

Purification of Barley Yellow Streak Mosaic Virus and Detection by DAS-ELISA and ISEM Using Polyclonal Antibodies

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ABSTRACT

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A purification procedure was developed for barley yellow streak mosaic virus (BaYSMV), the causal agent of a disease in barley and wheat. The procedure utilizes differential and density gradient centrifugation in Percoll gradients and yields highly concentrated virus preparations. These preparations were infectious in *Nicotiana benthamiana*. Electron microscopy of non-glutaraldehyde-fixed virus particles negatively stained or detergent-treated revealed the presence of a finely granular viral nucleocapsid and an envelope presumed to be lipoidal in nature. Internal cross striations characteristic of rhabdovirus particles were not observed. Concentrated virus preparations were used to produce rabbit polyclonal antibodies that were useful in enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) for the detection of the virus in barley, wheat, *N. benthamiana*, as well as in its vector, the brown wheat mite *Petrobia latens*. The virus was detected by ELISA and ISEM in the Pocatello Valley, Idaho, in 1992 and 1993, verifying our 1991 detection of the virus in the same valley. Leaf extracts from BaYSMV-infected barley or *N. benthamiana* plants injected via hypodermic needle and syringe were infectious, causing characteristic disease symptoms in barley. By contrast, viral nucleic acid preparations used to rub-inoculate *N. benthamiana* plants were non-infectious.

In 1982, a viruslike disease of barley (*Hordeum vulgare* L.) was discovered in Montana by Robertson and Carroll (14-16). Whereas some diseased plants exhibited distinctive chlorotic leaf-banding symptoms, most plants expressed streaking, striping, and mosaic leaf symptoms indistinguishable from those produced by barley stripe mosaic virus (BSMV) or wheat streak mosaic virus (WSMV). Ultrathin section electron microscopy of diseased leaves revealed the presence of novel, enveloped, filamentous, viruslike particles (VLPs) that were subsequently determined to be the causal agent of the disease. The virus was named barley yellow streak mosaic virus (BaYSMV) (17). In thin sections, BaYSMV particles average about 64 nm in diameter and range from 127 nm to over 4,000 nm in length (17). Many single VLPs and groups of intracellular VLPs are confined to membrane-bound cavities of the endoplasmic reticulum (17). The virus is transmitted from barley to barley by the brown wheat mite, *Petrobia latens* Müller (16,17). The virus can also be mechanically transmitted from barley or *Nicotiana benthamiana*

Domin. to *N. benthamiana* (18). Due to their shape, size, and somewhat labile nature, BaYSMV particles are difficult to purify from barley or *N. benthamiana*. Previous efforts to purify the virus by centrifugation in sucrose step gradients resulted only in partial purification (18). The two green bands that formed in the sucrose gradients at the 30/40% and 40/60% interfaces were infectious when mechanically inoculated onto *N. benthamiana*, but host contaminants in the bands prevented the determination of relative virus particle concentration therein. Moreover, virus isolated by this procedure (18) failed to elicit polyclonal antibodies specific to BaYSMV in rabbits (T. W. Carroll, unpublished). Past methods used for BaYSMV identification and disease diagnosis were limited to the equivocal symptom observation, laborious and slow thin section electron microscopy, and/or mite transmission assays. In this paper, we describe a purification procedure for BaYSMV particles that has made possible the production of polyclonal antibodies for use in serological diagnostic tests for the virus.

MATERIALS AND METHODS

Virus isolate. The BaYSMV isolate used in all experiments was originally recovered from viruliferous brown wheat mites collected from an infected barley field near Valier, Montana. The isolate has been maintained continuously in the barley cultivar Klages by serial mite transfers and in *N. benthamiana* plants by serial mechanical inoculations in the greenhouse.

All plants were grown at 24°C. Natural lighting was supplemented with fluorescent lights to provide a 16-h day. Healthy barley and *N. benthamiana* were grown as negative controls under the same conditions. Carborundum-dusted *N. benthamiana* plants with 4 to 6 partially expanded leaves were mechanically inoculated with cotton swabs dipped in leaf extracts from symptomatic barley and/or *N. benthamiana* plants. The inocula were prepared in extraction buffer (100 mM NaH₂PO₄/Na₂HPO₄ phosphate buffer pH 7.2 + 10% sucrose, wt/vol) in a chilled mortar and pestle. Cotton swabs were used to apply the extract onto the upper epidermis of the leaves. Plants were then kept in the greenhouse for 2 to 3 weeks for symptom observation.

