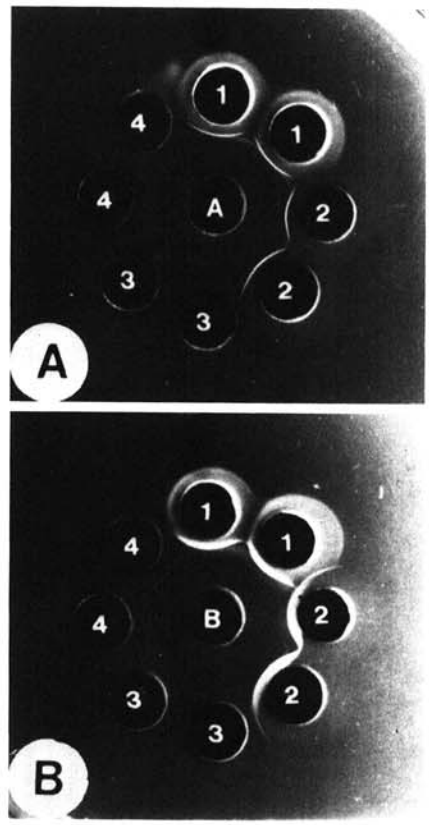

Fig. 1. Immunodiffusion tests with cucumber mosaic virus (CMV) antisera AsI **(A)** and AsII **(B)** in the central wells. The outer wells were filled with purified virion preparations of CMV-LQ subgroup Ib (1), CMV-Cyn subgroup II (2), PSV-74-23 (3), and TAV-Till (4) at 1 mg/ml in phosphate-buffered saline.

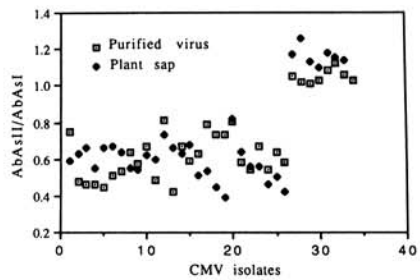

Fig. 2. Serotyping of 32 cucumber mosaic virus (CMV) isolates from California and two from France by protein A sandwich-enzyme-linked immunosorbent assay. The ratio of the absorbance produced by each isolate with AsII and with AsI separates 26 CMV-I isolates (below 0.9) from eight CMV-II isolates. The CMV isolates are in the same order as in Table 1; the last isolate (CMV-Cyn) was not tested in plant sap.

Table 2. Characterization of cucumoviruses by thermosensitivity and host reaction

Cucumovirus or subgroup	Thermo-sensitive[a]	Host reaction[b]		
		Cucurbita pepo	*Nicotiana glutinosa*	*Vigna unguiculata*
CMV-Ia	No	Mosaic	Mosaic	Mosaic
CMV-Ib	No	Mosaic	Mosaic	Nec LL
CMV-II	Yes	Mosaic	Nec	Nec LL
PSV	No	Nil	Mosaic	Mosaic
TAV	Yes	Nil	Nec	Nec LL

[a]Thermosensitive isolates caused symptoms on indicator plants at 25 C but not at 32 C.
[b]Symptoms: Nec = systemic necrosis; Nec LL = necrotic local lesion; Nil = no symptoms.

between CMV-I and CMV-II subgroups but that were indistinguishable among all isolates within each subgroup. All isolates in the CMV-II subgroup produced patterns similar to isolate 2 (Fig. 4, lane II/2). All isolates in the CMV-I subgroup produced patterns like isolates 7, 10, 17, and 28 (Fig. 4, lanes Ia/7 to Ib/28). Capsid protein digestion was more complete with protease V8 (Fig. 4D), but partial digestion with papain (Fig. 4B) and chymotrypsin (Fig. 4C) produced peptides that better discriminated the cucumoviruses. Partial digestion with papain produced patterns with two major bands below the main enzyme band for the CMV-II isolates (Fig. 4B, lane II/2), and three major bands for CMV-I isolates (Fig. 4B, lanes Ia/7 to Ib/28; in this gel only two bands showed in lane Ib/28). Peptide maps produced by PSV and TAV partially digested by papain (Fig. 4B, lanes P and T) were distinct from each other and from CMV isolates. Partial digestion with chymotrypsin produced patterns with three major bands for CMV-I and CMV-II isolates and one major band for PSV and TAV (Fig. 4C). Digestion with protease V8 produced two major bands for all cucumoviruses (Fig. 4D). The slower migrating peptide that comigrated with a minor enzyme band was more intense for CMV-II isolates (Fig. 4D, lane II/2) than for CMV-I isolates (Fig. 4D, lanes Ia/7 to Ib/28). Peptide maps produced by PSV and TAV digested by protease V8 were indistinguishable from each other or from CMV-II isolates.

