Purification and Partial Characterization of Beet Yellow Stunt Virus

R. R. REED and B. W. FALK, Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

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Beet yellow stunt virus (BYSV), a suspected closterovirus, was compared with beet yellows virus (BYV), the type member of closterovirus subgroup I. Virions of BYSV and BYV were purified from herbaceous hosts. Purified virions of BYSV cosedimented in sucrose gradients with those of BYV, and the virions of each virus were indistinguishable by electron microscopy. The virions of BYSV and BYV are composed of a single major capsid protein subunit with molecular weight of approximately 24,500 (BYSV) or 24,000 (BYV) and a single species of single-stranded RNA with molecular weight of approximately 6.1 million (BYSV) or 5.5 million (BYV). Immunoblot analysis using antisera to both viruses showed that BYSV capsid protein is serologically unrelated to that of BYV. Double-stranded RNA (dsRNA) analysis confirmed results of virion single-stranded RNA analysis, in that BYSV-infected *Chenopodium capitatum* had a major dsRNA species with molecular weight of about 13 million, slightly larger than the dsRNA of BYV and slightly smaller than the dsRNA of citrus tristeza virus, another well-characterized closterovirus. Based on the properties described, BYSV is distinct from BYV yet sufficiently similar to it to warrant inclusion in closterovirus subgroup I.

Beet yellow stunt virus (BYSV) causes a destructive disease of lettuce (*Lactuca sativa* L.) and sugar beets (*Beta vulgaris* L. subsp. *vulgaris*) in California (9). The natural host of BYSV is sowthistle (*Sonchus oleraceus* L.), and under field conditions BYSV is transmitted by the sowthistle aphid (*Hyperomyzus lactucae* L.) in a semipersistent manner (8). The incidence of BYSV in both lettuce and sugar beets is generally low, but in areas where sowthistle growth is left unchecked, lettuce crop losses as high as 85% caused by BYSV infection have been reported (9).

Duffus (8) first described BYSV in 1964 and determined that it is distinct from beet yellows virus (BYV) on the basis of host range studies and aphid transmission data. In 1970 Hoefert et al (14) published results of electron microscopy studies of plants infected by BYSV. Their micrographs documented ultrastructural changes in host tissue similar to those produced by BYV, the type member of the closterovirus group (1). The viruslike particles seen in infected cells were typically "closteroviruslike," being very long, flexuous rods approximately 1,250 nm long and 12 nm in diameter, and are indistinguishable from those of BYV (10).

In a recent review of the closteroviruses, Lister and Bar-Joseph (18) commented that the closterovirus group is ill defined and probably contains some members that would be more appropriately assigned elsewhere and excludes some that should be included in the group. Physicochemical characteristics that seem to be similar among viruses in the closterovirus group include coat protein subunit molecular weight, amino acid composition of the coat protein, and the production of specific intracellular inclusions (1). Closteroviruses are further separated into two subgroups on the basis of particle length and aphid transmissibility or nontransmissibility (1,18). Still, some authors persist in considering any virus with threadlike particles to be "closteroviruslike" (18).

BYSV has general characteristics typical of closteroviruses. However, the geographic, host, and vector ranges of BYSV overlap with those of the type member of the group, BYV. These two viruses also cause very similar symptoms in some plant species. The virions of BYSV have never been purified, and the physicochemical and serological properties of BYSV have not been compared with those of BYV or other characterized closteroviruses.

In this study, we report results of side-by-side comparisons of BYV and BYSV. BYSV is shown to be physicochemically and serologically distinct from BYV and thus readily separable by diagnostic methods. The viruses are, however, sufficiently similar to warrant the inclusion of BYSV within closterovirus subgroup I.

MATERIALS AND METHODS

Virus maintenance. Isolates of BYV and BYSV were obtained from J. E. Duffus (USDA, Salinas, CA). BYV was transferred from sugar beet to *Chenopodium capitatum* (L.) Asch. using green peach aphids, *Myzus persicae* (Sulzer),

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that had been maintained on healthy radish (*Raphanus sativus* L.). Nonviruliferous aphids were allowed a 24-hr acquisition access on the virus source (detached leaves in petri plates) and then a 48-hr inoculation access on healthy *C. capitatum*. Plants were then sprayed with 0.1% permethrin (Pounce) and placed in an insect-free greenhouse until symptoms developed, about 2–3 wk. *C. capitatum* plants displaying typical symptoms were then used as source plants for transmission of BYV to *Tetragonia tetragonioides* (Pallas) O. Ktze. (*T. expansa*), the maintenance and purification host.