Virus purification. Most experiments were conducted with 50 to 200 g of fresh *N. benthamiana* plants 15 to 20 days after inoculation. Symptomatic leaves were homogenized in a blender using three volumes (1 g per 3 ml) of cold extraction buffer. This initial extract was filtered through four layers of cheesecloth and a layer of Miracloth (Calbiochem, La Jolla, Calif.) before the addition of 5% (wt/vol) activated charcoal (Darco G 60, MCB, Cincinnati, Ohio). After stirring for 20 min at 4°C, the mixture was filtered again as described above and centrifuged at 3,000 × g for 10 min in a Sorvall SS-34 or GSA rotor. The following steps were conducted at 4°C. The supernatant was then centrifuged in the same rotor at 27,000 × g for 30 min. The resulting dark green pellet was resuspended in extraction buffer (1/10th of the initial extract volume) and vacuum filtered through a 47-mm disk of Extra Thick Glass Fiber (Gelman Sciences, Ann Arbor, Mich.). Eighteen milliliters of the filtrate was then layered on top of a 5-ml phosphate-buffered 35% (wt/wt) sucrose cushion and centrifuged at 100,000 × g for 30 min in a Beckman Type 30 rotor. Pellets were resuspended in 1/30th of the initial extract volume and vacuum filtered again through a glass fiber filter disk. Five milliliters of the filtrate (extracted from 50 g of green tissue) was thoroughly mixed with 15 ml of 100% Percoll (Pharmacia, Upsala, Sweden) and centrifuged at 100,000 × g for 20 min in a Beckman 80 Ti rotor. The resulting gradient was fractionated using a Density Gradient Fractionator (ISCO, Lincoln, Nebr.), and fractions enriched with virus particles (as determined by electron microscopy)

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were pooled, diluted with buffer, layered on top of 5 ml of a 50% (wt/wt) sucrose cushion, and centrifuged at 100,000 × g for 1 h. The interface was collected, diluted with buffer, and centrifuged at 100,000 × g for 30 min in a Beckman Type 30 rotor. The final pellet, which appeared clear to pale green, was resuspended in 1/300th of the initial extract volume. The buoyant density of the virus in Percoll was determined using Pharmacia's density marker beads (13).

The infectivity of the resuspended final pellet was assayed by mechanically inoculating young *N. benthamiana* plants as described above. Tissues obtained from healthy plants were processed in the same manner and used as controls.

Treatment of purified BaYSMV with detergents. To determine the nature of the putative envelope surrounding each BaYSMV particle (17), purified virus preparations were incubated with either the nonionic detergent Triton X-100 or the anionic detergent sodium dodecyl sulfate (SDS) (Sigma, St. Louis, Mo.). The detergent concentrations used were 0.5 and 1% and the incubations were done at room temperature for 5 min.

Electron microscopy. Electron microscopy was used to evaluate the structural integrity of virus particles throughout the various steps of the purification procedure. Formvar-coated grids were prepared as described by Davison and Colquhoun (4). Collodion (0.5% in amyl acetate)-coated grids were prepared similarly. Specimens were prepared for electron microscopy by floating formvar- or collodion-coated copper grids on a drop of leaf extract or virus preparation from the purification procedure for 5 min. The grids were then rinsed with a few drops of distilled water and stained by floating them for 1 min on a

drop of 4% uranyl acetate (UA) or 2% phosphotungstic acid (PTA) in distilled water. After a final rinse, grids were dried in a petri plate at room temperature and examined using a Zeiss EM 10 CA electron microscope. Measurements of virus particles were taken from prints using a Zeiss Interactive Digital Analytical System (ZIDAS).