Patterns of dsRNA. Silver staining resolved slight differences among patterns of dsRNAs 1 and 2 (Fig. 5A) better than ethidium bromide staining, which was superior for RNA 5 (Fig. 5B). Three major dsRNA patterns of RNAs 1 and 2 corresponding to the subgroups were detected with all the CMV isolates. The CMV-Ia isolates produced well-separated bands for RNA 1 and 2, but the RNA 2 was faint, as shown for isolate 7 (Fig. 5, lane Ia/7). In contrast, CMV-Ib isolates produced distinct, separated RNA 1 and 2 bands that were equally dense (Fig. 5, lanes Ib/10 to Ib/28). The CMV-II isolates produced a faint RNA 1 band, which migrated close to RNA 2, making it difficult to discern easily (Fig. 5, lane II/2). A fifth RNA with low molecular weight (possible satellite RNA) was observed only with CMV isolates 14 (not shown) and 28 (Fig. 5B, lane Ib/28). The dsRNA patterns of PSV 74-23 (Fig. 5, lane P) could not be distinguished from patterns of CMV-Ib isolates. TAV-Till produced patterns similar to CMV-II patterns in other experiments (*unpublished data*).

DISCUSSION

These results strongly confirmed the existence and characteristics of the two major subgroups of CMV, both of which were found among 30 isolates from California. The subgroups were verified by comparison with CMV-LQ and CMV-Cyn that represented the two serotypes, originally described as DTL and ToRS (8), and the two types of thermal reaction (26). All the isolates from California were uniform within the subgroup for other characteristics that have also been used to divide other sets of isolates, e.g., electrophoretic mobility of capsid subunits and peptide mapping patterns (11). Representative isolates of two other cucumoviruses, TAV and PSV, could be distinguished from each other and from CMV by these same characteristics.

We prefer to follow the nomenclature of Rizzo and Palukaitis (32) and to designate the two major subgroups as CMV-I and CMV-II, with CMV-I further divided into CMV-Ia and CMV-Ib on the basis of systemic versus local infection of *V. unguiculata*. This system of nomenclature avoids the confusion of naming subgroups with an alphabetical letter, which usually has been used to identify specific isolates.

The choice of method to identify subgroups from the field depends upon the facilities available. Serological tests using PAS-ELISA or DAS-ELISA (21) are the most rapid and can detect single or mixed infections directly in field samples. The thermosensitivity test, which has been reported only in France (26), is simple and rapid but requires two growth chambers. In this test, *C. quinoa* was more sensitive to all isolates than *V. unguiculata*, but use of the latter host permitted identification of the CMV-Ia isolates at the same time.

The CMV-I isolates were highly variable biologically and physically. They were the predominant subgroup in peppers and other hosts in our collections from the Central Valley and the Santa Clara Valley. This pattern was different from those in the Provence region of France or in New York, where both subgroups were found equally commonly on peppers, and mixed infections were detected by direct serological testing of field samples (21,31). The prevalence of CMV-I isolates in the Central Valley of California and on warm season pepper crops in the southern Santa Clara Valley may be related to the thermoresistance of these isolates. A similar tendency for thermoresistant isolates to predominate during the summer was noted in Provence (31).

The use of host range and symptomatology to define strains is not practical (1,25). If many hosts are used and small symptomatic differences are noted, each isolate will virtually constitute a strain. On the other hand, the symptomatology observed on three indicator plants consistently identified the three subgroups of CMV and the three cucumoviruses that were recognized by other characteristics (serotype, thermosensitivity, and protein and nucleic acid banding patterns).