BYSV was transferred from sowthistle to *C. capitatum* using *M. persicae* in the same manner, including treatment with insecticide, as described for transmission of BYV. *C. capitatum* plants were placed in an insect-free greenhouse until typical symptoms of beet yellow stunt developed (3–5 wk). All subsequent transfers of BYSV were from infected *C. capitatum* to healthy *C. capitatum* using *M. persicae*.

Virion purification. Virions of both BYV and BYSV were purified by a modification of the method for BYV reported by Kassanis et al (15). Two buffers were required: the extraction buffer was 0.1 M ammonium acetate buffer (0.1 M ammonium acetate, 0.01 M ethylenediaminetetraacetic acid [EDTA], 0.1% β-mercaptoethanol (2), pH 7.0), and the resuspension buffer was 0.01 M sodium borate, pH 7.8. Tissues were processed with a sap expresser (Piedmont Mechanical and Tool, Six Mile, SC). Extraction buffer ratios of 3 ml and 5 ml of buffer per gram of tissue were used for BYV and BYSV, respectively. In all other respects, the virion purification procedure was identical for the two viruses.

Leaves, stems, and in some cases washed roots were passed through the rollers of the sap expresser. Buffer was kept cold and was added directly to the rollers. Expressed sap was collected in a plastic container lying on a bed of ice directly under the rollers. Debris was removed by centrifugation at 9,000 *g* (max) in a Sorvall GSA rotor for 10 min. Residual debris was removed by passing the supernatant through two layers of cheesecloth. The resulting supernatant was adjusted to 2.5% (v/v) Triton X-100 and was gently stirred for 30 min at 4 C. The adjusted supernatant was centrifuged for 1.5 hr at 36,000 *g* (max) in either a Sorvall SS-34 rotor or a Beckman 45-Ti rotor.

The supernatant was discarded, and the pellets in each tube were covered with cold borate buffer. The volume of borate buffer added was approximately 10% of the initial volume of the centrifuge tube. Pellets were resuspended at 4 C for 72 hr, allowing the virions to slowly come into solution. Any unsuspended pellets were then brought into suspension by gentle shaking. The preparations were pooled and centrifuged at 9,000 *g* (max) for 10 min in an SS-34 rotor to remove residual debris. The supernatant was carefully decanted and recentrifuged for 1.5 hr at 36,000 *g* (max). The pellets were resuspended in borate buffer and left at 4 C for 72 hr. The preparations were then gently swirled, pooled, and centrifuged at 9,000 *g* (max) for 5 min in an SS-34 rotor to remove plant debris.

Virions were further purified by rate zonal centrifugation in 5–40% (w/v) sucrose density gradients in 0.01 M borate (pH 7.8) for 2.5 hr at 25,000 rpm in an SW-27 rotor. To compare relative sedimentation profiles of BYV and BYSV, opposing gradients were loaded with freshly extracted virion preparations and centrifuged simultaneously. Gradients were fractionated, and fractions containing virions were pooled. Centricon 30 protein concentrators (Amicon, Danvers, MA) were used to remove sucrose and concentrate the virions. Each concentrator was loaded with a 2-ml gradient fraction. Concentrators were centrifuged at 5,000 *g* (max) as recommended by the manufacturer. When the volume was reduced to about 200 µl, cold 0.01 M borate buffer was added to bring the volume back up to 2 ml. This cycle was repeated three times to remove most of the residual sucrose. The final volume of 200 µl was collected and stored until needed at either 4 C or −20 C, depending on the analysis to be performed.

Electron microscopy. Purified virions of BYSV or BYV were placed on a Formvar-coated carbon-backed grid, stained with 1% phosphotungstic acid, and examined with a Zeiss EM 109 electron microscope.