Antisera production and evaluation. Polyclonal antibodies against BaYSMV were produced in a New Zealand White rabbit. The animal received four subcutaneous injections administered at biweekly intervals. Each injection contained purified virus obtained from 50 g of *N. benthamiana* leaves diluted in 0.5 ml of distilled water. Prior to the first injection, the virus suspension was emulsified in an equal volume of Freund's complete adjuvant (GIBCO, Grand Island, N.Y.). Freund's incomplete adjuvant was used in subsequent injections. The rabbit was bled 10 and 20 days after the third and fourth injections and each antiserum was evaluated in an unamended agar gel medium by the Ouchterlony double immunodiffusion test (12,20). To remove non-viral specific antibodies, the antiserum SC450 (from the final bleeding) was absorbed with the resuspended 27,000 × g pellet obtained from healthy *N. benthamiana* tissues essentially as described by Shepard (20). Immunoglobulin G (IgG) was purified from the absorbed antiserum with a protein-A column using Immunopure IgG Purification Kit (Pierce, Rockford, Ill.). Purified IgG was conjugated to alkaline phosphatase (Type VII-S, Sigma) as described by Clark and Adams (2).

Enzyme-linked immunosorbent assay. Double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA) and indirect ELISA were generally done ac-

ording to the standard protocols described by Clark and Adams (2) and by Koenig (9), respectively. Five antigen extraction buffers each containing phosphate-buffered saline (PBS) were compared (Table 1). To evaluate the efficacy of the tests in detecting the virus in its vector, virus-free as well as viruliferous mites were used in a number of tests as described for plants except that PBS was used as extraction buffer. The reaction was evaluated colorimetrically by measuring the absorbance at 410 nm (A_{410}) using a Minireader II spectrophotometer (Dynatech Laboratories Inc., Alexandria, Va.).

In all direct and indirect ELISA the following controls were included in each plate: (i) extracts from healthy tissues as negative controls (duplicated in 10 wells); (ii) extracts from virus-infected tissues as positive controls; (iii) the extraction buffer; and (iv) extracts from plants infected with BSMV, tobacco mosaic virus (TMV), WSMV, and a Montana isolate of barley yellow dwarf virus (BYDV-MT-RMV), all in duplicate wells.

Only samples with (A_{410}) values greater than three times the absorbance of healthy controls were considered positive.

Immunosorbent electron microscopy. The protocol used for immunosorbent electron microscopy (ISEM) was based on that described by Derrick and Brlansky (5). Formvar-coated grids were floated on a drop of IgG from the absorbed antiserum SC450 (diluted 1:500) for 30 min at room temperature. IgG-coated grids were then rinsed by floating them three times on drops of buffer (50 mM Tris-HCl pH 7.2) and three times on drops of distilled water. Grids were then floated on plant extracts for 30 min and then washed again as described above. The grids were stained by floating them on drops of 5% UA or 2% PTA in water for 1 min, washed, air dried, and visualized by electron microscopy as described above.

Inoculation of barley by injection. Leaf extracts from barley and *N. benthamiana* were injected into barley plants (cv. Atlas) at the two- to three-leaf stage with 27 G 1/2 Tuberculin needles (Becton-Dickinson, Rutherford, N.J.). Inoculum was prepared as described previously for mechanical inoculation except that it was filtered through two layers of Miracloth. About 500 µl of inoculum was injected into leaf veins with an average of 5 to 7 inoculation points on each plant. Injected plants were grown in the greenhouse and observed for symptoms. All symptomatic and some asymptomatic plants were examined by electron microscopy as described above.

Serological detection of BaYSMV in Idaho field samples. In 1992, 125 barley plants exhibiting symptoms characteristic of BaYSMV infection were collected from the Pocatello Valley, Oneida County, Idaho, near the Utah border and examined

Table 1. Comparison of different extraction buffers used for the detection of barley yellow streak mosaic virus (BaYSMV) in leaf extracts of barley and *Nicotiana benthamiana* by direct double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA)^a

Extraction buffer	Absorbance at 410 nm ^b						
	Buffer	Barley			<i>N. benthamiana</i>		
		Healthy	Infected	Ratio	Healthy	Infected	Ratio
PBS ^c	0.000	0.035	0.560	16.0	0.015	0.725	48.3
PBS + 5% T ^d	0.005	0.100	0.750	7.5	0.053	0.840	15.8
PBS + 5% DNM ^e	0.001	0.220	0.835	3.8	0.065	0.780	12.0
PBS + 2% PVP ^f	0.048	0.180	0.625	3.5	0.058	0.633	10.9
PBS + 5% BSA ^g	0.017	0.065	0.750	11.5	0.035	0.760	21.7

^a Wells were coated with 1 µg/ml SC450 immunoglobulin G (IgG) preparation in coating buffer, loaded with 200-µl samples extracted with 10× volume (g/ml) of each extraction buffer, and with 1:2,000 dilution of IgG-alkaline-phosphatase conjugate. The IgG preparation was obtained from SC450 antiserum absorbed with concentrated healthy protein.