Serological tests have been used to separate cucumoviruses and the subgroups. The cucumoviruses were classified into five categories by immunodiffusion tests: CMV-ToRS, CMV-DTL, TAV, PVS-V, and PVS-W (8). Indirect

Table 3. Absorbance values obtained in a protein A sandwich-enzyme-linked immunosorbent assay with cucumoviruses

Antigen[a]		Antiserum[b]			
Virus	Isolate no.	AsI	AsII	AsPSV	AsTAV-Till
CMV-Ia	7	0.74	0.24	0.06	0.06
CMV-Ib	10	0.78	0.29	0.06	0.05
CMV-Ib	17	0.76	0.33	0.05	0.07
CMV-Ib	28	0.80	0.27	0.06	0.05
CMV-II	2	0.70	0.78	0.08	0.05
PSV	74-23	0.33	0.24	0.31	0.11
TAV	Till	0.45	0.13	0.07	0.82
PBS buffer		0.05	0.04	0.06	0.05

[a] Antigens were purified virions at 10 μg/ml in phosphate-buffered saline (PBS) buffer (0.137 M NaCl, 0.0015 M KH_2PO_4, 0.016 M Na_2HPO_4, and 0.0027 M KCl).

[b] Antisera AsI (CMV-LQ VII) and AsII (CMV-R) were furnished by H. Lot, Montfavet, France; AsPSV was provided by S. Tolin, Blacksburg, VA; and AsTAV-Till was from the collection at the University of California, Davis.

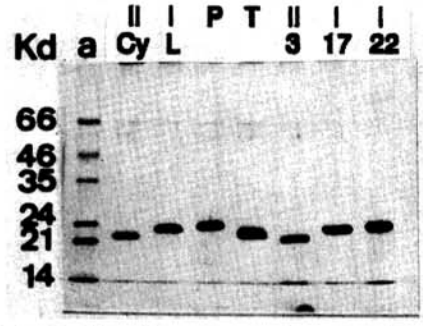

Fig. 3. Migration pattern of cucumovirus capsid proteins (0.4 μg/well) in 12% polyacrylamide gel. Protein markers (Sigma SDS-7) at 0.46 μg protein (lane a); cucumber mosaic virus in lanes marked by subgroup and isolate number (as in Table 1 except Cy = isolate Cyn, L= isolate LQ); peanut stunt virus (lane P) and tomato aspermy virus (lane T).

ELISA detected relatively distant relationships (serological differentiation indices of 3–8) of cucumoviruses that were not detected by the direct ELISA (9). On the other hand, the two serotypes of CMV were demonstrated by direct DAS-ELISA (11), using the same antisera that were ineffective in immunodiffusion tests (12). The PAS-ELISA has the advantages of using unfractionated antisera and of having the reduced specificity of an indirect ELISA; it was used to quantify serological relationships among tobamoviruses (18) and was a simple, reliable method for grouping CMV isolates. Our data may have resulted from the nature of the two antisera used, especially antiserum AsII. The CMV-II isolate R to which AsII was prepared had several epitopes in addition to those that were common to both CMV-I isolate LQ and CMV-II isolate R. There were no similar epitopes specific to CMV-I and not found in CMV-II isolate R. This interpretation was supported by the PAS-ELISA test and the gel-diffusion tests shown here and in Devergne and Cardin (7).

Intact virions of three strains of CMV and one of PSV differed in mobility in PAGE, with CMV-II isolate S migrating most rapidly and CMV-I isolate D most slowly (23). In contrast, the capsid proteins of several CMV-I isolates migrated faster in PAGE than those of CMV-II isolates (11). Edwards and Gonsalves (11) attributed their results to the larger number of amino acids in the capsid proteins of CMV-II isolate S and TAV than in CMV-I isolate D and PSV (20). Our data contradict theirs, because the capsid proteins of our CMV-II isolates and TAV migrated faster than those of CMV-I isolates. Perhaps a small peptide was cleaved from the capsid protein of a different subgroup during purification and/or PAGE in each laboratory.

The peptide mapping of the intragel digested CMV capsid protein produced distinct patterns for each subgroup as reported by Edwards and Gonsalves (11). The two CMV subgroups would already have been recognized during isolation of capsid protein, but peptide mapping may be useful for separating CMV from the other cucumoviruses.

Analysis of cucumovirus RNAs in PAGE has revealed variability in sizes and composition (17,20,23). The development of better techniques for isolation and analysis of dsRNAs (10,34) made possible the use of this method not only for virus identification, but also for characterization of virus isolates. Qualitative (position of dsRNAs in the gel) and quantitative (intensity of certain bands) differences were observed in dsRNA patterns of citrus tristeza virus isolates, but seasonal and host variation affected the total dsRNA recovery and the interpretation of the results (27). In the present study, we used the same host grown in a greenhouse and minimized that kind of problem. The banding patterns obtained in several experiments were consistent and represented defined viral characteristics. This method has potential for a better classification of cucumoviruses.

