Purification, Characterization, and Serological Analysis of Maize Stripe Virus in Taiwan

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ABSTRACT

An isolate of maize stripe virus (MSV) was identified in Taiwan (MSV-T). The 34.7-kDa capsid protein and three noncapsid proteins (19.8, 20.4, and 22.5 kDa) purified from MSV-T-infected corn plants were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and used as antigens for comparison with the capsid protein (35.0 kDa) and noncapsid protein (19.8 kDa) of maize stripe virus from Florida (MSV-FL). The antiserum to the 19.8-kDa capsid protein of MSV-FL reacted with the plants infected with MSV-T and purified MSV-T in indirect and double-antibody sandwich (DAS) enzyme-linked immunosorbent assays (ELISA); the antiserum to the 19.8-kDa noncapsid protein of MSV-FL reacted more strongly in indirect ELISA than in DAS-ELISA. Analysis by Western blots showed that the MSV-FL antiserum reacted only with the capsid proteins from MSV-T, MSV-FL, and rice stripe virus (RSV); the noncapsid protein antiserum to MSV-FL reacted only with the noncapsid proteins of MSV-FL and MSV-T. The MSV-T virions were seen as fine-stranded particles with a helical structure, measuring about 12 nm in diameter and up to 709 nm long. The biology and characteristics of MSV-T confirm that this is a member of the tenuivirus group.

A virus-like disease of corn was first discovered in central Taiwan in 1987 (2). Infected plants are severely stunted with chlorotic stripes along the veins. The chlorotic bands and/or total discoloration form progressively on the upper leaves. The causal agent is transmitted by the corn planthopper Peregrinus maidis (Ashmead) in a persistent manner (2). This disease is most prevalent in white corn during winter months, especially where corn is grown in a small area and little or no insecticide is applied. In addition to infecting various cultivars of corn, the virus also is reported to infect wheat (25). The symptomatology, vector specificity, transmission characteristics, and host range of the virus resemble those described for maize stripe virus (MSV) in Florida (MSV-FL) (8,22), Australia (11), and East Africa (17). A preliminary result of purification of MSV from Taiwan was reported earlier (4). This paper reports on the purification, physicochemical properties, and serology of a Taiwan isolate of MSV (MSV-T).

MATERIALS AND METHODS

Virus culture and maintenance. MSV-T was maintained on white corn (Zea mays L. ‘Tai-nung 351’) in a greenhouse by periodic transfers to new plants from the corn planthopper P. maidis. Virus-free planthoppers were reared in an insect-rearing room at 25-30 C and 24 hr of light/day in aluminum frame cages covered with nylon nets. A group of second and third instar P. maidis nymphs were given 2 days’ acquisition access feeding on infected plants and held on healthy corn plants for another 2 wk to allow for incubation of virus in the vector. Three to five corn planthoppers were placed on each healthy corn seedling for a 2-day inoculation access period, and the inoculated corn seedlings were kept in a greenhouse for symptom development. Leaves from 3- to 4-wk-old plants were used for all experiments. Healthy P. maidis were periodically sampled and tested for accidental contamination.