^b Readings were recorded after 1 h of enzyme-substrate reaction at room temperature. Each buffer reading is the average of two duplicate wells. Each healthy reading is the average of 10 duplicate wells.

^c PBS = Phosphate-buffered saline (140 mM NaCl, 1.5 mM KH₂PO₄, 10 mM Na₂HPO₄, 2.7 mM KCl, 3 mM (0.02%) Na₂CO₃ pH 7.4).

^d T = Tween 20.

^e DNM = dried nonfat milk.

^f PVP = polyvinylpyrrolidone.

^g BSA = bovine serum albumin.

for BaYSMV by ISEM, ELISA, and thin section electron microscopy. More samples were also collected from the same area in 1993 and examined similarly.

Infectivity of viral nucleic acid. All glassware, plasticware, and solutions were treated with diethylpyrocarbonate (DEPC) as described by Sambrook et al. (19). Both the final pellet and the 27,000 × g pellet obtained from the purification procedure described above were used for the extraction of viral nucleic acid as reported by Robertson and Carroll (18) except for the addition of an RNA washing step as described by Logemann et al. (10). The final pellet was rinsed once with 70% ethanol, vacuum dried, and resuspended in RNase-free, cold, Tris-HCl buffer, pH 7.2. These nucleic acid preparations were applied to the surface of young *N. benthamiana* leaves using DEPC-treated cotton swabs. To confirm that the initial extracts as well as the 27,000 × g pellet (from which the nucleic acid was extracted) contained infectious virions, aliquots from those two fractions were used to inoculate additional *N. benthamiana* as described above.

RESULTS

Virus purification. Based on the number of particles counted in a defined electron microscope field of view, virus particles were concentrated 1,000-fold from infected plants by the use of differential and density gradient centrifugation in Percoll (Fig. 1). Virus particle length in the initial extract ranged from 194 nm to 3,081 nm with an average of 1,155 nm ($n = 100$ virus particles measured, standard deviation ± 574), and the length ranged from 370 nm to 2,053 nm with an average of 1,006 nm ($n = 100$ virus particles, standard deviation ± 367) in the final pellet, indicating that the purification procedure did not result in any significant fragmentation of virus particles.

High magnification views of negatively stained, unfixed, individual virus particles revealed the presence of a surrounding envelope, a granular-appearing viral nucleocapsid, and no internal striations (Fig. 2).

Percoll was the only suitable centrifugation medium that resulted in any significant concentration of intact virus particles. Previous attempts to purify the virus using other centrifugation media such as cesium chloride, cesium sulfate, and potassium tartrate (all gradients were 0 to 40%, wt/wt) resulted in significant virus particle losses and/or degraded virus unrecognizable as discrete particles by electron microscopy (J. S. Skaf and T. W. Carroll, unpublished). The use of activated charcoal was helpful in producing a final pellet that contained minimal amounts of contaminating host material. Also, the addition of 5% celite to the initial extract was helpful, too, although the results obtained were inconsistent, especially during large-scale puri-

fications in which more than 30 g of celite was added.

The virus buoyant density in Percoll ranged from 1.06 to 1.07 g per cm³ (average 1.064 g per cm³).

The final pellet obtained using this protocol contained infectious virus. Two separate infectivity assays induced symptoms characteristic of those caused by BaYSMV in 24 of 40 (60%) and 20 of 40 (50%) *N. benthamiana* plants inoculated. BaYSMV long filamentous particles were observed in all symptomatic plants examined by electron microscopy.

When samples of the same purified virus preparation were deposited on the two different coating membranes, 10 to 20 times more virus particles were observed on formvar-coated grids.

Treatment of purified BaYSMV with detergents. The envelope that surrounds individual virus particles was removed by detergent treatment even at the lower concentration (0.5%). Generally, detergent treatments resulted in virus particles unrecognizable as such by electron microscopy. In a few cases, however, partially disintegrated virus particles having a beaded appearance were seen (photo not shown).