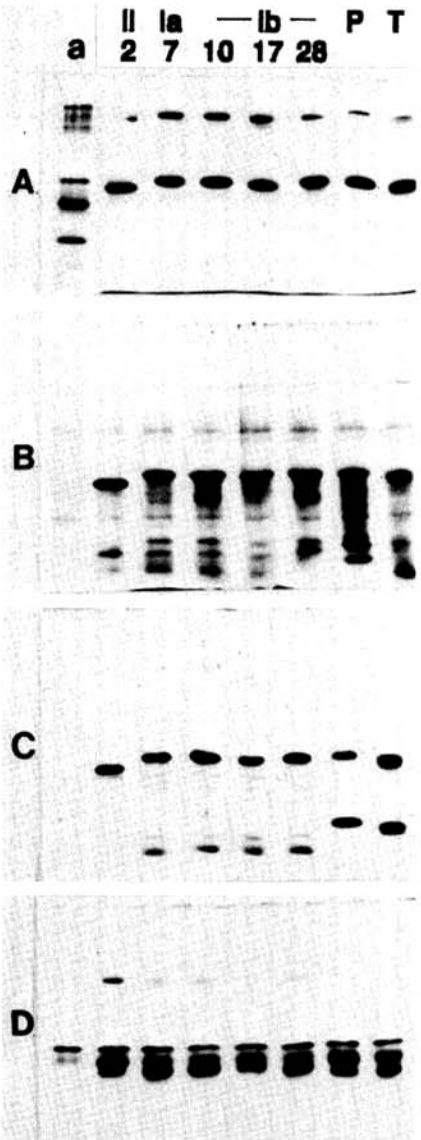

Fig. 4. Peptide maps of capsid proteins of selected cucumoviruses in a 15% polyacrylamide gel. Capsid proteins (0.4 μg) of cucumber mosaic virus in lanes marked by subgroup and isolate number (as in Table 1), peanut stunt virus (lane P), and tomato aspermy virus (lane T). **(A)** Capsid proteins without protease treatment; protein markers (Sigma SDS-7) at 0.46 μg in lane a. **(B)** Capsid proteins treated with 0.75 μg of papain; papain alone in lane a. **(C)** Capsid proteins treated with 0.125 μg of chymotrypsin; chymotrypsin alone in lane a. **(D)** Capsid proteins treated with 0.058 μg of protease V8; protease V8 alone in lane a.

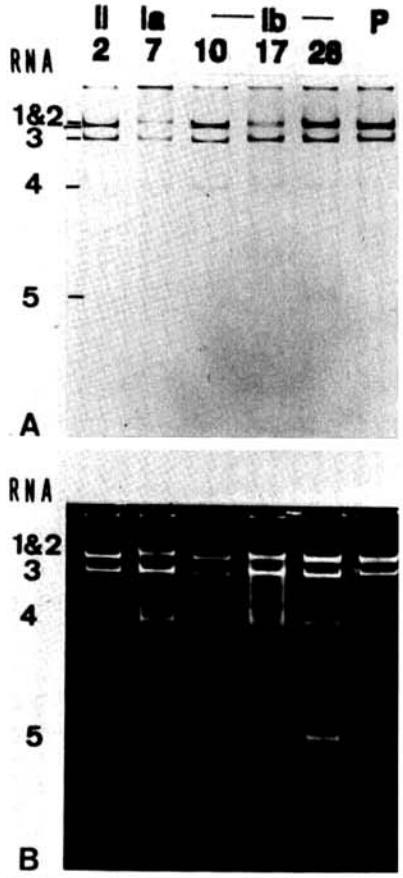

Fig. 5. Double-stranded RNA patterns of cucumoviruses in a 5% polyacrylamide gel stained with silver **(A)** or ethidium bromide **(B)**. Cucumber mosaic virus in lanes marked by subgroup and isolate number (as in Table 1) and peanut stunt virus in lane P.

ACKNOWLEDGMENTS

The first author thanks EMBRAPA (Brazilian Enterprise for Agricultural Research), which financed his studies in the United States. The California Pepper Improvement Foundation kindly provided partial financial support for this study. The authors thank H. Lot and S. Tolin for gifts of antisera and virus cultures and B. Falk for assistance with aphid transmission trials.

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