Comparison of Techniques for Purification of Maize Dwarf- and Sugarcane Mosaic Viruses

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ABSTRACT

A purification system was developed that is applicable to most known maize dwarf mosaic and sugarcane mosaic virus strains, members of the potatoe virus Y group. It incorporates acidification of pH 4.7 early in the clarification process and the use of a smaller concn of chloroform (3%) than previously reported. These viruses characteristically occur in low titer and are thus more effectively purified when the elapsed time between clarification and the final product extraction is minimized. The relative merits of this procedure and three previously published procedures are compared and discussed.

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Purification of a virus is prerequisite to detailed study of its physical, chemical, and serological properties. Maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus strains (SCMV), both members of the potato virus Y group (2), have been obtained in various degrees of purity (1, 4, 5, 6, 8, and 10).

Some techniques yield highly purified virus, but result in loss of major amounts of virus, whereas others retain more virus but yield a less homogeneous virus preparation.

A problem most often encountered in obtaining accurate, repeatable, and comparative data among different purification techniques is the difficulty of doing them all simultaneously. An investigator often states that several techniques and (or) sets of conditions were tried and proven unsatisfactory. The most satisfactory results are published as the best procedure. Usually the purification techniques were not compared in a valid manner; i. e., most were done sequentially rather than simultaneously. A sequential series introduces small variations in day-length, temp, time of day, matching standardizations, host physiology (age), various buffer preparations, lack of manpower, etc.; small variables that cumulatively cause large errors. The starting infected material must be identical to be comparable. Assay plants must be uniform at each stage tested, and the methodology for comparing them is most repeatable (uniform) when done by one operator.

We compared three purification procedures (5, 8, and 10) with one we developed (hereafter referred to as the Tosic procedure) that is a modification of several published purification procedures. Key aspects of this new procedure are: (i) a low pH treatment of the clarified sap, and (ii) the use of a smaller concn of chloroform than previously reported. Our aims were to develop a purification technique that might be equally effective for all maize dwarf and sugarcane mosaic viruses, and to compare the relative merits of several techniques on all these viruses produced in a uniform host and processed in a single laboratory.

MATERIALS AND METHODS.—MDMV strains A (Johnsongrass strain, Ia. 65-74, isolated from Johnsongrass near Hamburg, Ia.) and B (noninfectious on Johnsongrass, Ia. 66-188 isolated from commercial sweet corn near Dubuque, Ia.) were our isolates. SCMB strains, A, B, D, H, and I were obtained from A. G. Gillaspie, Jr., U.S. Sugarcane Field Station, Houma, La., and the MDMV-Jg strain was obtained from R. J. Shepherd, Davis, Calif. All viruses were purified from either 'Seneca Chief' or 'Golden Bantam' sweet corn. Systemic assays were made in the same host. Plants in the 2- to 3-leaf stage were inoculated by rubbing Carborundum (600-mesh)-dusted leaves with a pestle moistened with infected plant sap diluted with 0.01M potassium phosphate buffer, pH 7.0.

Plants with mosaic symptoms were harvested 3 wk after inoculation. Leaf tissue (250-300 g) was stored 12-16 h at 4C before division into 50-g units. Leaves were cut into 1-cm lengths, homogenized in a blender at 4C in four vol of 0.01M PO_4 buffer, pH 7.0; then the resultant pulp was homogenized three times using one-third of the sap-buffer each time, and finally pooling the complete sample. The temp was maintained at about 5C by blending with prechilled buffer in a cold room or in a blender with its container immersed in ice. The blended material was then squeezed through gauze and treated as shown in Fig. 1. The other three purification procedures with which this one was compared have been outlined previously (5, 8, and 10).

All samples were coded; thus, the identity of the virus isolate was unknown until the data were analyzed.

The four comparative purifications were done at 4C or maintained on ice, and were started within 1-2 h of each other.

Final preparations were analyzed by (i) a systemic infectivity assay, (ii) particle counts using a HU11C Hitachi electron microscope, (iii) ultraviolet (UV) absorption with a Beckman DB spectrophotometer,

Fig. 1. Diagrammed purification procedure used for maize dwarf mosaic and sugarcane mosaic virus isolates.