Purification of nucleoprotein. The purification of MSV-T nucleoprotein was essentially the same as described by Gingery et al (9) and Falk and Tsai (6) with some modifications. Symptomatic leaves were collected and stored at -75 C until used. About 500 g of frozen leaves was triturated in a blender in three volumes of cold extraction buffer (0.1 M potassium phosphate buffer [PPB], 0.01 M EDTA, 0.5% 2-mercaptoethanol, 200 µg/ml of bentonite [pH 7.2]). The homogenate was squeezed through four layers of cheesecloth, clarified by adding carbon tetrachloride to a final concentration of 20%, and stirred for 10 min. The emulsion was centrifuged in a rotor at 5,900 g for 10 min (Hitachi RPR 12-2). The supernatant was centrifuged through a 20% sucrose (w/v) cushion in IX saline sodium citrate solution (SSC; 0.15 M sodium chloride, 0.015 M sodium citrate [pH 7.0]) at 96,000 g in a rotor for 2.5 hr (Hitachi RP 30-2). The resulting pellets were resuspended in IX SSC and kept overnight at 4 C. The suspension was then treated with 1% Triton X-100 and centrifuged in a rotor at 7,700 g for 10 min (Hitachi RPR 20-2). The supernatant was subsequently layered on 10-40% (w/v) linear sucrose density gradients in SSC buffer and centrifuged at 155,650 g in a Hitachi RPS 40 T rotor for 90 min. The nucleoprotein band was removed and diluted in 0.01 M PPB and centrifuged at 243,000 g for 1 hr. Pellets were resuspended in 0.1X SSC for electrophoresis analysis or serological assay. For electron microscopy or serology studies, the partially purified virus preparation was subjected to another cycle of isopycnic centrifugation in a 55% Cs2SO4 (w/w) gradient at 62,300 g in a Hitachi 65 T rotor for 20 hr and concentrated as described above.

Pathogenicity of purified virus. The pathogenicity test was performed by injection and membrane feeding of the insects with purified virus. The technique used for injecting insects was similar to the methods described by Hsieh and Roan (13) and Tsai and Zitter (23). A group of 120 fourth or fifth instar nymphs was first anesthetized with CO2, and about 0.02 µl of inoculum (A260 = 1.0) was injected into each insect through the intersegmental membrane between thoracic segments. An equal number of healthy P. maidis were injected with 0.01 µl of PPB (pH 7.2) as controls. For the membrane feeding test, a group of 60 third and fourth instar nymphs was first starved for 2 hr and then placed in a 6-cm glass ring (5-cm diameter) with a screen covering one end. Stretched Parafilm membranes covered the other end. About 3 ml of purified virus preparation was placed between the stretched membranes. The insects were given a 24-hr acquisition access feeding at 24±1 C. As a control, a group of 60 third and fourth instar nymphs was fed on sterilized water. After injection and acquisition access feeding, the insects were held on healthy corn for the incubation period, which was followed by individual inoculation on healthy corn seedlings.

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Purification of noncapsid protein. The noncapsid protein associated with MStV-T was concentrated by the differential pH method (6,9). About 10 g of fresh tissue was ground in a blender in two volumes of phosphate-citrate buffer (0.2 M K2HPO4 and 0.1 M citric acid), pH 5.0. The macerate was squeezed through four layers of cheesecloth and kept overnight at 4 C. The filtrate was centrifuged at 12,000 g for 30 min (Hitachi rotor RPR 20-2). Pellets were resuspended in 5 ml of phosphate-citrate buffer, pH 7.0, and centrifuged for 10 min at 12,000 g. The supernatant was mixed with an equal volume of phosphate-citrate buffer, pH 5.0, and kept overnight at 4°C to allow for maximum recrystallization. The previous steps were repeated three to five times, and proteins were resuspended in sterilized distilled water and kept at 4 C.

Protein analysis. Molecular weights were estimated by the method of Laemmli (19). Purified capsid and noncapsid proteins were denatured in loading buffer containing 2% sodium dodecyl sulfate (SDS), 10% Ficol, 60 mM Tris-HCl (pH 8.8), 2 mM EDTA, and 0.1% bromophenol blue and heated for 10 min at 65 C. Electrophoresis was carried out in 10–15% polyacrylamide slab gels (polyacrylamide/bisacrylamide, 30:0.8, w/w) run for 6–8 hr at 100 V, 9.5 cm long × 13 cm wide, using a running buffer (0.05 M Tris-HCl pH 8.3, 0.38 M glycine, and 0.1% SDS). After SDS-polyacrylamide gel electrophoresis (SDS-PAGE) the gels were stained with brilliant blue R. Molecular weights were estimated relative to phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and L-alactalbumin (14,400) (Pharmacia, no. 17-0446-01).