Analysis of virion proteins and nucleic acids. To prepare virion proteins of BYV and BYSV and molecular weight marker proteins (Bio-Rad Laboratories, Richmond, CA) for electrophoresis, preparations (0.1 mg/ml) were mixed with an equal volume of disruption buffer (150 mM Tris, 10% glycerol [v/v], 5% β-mercaptoethanol [v/v], 2% sodium dodecyl sulfate [SDS] [w/v], pH 6.8) and boiled for 3 min. Bromphenol blue was added as a tracking dye, and samples were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (16) in a mini-slab unit (Idea Scientific, Corvallis, OR) at constant voltage of 200 V for 50 min. The 0.8-mm-thick gel was composed of a 10-mm-high 5% stacking gel and a 55-mm-high 12% resolving gel. When electrophoresis was complete, proteins were visualized by silver staining (20). Molecular weights of virion proteins were calculated by comparing mobilities with standards analyzed in the same gel.

Nucleic acids were extracted from purified BYV and BYSV virions by combining 1 vol of the purified virion preparation with 1 vol of RNA extraction buffer (0.2 M Tris-HCl, 0.02 M EDTA, 1.5% SDS, 1.5% *N*-lauroylsarcosine, pH 8.0) and 0.1 vol of purified bentonite (13). This mixture was kept at room temperature for 30 min, with occasional mixing by gentle inversion of the tubes. After 30 min, 2 vol of water-saturated phenol (pH 8.0) was added. The sample was gently inverted until the solution was uniformly cloudy and was then centrifuged for 10 min at 10,810 *g* (max). The aqueous phase was reextracted with 2 vol of phenol and 1 vol of chloroform-pentanol (25:1), and the nucleic acids were precipitated with ethanol and stored at −20 C. When needed for analysis, precipitates were pelleted by centrifugation for 15 min at 10,810 *g* (max), then washed several times with 70% ethanol to remove

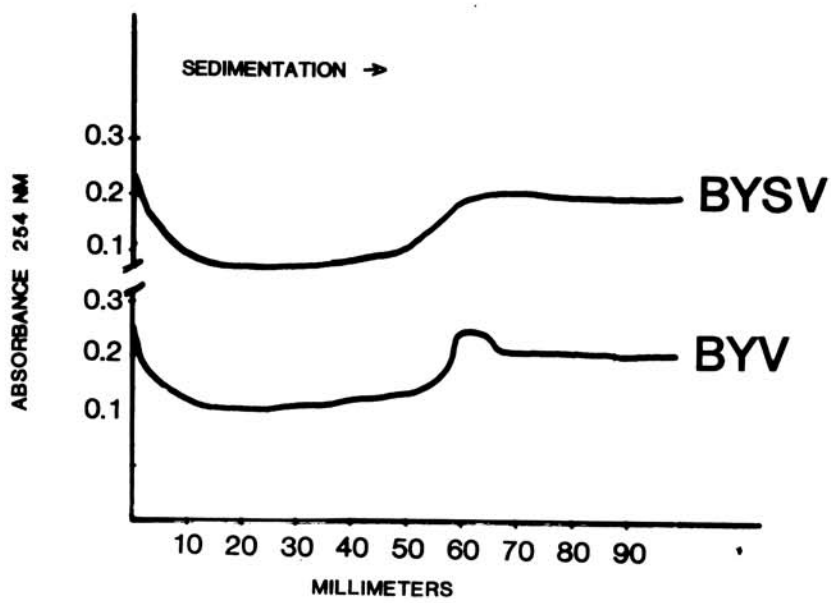

Fig. 1. Sucrose density gradient profile showing the relative sedimentation of virions of beet yellows virus (BYV) and beet yellow stunt virus (BYSV) in 5–40% (w/v) sucrose gradients. Note the presence of large shoulders of 254 nm absorbance, particularly for BYSV.

residual salts before a final resuspension in sterile distilled water.

Electrophoresis of virion nucleic acids was performed under denaturing conditions using the glyoxal-dimethyl sulfoxide system of McMaster and Carmichael (19), with the modification that buffers and the gel contained 0.02 M sodium Hepes buffer, pH 7.0, and 1 mM EDTA. In addition, nucleic acids were not mixed in a vortex; rather, the mixture of nucleic acids and glyoxal-dimethyl sulfoxide was gently drawn up into a sterile plastic pipet tip 10 times, then incubated at 55 C for 60 min. Nucleic acid standards of known molecular weight (1 Kb DNA ladder, Bethesda Research Laboratories, Gaithersburg, MD; and Hind III-digested lambda DNA) were prepared in an identical manner.