Infected sap expressed through double layer of gauze
- centrifuge 1,000 *g* 10 min

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Discard pellet
- acidify by dropwise addition of 1.0 N HCl to pH 4.7 with constant stirring
- centrifuge 2,500*g* 10 min

↓

Discard pellet
- add chloroform to a final concentration of 3% (v/v)
- blend at high speed 2 min in Waring Blendor
- centrifuge 2,500*g* 10 min

↓

Discard pellet
- filter through two layers of gauze
- centrifuge 7,500*g* 15 min

↓

Discard pellet
- centrifuge 35,000*g* 3 h

↓

Discard supernatant
- suspend pellet in 0.01 M PO_4, pH 7.0
- centrifuge 2,500*g* 10 min

↓

Discard pellet
- centrifuge through 30% sucrose in 0.01 M PO_4, pH 7.0, 63,000*g* 3 h

↓

Discard supernatant
- resuspend pellet in 0.01 M PO_4, pH 7.0 (or 0.05 M borate, pH 7.0)
- centrifuge 2,500*g* 15 min

↓

Discard pellet
- optional: layer viruses (2 ml) on 7:7:7:4 ml of 50:40:30:20% sucrose, respectively, and centrifuge 90 min at 42,000*g* in Spinco SW 25 rotor
- Remove broad band 7-27 mm below meniscus, dilute with equal vol. buffer
- optional: centrifuge recovered virus 63,000*g* 90 min

↓

Discard supernatant
- suspend virus in borate or phosphate buffer

and (iv) measuring the protein content of the final preparation with the biuret test (3).

The viruses were assayed by diluting the final purified preparation relative to the amount of starting material, and inoculating ten 10-day-old sweet corn seedlings. Readings of systemic symptoms were made 3 wk later.

Particle counts were made by diluting the virus samples to known values, and by atomizing these samples onto Formvar-coated grids. Preparations were either negatively stained with neutral Na-phosphotungstate for immediate analysis, or dried and shadowed with platinum-palladium (80:20) applied at an angle of 20°. A standard quantity of polystyrene balls (88-nm diam) were added to the preparations to quantitate the number of virus particles per preparation. Virus particles and polystyrene balls in at least 12, and often up to 30, electron microscope viewing fields were counted and averaged to give the ratios found in Table 3.

Ultraviolet absorption data were obtained by measurement on a Beckman DB recording spectrophotometer, and the 260/280 ratios were calculated. Protein content was determined by the biuret test (3). After the experiment was completed, we repeated it 2 wk later, with nearly identical results. Data reported here are averages of the two experiments.

We reduced operational errors by having each author do one of the four purification procedures. Then, for all samples from these 4 purifications, a pair of us did all infectivity assays, one of us did all the particle counting, a pair of us did all spectrophotometric analyses, and a pair of us did all protein analyses.

RESULTS AND DISCUSSION.—We did not attempt to modify in any way the purification procedures reported by others (5, 8, and 10). The purification procedures compared here each have advantages and disadvantages. The Shepherd and Tosic systems involve less time than the others. The Sehgal system seemed particularly well adapted to maintaining infectivity of MDMV-B (Table 1). We had suspected this for some time following Sehgal and Jean's publication (8), and the data presented here confirmed that fact. The variability that we found shows that the Sehgal system (8, 9) may yield slightly more consistent 260/280 ratios than do the other purification methods (Table 2). For instance, we could neither detect infectivity nor find virus

TABLE 1. Dilution end points for systemic infectivity of selected strains of maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) on sweet corn after purification by four different procedures

Virus strain	Dilution end point[a] Purification procedure: Shepherd (10)[b]	Sehgal & Jean (8)	Langenberg (5)	Tosic
MDMV-A	2.5	1	1	2
-B	2	4	2	2
SCMV-A	2	2	3	2
-B	2.5	3	3	3
-D	3	2	1	1
-I	3	2	0	0

[a]Each value is the negative exponent of $1 \times 10^{-(\ )}$ (For instance 2.5 is $1 \times 10^{-2 \cdot 5}$ 4 is 1×10^{-4}, etc).

[b]Numbers refer to references in the Literature Cited section.