Antisera production and evaluation. The native (nonabsorbed) antiserum SC450 reacted in the double immunodiffusion test with both virus and host antigens in tissue extracts of infected barley and *N. benthamiana*. Antibodies produced against contaminating healthy plant protein were successfully eliminated by absorbing the native antiserum with healthy

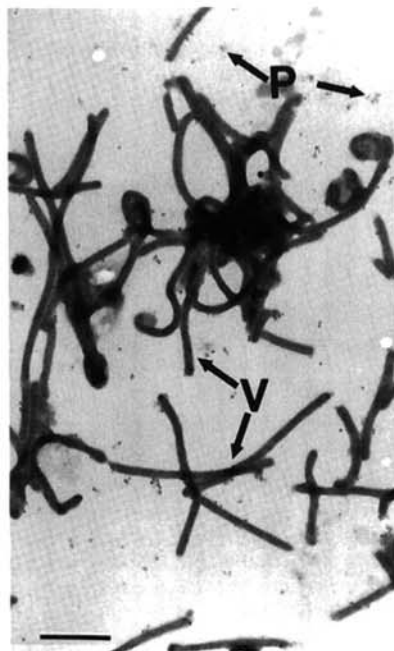


Fig. 1. Electron micrograph of a purified preparation of barley yellow streak mosaic virus (BaYSMV). Virus (V) particles positively stained with uranyl acetate. (P) contaminating Percoll particles. Bar = 500 nm.

N. benthamiana antigen without affecting the titer.

ELISA. Results of the initial work using nonabsorbed polyclonal antibodies in standard DAS-ELISA resulted in a high background reading with the healthy control. The use of purified IgG from antiserum SC450 absorbed with concentrated host protein and the selection of specific extraction buffers for both direct (Table 1) and indirect ELISA (data not shown) improved the results of the assays.

For direct DAS-ELISA, the best results were obtained when PBS was used as antigen extraction buffer. PBS was considered superior to other satisfactory buffers (e.g., PBS + 5% bovine serum albumin [BSA]). The ratio of A₄₁₀ readings of infected to healthy barley was 16× and 11.5×, respectively, and for *N. benthamiana* the ratio was 48.3× and 21.7× for PBS and PBS + 5% BSA, respectively (Table 1).

Indirect ELISA gave unsatisfactory results. The A₄₁₀ of infected to healthy plants was inconsistent between barley and *N. benthamiana* when the same extraction buffer was used. In addition, high buffer readings were observed in the case of PBS and PBS + 2% polyvinylpyrrolidone (data not shown). In both tests there was no difference in the results when extracted samples were incubated at 4°C or at room temperature.

Satisfactory results were obtained by direct DAS-ELISA when mites were extracted with PBS. In five different experiments, when one mite was used, the average ratio of A₄₁₀ of the viruliferous to nonviruliferous mite was 10× ($n = 25$

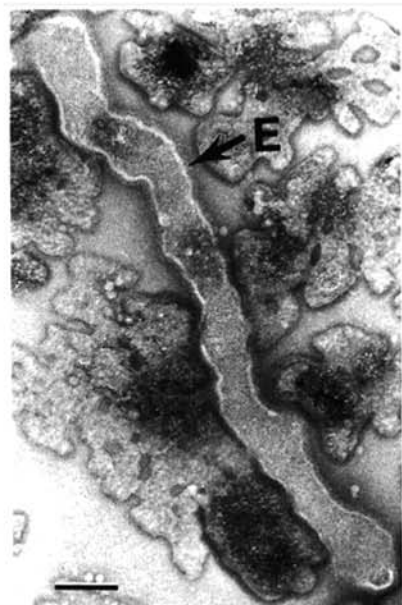


Fig. 2. Electron micrograph of an unfixed barley yellow streak mosaic virus (BaYSMV) particle negatively stained with PTA. The viral nucleocapsid is finely granular in appearance and no internal cross striations are visible. (E), the putative virus envelope. Bar = 100 nm.

mites per experiment, $A_{410} = 0.560$ and 0.056 , respectively). When three mites were used the average ratio was $11 \times$ ($n = 25$ mites per experiment, $A_{410} = 0.610$ and 0.054 , respectively).