Electrophoresis was done at room temperature in horizontal slab gels (10 cm long, 6.5 cm wide, and 3 mm thick) of 0.86% agarose for 2 hr at constant voltage of 100 V. Gels were subsequently soaked in 50 mM NaOH for 15 min to remove glyoxal, then in 0.5 M ammonium acetate for 15 min and 0.1 M ammonium acetate for 1 hr before staining in ethidium bromide (10 ng/ml). Nucleic acids were visualized by exposing the gel to ultraviolet radiation (305 nm) and were photographed using Polaroid 665 positive-negative film. Molecular weights of viral RNAs were estimated by comparing electrophoretic mobilities with molecular weight standards.

Serological analysis. Antiserum to partially purified BYSV was kindly provided by J. E. Duffus. Antiserum to BYV was produced in New Zealand white rabbits. Two sources of antigen were used: sucrose density gradient-purified virions and gel-purified coat protein subunits. Protein subunits were visualized in SDS-PAGE gels and electroeluted into dialysis tubing as described by Falk and Tsai (12). Buffers were exchanged by dialyzing the protein subunits against phosphate-buffered saline adjusted with 0.025% (v/v) SDS for 24 hr at 4 C. Equal volumes of antigen (either gradient-purified virions or gel-purified subunit protein) and Freund's adjuvant were mixed, and the rabbit was injected subcutaneously about once every 10 days for 40 days with approximately 300 μg of antigen in 900 μl of emulsion. Freund's complete adjuvant was used for the first injection, and incomplete adjuvant was used for all subsequent injections. Bleedings began 2 wk after the final injections and continued once every 2 wk for 8 wk. We used sera from the first three bleedings in these studies.

SDS-PAGE-separated virion capsid proteins of BYV and BYSV were analyzed serologically using standard immunoblotting techniques. Purified virions were dissociated and capsid proteins were resolved in SDS-PAGE as described above. Total protein extracts from BYV- and BYSV-infected and healthy plants were also used. These extracts were made by grinding 0.25 g of infected or healthy leaf tissue in liquid nitrogen. Disruption buffer (1.5 ml) was added to the powdered tissue, and the slurry was transferred to a 1.5-ml microfuge tube and placed in a boiling water bath for 3 min. Electrophoretic analysis with subsequent silver staining was done as for purified disrupted virions.

Western blotting and immunological analysis of SDS-PAGE-analyzed proteins were done as described by Burnette (4) using a transblot cell (Bio-Rad Laboratories). After transfer, nitrocellulose membranes were washed and probed as previously described (11), except that alkaline phosphatase-congugated secondary antibody was used. BYV and BYSV antisera were diluted in TBST (20 mM Tris, 500 mM NaCl, 0.3% Tween 20, pH 7.5) (Bio-Rad Laboratories) to concentrations of 1:100 and 1:20, respectively, and used as primary antibodies. Alkaline phosphatase-conjugated goat-antirabbit antibodies were diluted in TBST 1:2,000 and used as the secondary antibody. The substrate was 5-bromo-4-chloro-3-indolyl in nitro blue tetrazolium, prepared as described by the manufacturer (Bio-Rad Laboratories). Blots were developed for 30 min then photographed using 35-mm Kodolitho #2556 type 3 high-contrast film (Eastman Kodak, Rochester, NY).

Analysis of double-stranded RNA.

Fig. 2. Silver-stained virion proteins of beet yellows virus (lane 1) and beet yellow stunt virus (lane 2) after analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis was at 200 V for 50 min. The darker staining, slower moving band in each lane represents the major capsid protein. The less intensely staining band with greater mobility is thought to be an in vitro or in vivo digestion product of the capsid protein subunit. The locations of molecular weight markers are shown at left: lysozyme (14,400), soybean trypsin inhibitor (21,500), carbonic anhydrase (31,000), ovalbumin (45,000), and bovine serum albumin (66,200).