TABLE 2. Ultraviolet absorption ratios (260/280) calculated from spectrophotometer readings of maize dwarf mosaic (MDMV) and sugarcane mosaic (SCMV) virus strains purified by four different procedures

Virus strain	260/280 ratio Purification Procedure: Shepherd (10)[a]	Sehgal & Jean (8)	Langenberg (5)	Tosic
MDMV-A	0.25	1.20	1.17	1.14
-B	0.28	1.20	1.26	1.12
SCMV-A	0.28	1.18	1.20	1.22
-B	0.29	1.22	1.11	1.32
-D	0.33	1.18	1.19	1.13
-I	0.29	1.22	1.26	1.14

[a]Number refers to references in the Literature Cited section.

particles of SCMV-I after purification by the Tosic method, yet 260/280 ratios of these preparations were comparable to other isolates purified by this same procedure. We did not attempt to determine the relative "purity" of the product from each purification method compared among the purification methods. This variance is even more noticeable in the total protein content determinations. We feel, however, based on electron microscopic observations, that Langenberg's (5) purification yielded very clean final preparations of viral particles. Our method (Tosic) is nearly comparable, but we did not test this in a more definitive way.

Attempting to compare between the total protein data and the infectivity data may be criticized since we do not have comparable purities of the final product from all purification methods. There does not seem to be a particular advantage of any one purification procedure over another based on infectivity alone (Table 1).

Any meaningful or significant comparisions between particle numbers and the infectivity data are probably not valid until the specific infectivity of each of these virus isolates is determined. Such a comparison of our data leads to widely varying conclusions. If such a comparison is valid here, it seems like SCMV-B and -D have the highest infectivity per unit of virus particles. The variability which is typically reflected here for MDMV-A is surely related to its lack of stability in vitro. One final

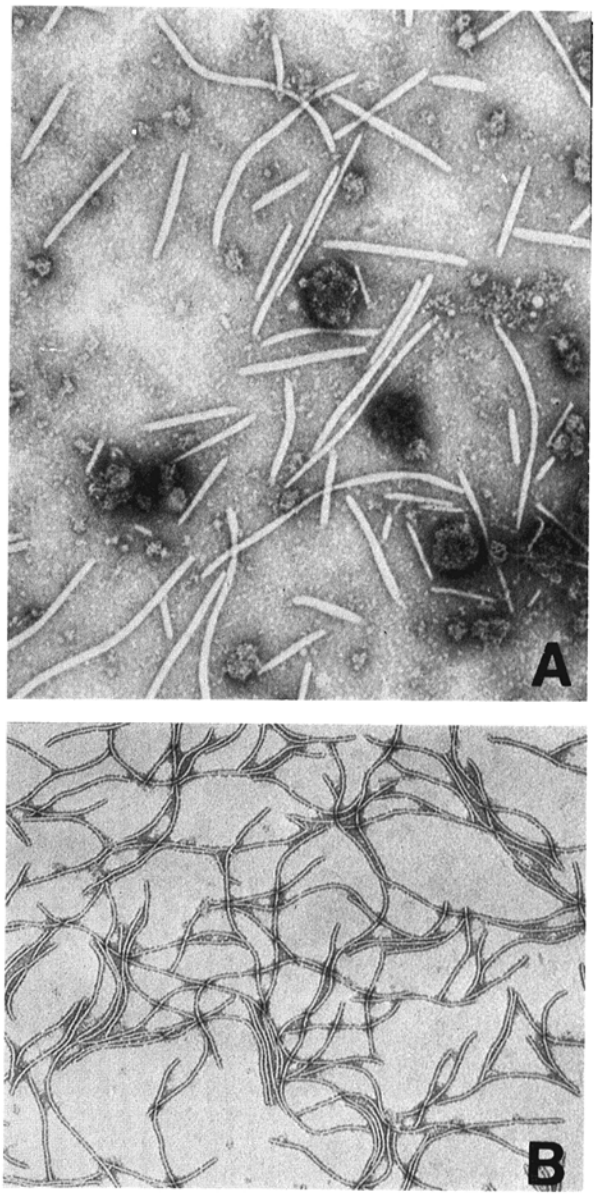

Fig. 2. Sugarcane mosaic virus strain D negatively stained in 2% Na phosphotungstate, pH 7.0, which had been treated during purification with chloroform (25-50% final concentration). Note the "swelling" of virus particles (A). Compare with virus particles which we consider normal (B) which were treated with 3-5% chloroform. Swelling was observed in purified preparations of all virus strains included in this study where percentages of chloroform 25%, or greater, were used. Magnification × 22,800.

caution: one must consider the relatively lower sensitivity of the systemic assay system employed here, compared to that of a good local lesion assay system.