Serological assays. Separate leaf samples from MStV-T-infected and healthy plants homogenized with phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween and purified preparations adjusted to a concentration of A260 = 1.0 were tested by direct ELISA as described by Clark and Adams (5). Antisera to the Florida isolate of MStV (MStV-FL) 35.0-kDa capsid protein and 19.8-kDa noncapsid protein were used throughout this study (6,14,15). The ELISA plate was coated with immunoglobulin G (IgG) at 1 μg/ml, and IgG-alkaline phosphatase conjugate was diluted 1,000 times. Indirect ELISA was done according to the methods of Vollert et al (24) and Koenig (18). Plates were coated with purified or extracted antigen in coating buffer. Plates were washed, and purified IgG-Ag was added at 1 μg/ml in PBS-Tween that was added for 3 hr. After washing, antirabbit IgG-alkaline phosphatase from goat (Boehringer Mannheim, Indianapolis, IN) at 1:8,000 dilution in PBS-Tween (PBST) containing 0.1% Tween-40 was added. Plates were washed and nitrophenyl phosphatase (0.6 mg/ml) in diethanolamine buffer was added. Reactions were photometrically evaluated at 405 nm with a microplate reader (model EL-311, BioTek Instruments, Burlington, VT).

Western blot analysis was done by the method of Burnette (1). Blots were washed for 30 min in 6 M urea-PBST followed by three 10-min washes in PBST. Antiserum dilutions in PBST of 1/250 for the 35.0-kDa and 1/500 for the 19.8-kDa antisera were added to the washed blots and incubated with shaking for 2 hr. Blots were then given three washes of 10 min in PBST and then incubated in protein A conjugated with horseradish peroxidase diluted 1/3,000 in PBST for 2 hr. Blots were again washed, 50 ml of substrate (0.1 mg/ml dianisidinobenzidine in 0.02% hydrogen peroxide) was added, and blots were kept at 24 ± 1°C for 3–5 min. Blots were removed from the substrate, briefly rinsed in distilled water, allowed to dry, and photographed.

Electron microscopy. Purified preparations of MStV-T and healthy corn leaves were used for electron microscopy. One drop of purified preparation of MStV-T and one drop of 0.1% bacitracin were mixed on a Parafilm membrane, and one drop of 2% glutaraldehyde was added. The mixture was subsequently transferred to a carbon-coated Formvar membrane on grid. After 1 min, the unabsorbed mixture was removed by filter paper, and the grid was washed with 1–2 drops of distilled water and stained with 2% uranyl acetate, pH 4.3. The stained specimens were examined in a JEOL 200 CX electron microscope at 80 kV. The size measurements were based on those of tobacco mosaic virus particles.

RESULTS

Purification of nucleoprotein. Purified nucleoprotein formed a single opaque band typical of a virus-containing zone obtained by CsSO4 density gradient centrifugation. The ultraviolet absorption spectra had a maximum absorbance at 259 nm and a minimum at 238 nm. The A260/A230 ratio was 1.40 ± 0.13. The yields of purified MStV-T were 36.7–127 μg per gram of MStV-T-infected tissue, depending upon the season. No proteins similar to the virus capsid and noncapsid proteins were observed in corresponding preparations from healthy control plants.

Pathogenicity of purified virus. Of a total of 120 fourth and fifth instar nymphae injected with purified MStV-T, 78 insects survived after 2 wk. They were caged singly on corn plants, and 17 became infective. In the same experiment, 120 nymphae were injected with sterile water, and 75 of these insects survived after 2 wk. None of the 75 test plants were infected. In membrane feeding tests, 5 of 60 (8.3%) insects became infective, and none of the 60 control insects were infective.

Purification of noncapsid protein. Large quantities of noncapsid protein were purified from the MStV-T tissue. Two or three bands formed in the sucrose gradients. The ultraviolet absorption spectra had a maximum at 270–279 nm and a minimum at 250–255 nm.