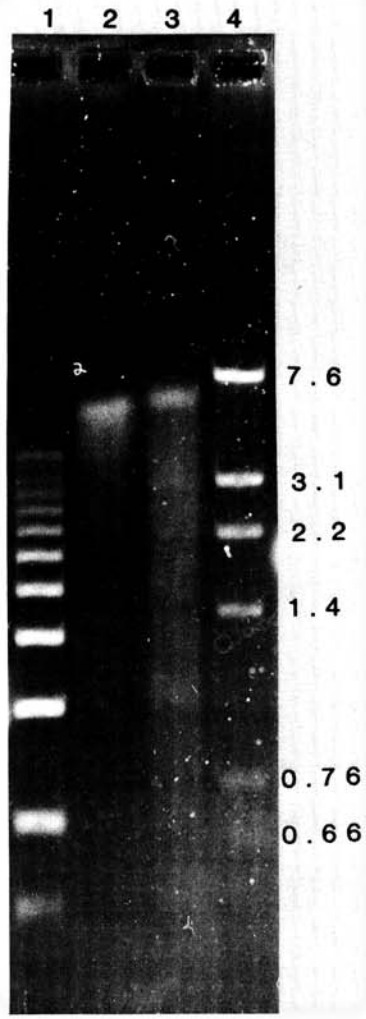

Fig. 3. Agarose gel (0.86%) electrophoresis of the virion single-stranded RNAs (ssRNAs) of beet yellows virus (BYV) and beet yellow stunt virus (BYSV). Nucleic acids were glyoxalated and electrophoresed in Hepes buffer at 100 V for 2 hr with one change of buffer after 80 min. Lane 1 contains 1 Kb DNA ladder, lane 2 contains BYV virion ssRNA, lane 3 contains BYSV virion RNA, and lane 4 contains bacteriophage lambda DNA digested with Hind III. Numbers at the right represent molecular weights ($\times 10^{-6}$) of bacteriophage lambda-digested DNA. Detection was by staining with ethidium bromide.

Double-stranded RNAs (dsRNAs) of citrus tristeza virus (CTV) and tobacco necrosis virus were kindly provided by R. Valverde. The dsRNAs were extracted from fresh leaf tissue (7 g) of virus maintenance plants and their healthy counterparts (*T. tetragonioides* for BYV, *C. capitatum* for BYSV) and purified by the CF-11 column method as described by Valverde et al (23). Samples were also treated by incubation at 37 C in 5 units of RNase-free DNase (Sigma, St. Louis, MO) (DNase stock at 2 μg/ml, an equal volume of proteinase K, and 1/15 vol of 0.45 M $CaCl_2$) per milliliter and 10 mM $MgCl_2$ (v/v) to remove residual DNA (22). Samples were stored either as an ethanol precipitate or in 1× electrophoresis buffer plus bromphenol blue as described by Valverde et al (23). The dsRNAs were analyzed by electrophoresis in 6% polyacrylamide gels (23). The dimensions of the polyacrylamide gels were the same as for protein gels, except the dsRNA gels were 65 mm long with no stacking gel. Electrophoresis times varied between 3.5 and 7 hr at 100 V constant voltage. Gels were stained in ethidium bromide as described earlier.

RESULTS

Virion purification. Virions of BYV and BYSV sedimented to nearly the same depth when analyzed in sucrose density gradients. In some gradients the virions of BYSV appeared to sediment slightly farther than those of BYV (Fig. 1). Preparations of both viruses showed significant virion aggregation, but this was always more pronounced for BYSV. Yields of virions were approximately 0.5–3 mg/100 g of tissue for BYV and 0.1–0.4 mg/100 g for BYSV using an extinction coefficient of 2.0 as reported by Bar-Joseph and Hull for BYV (2).

Electron microscopy. Electron microscopy of purified BYV and BYSV virions revealed very long, flexuous rods (*data not shown*). Both types of particles were estimated to be 13 nm in diameter. We did not attempt to measure particle length because of the extensive aggregation.

Analysis of virion proteins and nucleic acids. BYV and BYSV each showed one major and one minor protein upon analysis by SDS-PAGE. The molecular weight of the major protein was approximately 24,500 (BYSV) and approximately 24,000 (BYV) and of the minor protein approximately 23,800 (BYSV) and approximately 22,100 (BYV) (Fig. 2).

The virion RNAs migrated as single major species for both BYV and BYSV. The mobility of BYSV RNA was slightly less than that of BYV RNA. RNA molecular weights were estimated to be approximately 5.5 million for BYV and 6.1 million for BYSV using five glyoxalated DNA markers with molecular weights ranging from 1.4 million to 7.6 million (Fig. 3).