Acidification is a very important limitation to getting a clean preparation. The adjustment of pH to 4.7 (1) is critical and must be done carefully. Another variable that seemed almost equally important as determined by over a dozen purification attempts, was the concentration of chloroform. We found that the higher concentrations of chloroform (25-50% by volume) used by other workers were not necessary. A 3% concentration proved as effective as 50% in helping remove host contaminants. This low concentration seemed to reduce virus losses in initial clarification steps, although this was not proven conclusively. Also, chloroform concentrations above 20% seem to cause swelling of virus particles, detected by measuring the variability in various particle diameters (Fig. 2). This may not be a severe problem, but chloroform does reduce infectivity (4), therefore, this may be visible evidence of the first step of protein–RNA (7) bond-loosening which leads to loss of infectivity.

Although Bond and Pirone (1) lost 90% of the infectivity when virus preparations were ultracentrifuged a second time at high speed, the Tosic method did not produce this much loss.

Our new purification method, the Tosic procedure, was almost equally successful with all MDMV and SCMV strains except SCMV-I. Sweet corn is a poor host for SCMV-I, based on dilution end point infectivity assays of virus titer. This may explain partially the lack of success with SCMV-I. Good results may be obtained by purifying MDMV and SCMV with the Tosic method. This is especially useful in comparative work, rather than trying to find an optimum purification method for each strain. One author specifically says this method is good for only a given strain (5) while another doesn't specify (8), thus leaving it to future researchers to determine this by trial and error.

There should be more effort spent to specifically study SCMV-I and possibly -D. For these two strains, none of the procedures tested yielded particularly satisfying results. Each method has its relative merits, is a matter of choice, and is generally satisfactory. Langenberg's system seems to provide the purest preparation with fewest contaminants, but it has the distinct disadvantage of low yields (Table 3, 4). Although the Tosic method takes about the same amount of time as Shepherd's (10), it tends to yield more virus than Shepherd's (Table 3, 4). The advantages of slightly higher yields in some cases by the Sehgal (8) system are offset by it being more cumbersome and less consistent with batch-to-batch in our hands.

TABLE 3. Quantitation of final number of maize dwarf mosaic (MDMV) and sugarcane mosaic (SCMV) virus particles obtained following each of the four purification procedures

	Virus/polystyrene particle ratio			
	Purification procedure			
Virus strain	Shepherd (10)[a]	Sehgal and Jean (8)	Langenberg (5)	Tosic
MDMV-A	.6[b]	1.0		1.7
-B	1.1	0.9	0.02	3.6
SCMV-A	4.0	4.9	0.5	3.6
-B	0.2	0.5	0.04	0.3
-D	0.1	0.8	0.3	0.1
-I	0.9	1.9	0.2	0

[a]Number refers to references in the Literature Cited section.
[b]Ratio equals number of virus particles divided by number of polystyrene particles.

TABLE 4. Protein content of final purified preparations of maize dwarf mosaic (MDMV) and sugarcane mosaic mosaic virus (SCMV) strains[a]

	Protein (mg/ml)			
	Purification procedure			
Virus strain	Shepherd (10)[b]	Sehgal and Jean (8)	Langenberg (5)	Tosic
MDMV-A	0.35	1.55 (1.00)[c]	0.11	0.44
-B	0.19	1.61 (1.22)	0.09	0.41
SCMV-A	0.18	1.27	0.08	0.47
-B	0.15	1.50	0.06	0.65
-D	0.21	0.99	0.04	0.17
-I	0.31	0.65	0.02	0.25

[a]Protein contents determined by the biuret test (3).
[b]Number refers to references in the Literature Cited section.
[c]Values in parenthesis indicate that the data from the two experiments were not averaged because they were so variable, thus each of the two values is reported.

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