Protein analysis. One capsid protein with an estimated molecular mass of 34.7 kDa was obtained. Purified MStV-T was isolated by SDS-PAGE. With the noncapsid protein purified from MStV-T-infected leaves electrophoresed in the same gel, three proteins with molecular masses estimated at 19.8, 20.4, and 22.5 kDa were resolved (Fig. 1).

Serological tests. In the indirect ELISA with purified MStV-T (A260 = 1.0) as antigen, the reaction dilution end points against two MStV-FL capsid antisera were measured at 10^-4 (Fig. 2A). All antisera failed to react with healthy controls. Specific reactions to crude leaf extracts at dilutions up to 10^-4 were also recorded in the indirect ELISA (Fig. 3A).

In the DAS-ELISA with purified virus (A260 = 1.0) as antigen, specific reactions to two MStV-FL capsid antisera were obtained at dilutions up to 10^-4 (Fig. 2B); whereas, using crude sap of infected MStV-T leaves as antigen, positive reactions to two MStV-FL antisera were detected at dilutions of 10^-3 and 10^-4 (Fig. 3B).

The antisera to the 19.8-kDa noncapsid protein of MStV-FL was also used.
for detecting the MSTv-T by indirect ELISA and DAS-ELISA. The 19.8-kDa antiserum of MSTv-FIL gave very strong reactions with crude sap and the noncapsid protein of MSTv-T by indirect ELISA (Fig. 4) but gave a very weak reaction by DAS ELISA.

A serological relationship between MSTv-T and MSTv-FIL was also evident from immunoblotting tests on the 34.7-kDa capsid and 20.4- and 22.5-kDa noncapsid proteins obtained by SDS-PAGE. The antiserum of the 35.0-kDa capsid protein of MSTv-FIL only reacted with the antigen of the 34.7-kDa capsid protein of MSTv-T but not with the noncapsid protein. On the other hand, specific reactions only occurred between the antiserum of 19.8-kDa noncapsid protein of MSTv-FIL against the 20.4- and 22.5-kDa noncapsid proteins of MSTv-T but not the 34.7-kDa capsid protein of MSTv-T (Fig. 5). Western blot analysis using the antiserum to the 35.0-kDa capsid protein of MSTv-FIL against the nucleoprotein preparations from MSTv-T, rice stripe virus (RSV), and rice wilted stunt virus (RWSV) (3) showed that the capsid protein antiserum of MSTv-FIL had a specific reaction with the capsid proteins of MSTv-T and RSV but not with that of RWSV (Fig. 6). RWSV is suspected to be a phalanptus-transmitted tenuivirus (3). This indicates the existence of serological relationships among MSTv-T, MSTv-FIL, and RSV.

**Electron Microscopy.** Initial attempts to negatively stain the purified MSTv-T preparation with 2% uranyl acetate without fixation always led to severe aggregation and poor resolution of virus particles. However, when purified MSTv-T was first fixed with 2% glutaraldehyde and then stained with uranyl acetate, the MSTv-T particles consistently appeared to be helical structures about 12 nm (10-13 nm) wide and of various lengths. The longest circular filament was measured at about 709 nm (Fig. 7). Most of filaments were in the range of 150-300 nm (Fig. 8). Occasionally, a helical organization of the stretched region of the filamentous nucleoprotein was observed on some negatively stained specimens without fixation (Fig. 9). This suggests that the helical structure may be the virion morphology of MSTv-T, and the stretched filamentous structure may be an artifact of staining procedures.

**DISCUSSION**

With SDS-PAGE we consistently found a major polypeptide of 34.7 kDa. This 34.7-kDa capsid protein appears to be similar to that of MSTv-FIL (15). Both were detected primarily in the high-speed pellet fractions from infected tissue (6,9). The noncapsid protein purified from MSTv-T-infected roots leaves separated into either two or three bands by SDS-PAGE, corresponding to molecular masses of

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**Fig. 2.** Absorbance (405 nm) values for (A) indirect and (B) double-antibody sandwich enzyme-linked immunosorbent assays with purified virus preparations of MSTv-T adjusted to an initial concentration of 400 ml/ml reacting with 35.0-kDa capsid protein antiserum (x) and with another 35.0-kDa capsid protein antiserum (o) of MSTv Florida isolate (MSTv-FIL) at 1 µg/ml; extract dilutions purified by the same procedures from healthy plants reacting with antiserum to the 35.0-kDa capsid protein of MSTv-FIL (c).