Serological analysis. In immunoblots BYSV capsid proteins failed to react with BYV antiserum but did react with homologous antiserum. Also, purified BYV capsid proteins did not react with BYSV antiserum but did react with homologous antiserum (Fig. 4). Identical results were obtained for each virus when total proteins were analyzed by this method (*data not shown*).

Double-stranded RNA analysis. A single major and three minor dsRNAs were seen for BYV and BYSV (Fig. 5). When electrophoretic separation was only for 3.5 hr, the upper major dsRNAs had very similar mobilities, while the minor dsRNAs had distinctly different mobilities for BYV and BYSV. Upon longer electrophoretic separation (7 hr), the mobilities of the major dsRNAs were also different. The major dsRNA of BYV migrated slightly farther than the major dsRNA of BYSV. The major BYSV dsRNA migrated less than 1 mm farther than the dsRNA of CTV. Based on published CTV dsRNA molecular weight of 13.3 million (7,23), we estimate the molecular weight of BYSV dsRNA at approximately 12.5–13 million and BYV molecular weight somewhere below this value.

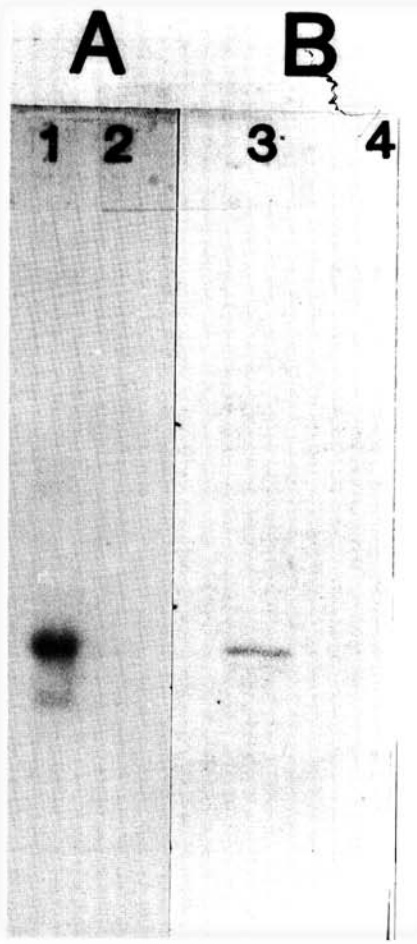

Fig. 4. Immunoblots of proteins of beet yellows virus (BYV) and beet yellow stunt virus (BYSV) analyzed by duplicate 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Each gel was loaded with the same concentration of disrupted purified virions as shown in Fig. 2. Electrophoresis was for 50 min at 200 V before transfer to 0.2 μm nitrocellulose membranes using a transblot apparatus at 50 V overnight at 4 C. Blot A was probed with BYV antiserum, blot B with BYSV antiserum. Lanes 1 and 4 are BYV, and lanes 2 and 3 are BYSV.