No reactivity was observed between the antiserum SC450 and BSMV, WSMV, BYDV-RMV-MT, and TMV. Their A_{410} readings were comparable to those of their respective healthy controls (data not shown).

Inoculation of barley by injection. For the first experiment, 11 of the 20 Atlas barley plants (55%) injected with BaYSMV extracted from barley and *N. benthamiana* showed symptoms characteristic of the disease about 6 weeks after inoculation. By contrast, none of the 20 barley plants inoculated by injection in a second experiment developed any symptoms. However, in a third experiment, one out of 50 injected barley plants became diseased. In the second and third experiments, plants were injected with virus extracted from infected *N. benthamiana* plants. In the first and third experiments, only new tillers developed symptoms characteristic of the disease. No symptoms were observed on injected leaves. The long filamentous virus particles were detected by electron microscopy in extracts obtained from all the injected symptomatic plants. Furthermore, these extracts were infectious when used to mechanically inoculate *N. benthamiana* plants.

Serological detection of BaYSMV in Idaho field samples. The large filamentous particles unique to BaYSMV were identified in all symptomatic barley samples collected from the Pocatello Valley, Idaho, in 1992 and 1993. The plants displayed typical mosaic, streaking, and necrosis symptoms and reacted positively in DAS-ELISA. In 1991, at least 400 ha of the barley cultivars Otis, Steptoe, and Korol were affected by the disease (21). In 1992, the disease incidence reached 100% in about 30% of the fields inspected with yield loss estimates ranging from 30 to 100%. In 1992 and 1993, no differences in disease incidence or severity were observed between the barley cultivars grown in that area. The plants were grown under dry land conditions and the brown wheat mite was abundant in many of the affected fields.

Infectivity of viral nucleic acid. BaYSMV nucleic acid was not infectious in our assays. None of the 60 *N. benthamiana* plants inoculated with viral nucleic acid extracted from either the $27,000 \times g$ pellet or from the final pellets showed symptoms of BaYSMV infection. By contrast, using the initial extract and the $27,000 \times g$ pellet as inocula resulted in symptoms characteristic of BaYSMV infection in 20 of 30 (67%) and 15 of 30 (50%) of the *N. benthamiana* plants inoculated, respectively. No symptoms were observed in any of the 20 *N. benthamiana* plants inoculated with nucleic acids ex-

tracted from healthy plant tissues.

DISCUSSION

Efforts to further determine biological, serological, and molecular characteristics of BaYSMV have been impeded by the lack of a purification procedure that consistently yielded a high concentration of virus particles. The critical step in the purification procedure described in this paper was the pelleting of the virus particles using a relatively low centrifugal force for a short time ($27,000 \times g$ for 30 min) in the early steps of the purification. The $27,000 \times g$ supernatant fluid contained very few (if any) virus particles as determined by electron microscopy. In the previously published protocol (18), pellets obtained using a higher centrifugal force ($50,000 \times g$ for 30 min) were discarded resulting in a complete loss of the virus. Another critical step in this protocol was the use of Percoll as a centrifugation medium because of the very short time (20 min) required to obtain linear gradients in it, compared with other media (13). Percoll was important in preserving the structural integrity of the virus particles, as ascertained by monitoring the particles throughout the various steps of the purification procedure by electron microscopy. Using two cycles of centrifugation in Percoll gradients helped reduce the amount of contaminating host materials but it also led to a reduction in the yield of virus particles, which often necessitated combining virus preparations obtained from several purifications. The highly concentrated virus preparations obtained using this protocol contained contaminating host material as well as Percoll, as evidenced by electron microscopy and the presence of antibodies against healthy plant antigen in the nonadsorbed antiserum. This contamination interfered with the use of spectroscopy to quantify virus yield in mg/kg terms. Activated charcoal used in the initial step of the purification procedure was helpful probably due to its ability to adsorb certain substances that would otherwise result in the disintegration and/or aggregation of the virus. Celite, which is commonly used to purify the enveloped rhabdoviruses (3,6-8,11), was unsatisfactory especially in large-scale purifications when more than 30 g was added to the initial extract. Virus particles were irreversibly trapped by the celite during the subsequent filtration step, which led to a significant reduction in the yield. When less celite was used, the loss of virus particles was reduced but the amount of contaminating healthy materials was increased.