**Fig. 3.** Absorbance (405 nm) values for (A) indirect and (B) double-antibody sandwich enzyme-linked immunosorbent assays with crude sap samples of MSTv-T reacting with 35.0-kDa capsid protein antiserum (x), another 35.0-kDa capsid protein antiserum (o), and 19.8-kDa noncapsid protein antiserum (∆) of MSTv-FIL at 1 µg/ml; healthy sap dilutions reacting with antiserum to the 35.0-kDa capsid protein of MSTv-FIL (c).
sequences for the noncapsid protein genes (14). We demonstrated that in the Western blotting analysis the antiserum reacted only with the proteins used as immunogens. The MSV-FL noncapsid protein antiserum reacted with purified MSV-T noncapsid protein (19.8 and 22.5 kDa) but did not react with MSV-T capsid protein. We also confirmed that indirect ELISA could readily detect MSV-T-infected plants when the noncapsid protein antiserum was used (6). Conversely, the MSV-FL capsid protein antiserum reacted only with purified capsid proteins of MSV-T and RSV. These results are in general agreement with the findings of Falk et al (7) and Gingery et al (10).

Gingery et al (9) characterized a fine-stranded, filamentous particle associated with MSV-FL about 3 nm in diameter and of various lengths. In the initial attempts to stain MSV-T preparations with uranyl acetate, we found only mass aggregations with very few virus particles. When purified MSV-T was promptly fixed with 2% glutaraldehyde just before staining with uranyl acetate, helical

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**Fig. 4.** Absorbance (405 nm) values for indirect enzyme-linked immunosorbent assays using the 19.8-kDa noncapsid protein antiserum of MSV-FL. Dilutions of noncapsid proteins of MSV-T purified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (A); sap dilutions of the MSV-T-infected () and healthy () corn plants.

**Fig. 5.** Western blot tests of the capsid and noncapsid proteins of MSV-T. (A) Sodium dodecyl sulfate-polyacrylamide (15%) slab gel showing capsid protein (C), noncapsid protein (N), and marker (M). (B) The blot was probed with the 35.0-kDa capsid protein MSV-FL. (C) The blot was probed with the 19.8-kDa noncapsid protein of MSV-FL.

**Fig. 6.** Western blot tests of the Taiwan isolate of maize stripe virus (MS), rice stripe virus (RS), and rice wilty stunt virus (RW). (A) Sodium dodecyl sulfate-polyacrylamide (15%) slab gel showing capsid proteins. (B) Blots probed with the 35.0-kDa capsid protein of MSV-FL.

**Fig. 7.** (A and B) Electron micrographs of purified preparations of the Taiwan isolate of maize stripe virus (MSV-T). Samples were fixed with 2% glutaraldehyde, negatively stained with 2% uranyl acetate, and recovered from Cs2SO4 isopycnic centrifugation at 62,300 g for 20 hr. Threadlike particles appear clumped. Scale bars = 100 nm.
Fig. 8. Length distribution of circular filamentous particles observed in purified preparations of MSTv-T prefixed with 2% glutaraldehyde and stained with 2% uranyl acetate.

Fig. 9. (A, B, and C) Electron micrographs of purified preparations of MSTv-T. Samples were negatively stained with 2% uranyl acetate without fixation. (B) is a higher magnification of the upper left part of (A); some helical structures (arrow) appeared to be constructed of a filament, about 12 nm in width. Scale bars = 100 nm.

On the basis of symptomatology, morphology, transmission characteristics, host range, physicochemical properties, and serology, we concluded that MSTv-T is serologically related to MSTv-FL and is a member of the tenuivirus group (8,9).

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LITERATURE CITED