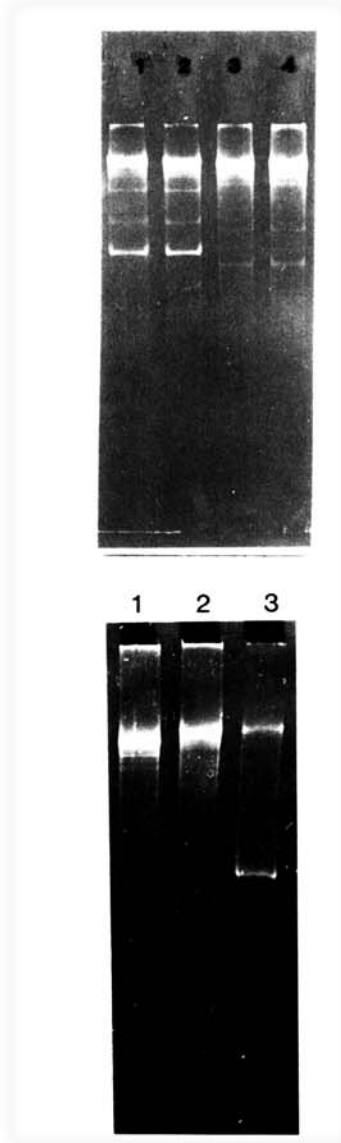

Fig. 5. Banding patterns (top) and relative mobility (bottom) of beet yellows virus (BYV) and beet yellow stunt virus (BYSV) double-stranded RNAs (dsRNAs) in 6% polyacrylamide gels. DsRNAs were extracted using a phenol/STE method and purified using the CF-11 column method as described by Valverde et al (23). One-half of the final eluent was treated with DNase as described in Materials and Methods; the other half was not. Upper panel: lane 1, BYSV; lane 2, BYSV after DNase treatment; lane 3, BYV; lane 4, BYV after DNase treatment. Lower panel: lane 1, BYV; lane 2, BYSV; lane 3, a combination of citrus tristeza virus (molecular weight 13.3 million) and tobacco necrosis virus (molecular weight 2.6 million). Electrophoresis was for 3.5 hr (top gel) and 7 hr (lower gel) at 100 V constant voltage. After electrophoresis, dsRNAs were detected by staining with ethidium bromide (10 ng/ml).

DISCUSSION

The physicochemical and serological studies reported here characterize BYSV and show that although BYV and BYSV are similar in several respects, they are distinct enough to warrant classification as two separate viruses. Their virions are long, flexuous rods. As pointed out by Bar-Joseph and Hull (2), such particle morphology is the hallmark of closteroviruses (*clostero* is Greek for "thread"). Our estimate of 24,000 for the molecular weight of the BYV coat protein subunit is in basic agreement with the data of others (2,5,6). We compared BYSV subunit protein with BYV and protein standards in sizing gels and estimated its molecular weight at 24,500. This estimate is within the range of protein subunit estimates for other members of the closterovirus group; for example, carnation necrotic fleck virus, 23,500 (3), and CTV, 23,000 (17).

In SDS-PAGE, the capsid proteins of BYSV and BYV always had a second, faster moving, minor protein component associated with the major protein subunit (Fig. 2). These bands were consistently associated with the virions. They were recognized by homologous antisera (Fig. 4) and were present in what appear to be nonstoichiometric ratios to the major protein band. Lee et al (17) noted similar bands for isolates of CTV and showed that the faster bands were either in vitro or in vivo digestion products of the major capsid protein. This is most likely also the case for the minor bands of BYSV and BYV.

Rybicki and Von Wechmar (21) demonstrated the utility of Western blot analysis in comparing serological relatedness within viral groups. Using this technique, we found that purified BYV and BYSV capsid proteins reacted only with homologous antisera. The serological data presented confirm earlier preliminary work by Duffus (9), which suggested a lack of cross-reactivity of BYV and BYSV using infected sap in precipitin tests. Tests also indicated that antiserum produced against virions of CTV (kindly provided by R. Lee, University of Florida) did not react with BYSV or BYV in Western blots, although it did react with CTV (*data not shown*). These results are consistent with the data summarized by Bar-Joseph et al (1), which show that some closteroviruses cross-react serologically but most do not.

BYSV and BYV were found to have ssRNAs of molecular weight 6.1 million and 5.5 million, respectively, based on comparisons with several standards analyzed in adjacent wells. BYV RNA was analyzed and compared with BYSV in all of our sizing gels and always migrated faster than the BYSV ssRNA. Our estimate of 5.5 million for the molecular weight of BYV ssRNA is higher than previous estimates by Bar-Joseph and Hull (2) (4.2–4.4 million), Carpenter et al (5) (4–4.8 million), and Chevallier et al (6) (4.15 million).

To confirm our ssRNA molecular weight estimates, we tried to compare the relative mobilities of viral dsRNAs. This approach assumes that the band with the lowest relative mobility in a dsRNA gel represents the replicative form of virion RNA. Therefore, this band is expected to be approximately twice the size of the single-stranded genomic RNA (7). As expected from our data on the ssRNAs of BYV and BYSV, the migration rate of the major BYV dsRNA is greater than that of the major BYSV dsRNA. Dodds and Bar-Joseph (7) also reported that dsRNA of BYV has a greater relative mobility in 6% polyacrylamide gels than dsRNA of CTV. They also estimated a molecular weight of 13.3 million for CTV dsRNA by measuring extended unbroken dsRNA molecules visualized by electron microscopy. BYSV migrated only slightly ahead of CTV (Fig. 5). This technique predicts a BYSV ssRNA molecular weight of around 6.5 million, which approximates the 6.1 million we estimated using glyoxalated DNA standards.