The partial removal of Percoll from the virus preparations by centrifugation through a 50% (wt/wt) sucrose pad presented a convenient alternative to the methods recommended by the manufacturer (13). High-speed centrifugation resulted in loss of virus particles in the Per-

coll pellet. Although gel filtration is recommended by the manufacturer, it failed to totally remove Percoll from rice transitory yellowing virus (RTYV) preparations (1).

The dimensions of virus particles obtained from the purification procedure described here are consistent with the previously published dimensions of virus particles in thin sections and crude plant extracts (15-17) indicating that this protocol resulted in highly concentrated virus preparations without fragmenting the long filamentous virus particles that were present in the initial plant extracts.

High purity of virus preparations had to be compromised by avoiding harsh protocols and additives (e.g., chloroform, 2-mercaptoethanol, detergents, etc.) so that structural integrity of the particles could be preserved. Avoidance of such additives has been recommended for enveloped viruses such as members of the Bunyaviridae (22).

This report documents that the concentrated long filamentous virus particles obtained using this purification protocol are infectious. Their infectivity provides reasonable proof that those particles are the causal agent of barley yellow streak mosaic disease. The envelope surrounding virus particles was readily removed when purified virus particles were treated with anionic or nonionic detergents, suggesting that the envelope has a lipid nature. The presence of the envelope was essential for preserving the structural integrity of the particles. The removal of the envelope resulted either in the partial or the total disintegration of the long filamentous particles giving them a beaded appearance, as previously reported for UA-stained BaYSMV particles (17).

The use of PTA to negatively stain unfixed, concentrated virus particles also caused the removal of the viral envelope and the disintegration of most virus particles. However, a few, apparently intact, negatively stained virus particles were observed. The negative stain revealed a finely granular nucleocapsid and no cross striations (Fig. 2). This, along with a similar observation of glutaraldehyde-fixed, negatively stained particles (17), indicates that BaYSMV is not a rhabdovirus.

This report describes the production of the first antiserum to BaYSMV. The presence of antibodies that reacted with healthy plant antigens in double immunodiffusion tests was expected due to the mild nature of the purification procedure used to obtain the virus preparations. However, these antibodies were removed by cross absorption with concentrated healthy *N. benthamiana* protein. The purified IgGs from the cross-absorbed antiserum were used in ISEM and ELISA to detect the virus in both infected plants and viruliferous mites.

The occurrence of BaYSMV in the U.S. was believed to be limited to ten counties in Montana until July 1991, when the presence of the virus in the Pocatello Valley, Idaho, was confirmed by ISEM and thin section electron microscopy (21). Subsequently, in 1992 and 1993, the virus was again detected in that valley by ISEM and ELISA. In that valley, barley is grown under dry land conditions that are conducive to the development of the disease (15). Also, the brown wheat mite was abundant in many of the fields visited, which explains the high disease incidence in many of the fields. It is a common practice to plant barley consecutively in the same fields. The symptoms caused by BaYSMV have been observed in that area for the last 5 years and the brown wheat mite found for the last 10 years. An interesting observation was that the disease incidence in the barley fields planted to safflower in the previous season was almost 60% lower than in the fields planted to barley. In the Pocatello Valley, safflower may very well be used in rotation with barley to reduce the mite populations and virus reservoirs, especially if environmental conditions conducive to high disease incidence persist. It is important, however, that experiments be conducted before recommending such a control measure.

Obtaining symptomatic barley plants via the injection of extracts from infected plants is the first reported demonstration of mechanical transmission of BaYSMV to barley. Although this method is not as efficient, practical, or consistent as using brown wheat mites, it provided an important step toward completing Koch's postulates. This approach should be further investigated to determine all the factors affecting the infectivity. Obtaining higher and more consistent percentages of infection is essential if injection is to be used in screening genotypes for resistance to BaYSMV.

The failure to obtain any symptomatic

plants using viral nucleic acid as inoculum can be attributed to (i) the degradation of the viral nucleic acid by nucleases on the surface of *N. benthamiana* leaves and/or (ii) the fact that the viral coat protein, envelope, and/or nonstructural proteins are required for infectivity.

The purification protocol and the antiserum to BaYSMV described in this paper are currently being used to study interactions between BaYSMV and its vector and host plants and to further characterize this virus at the molecular level.

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