Our data show that BYSV and BYV, although similar, are distinct viruses. They and CTV seem to have similar physicochemical properties, which may prove to be typical for aphid-transmitted closteroviruses.

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LITERATURE CITED

1. Bar-Joseph, M., Garnsey, S. M., and Gonsalves, D. 1979. The closteroviruses: A distinct group of elongated plant viruses. Adv. Virus Res. 25:93-168.
2. Bar-Joseph, M., and Hull, R. 1974. Purification and partial characterization of sugar beet yellows virus. Virology 62:552-562.
3. Bar-Joseph, M., and Smookler, M. 1976. Purification, properties, and serology of carnation yellow fleck virus. Phytopathology 66:835-838.
4. Burnette, W. 1981. "Western blotting": Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio-iodinated protein A. Anal. Biochem. 112:195-203.
5. Carpenter, J. M., Kassanis, B., and White, R. F. 1977. The protein and nucleic acid of beet yellows virus. Virology 77:101-109.
6. Chevallier, D., Engel, A., Wrutz, M., and Putz, C. 1983. The structure and characterization of a closterovirus, beet yellows virus, and a luteovirus, beet mild yellowing virus, by scanning transmission electron microscopy, optical diffraction of electron images, and acrylamide gel electrophoresis. J. Gen. Virol. 64:1-5.
7. Dodds, J. A., and Bar-Joseph, M. 1983. Double-stranded RNA from plants infected with closteroviruses. Phytopathology 73:419-423.
8. Duffus, J. E. 1964. Beet yellow stunt virus. (Abstr.) Phytopathology 54:1432.
9. Duffus, J. E. 1972. Beet yellow stunt, a potentially destructive virus disease of sugar beet and lettuce. Phytopathology 62:161-165.
10. Duffus, J. 1973. The yellowing virus diseases of beet. Adv. Virus Res. 18:347-386.
11. Falk, B. W., Morales, F. J., Tsai, J. H., and Niessen, A. I. 1987. Serological and biochemical properties of the capsid and major noncapsid proteins of maize stripe, rice hoja blanca, and Echinochloa hoja blanca viruses. Phytopathology 77:196-201.
12. Falk, B., and Tsai, J. 1986. The two capsid proteins of maize rayado fino virus contain common peptide sequences. Intervirology 25:111-116.
13. Fraenkel-Conrat, H., Singer, B., and Tsugita, A. 1961. Purification of viral RNA by means of bentonite. Virology 14:54-58.
14. Hoefert, L. L., Esau, K., and Duffus, J. E. 1970. Electron microscopy of *Beta* leaves infected with beet yellow stunt virus. Virology 42:814-824.
15. Kassanis, B., Carpenter, J., White, R., and Woods, R. 1977. Purification and some properties of beet yellows virus. Virology 77:95-100.
16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.
17. Lee, R. F., Calvert, L. A., and Hubbard, J. D. 1984. Characterization of the coat proteins of citrus tristeza virus. (Abstr.) Phytopathology 74:801.
18. Lister, R. M., and Bar-Joseph, M. 1981. Closteroviruses. Pages 809-844 in: Handbook of Plant Virus Infections and Comparative Diagnosis. E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, Amsterdam.
19. McMaster, G., and Carmichael, G. 1977. Analysis of single and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Natl. Acad. Sci. USA 74:4835-4839.
20. Morrisey, J. H. 1981. Silver stain for protein in polyacrylamide gels. A modified procedure with enhanced uniform sensitivity. Anal. Biochem. 117:307-310.
21. Rybicki, E., and Von Wechmar, B. 1982. Enzyme-assisted immune detection of plant virus proteins electroblotted onto nitrocellulose paper. J. Virol. Methods 5:267-278.
22. Tullis, R., and Rubin, H. 1980. Calcium protects DNase I from proteinase K: A new method for the removal of contaminating RNase from DNase I. Anal. Biochem. 107:260-264.
23. Valverde, R. A., Dodds, J. A., and Heick, J. A. 1986. Double-stranded ribonucleic acid from plants infected with viruses having elongated particles and undivided genomes. Phytopathology 76:459-465.