

Oat mosaic virusの純化ならびに諸性質について

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	宇杉,富雄 斎藤,康夫
発行元	日本植物病理學會
巻/号	47巻4号
巻号補足	
掲載ページ	p. 581-585
発行年月	1981年9月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Purification and Some Properties of Oat Mosaic Virus

Tomio USUGI* and Yasuo SAITO*

宇杉富雄*・斎藤康夫*: Oat mosaic virus の純化ならびに諸性質について

Oat mosaic virus (OMV), with its flexuous, rod-shaped particles about 600-750 nm long and 12-14 nm wide^{1,2,3)}, is transmitted through soil by the fungus, *Polymyxa graminis*⁴⁾. The virus is mechanically transmissible, but with some difficulties. Host range of the virus is restricted to *Avena* species⁴⁾. Virus-infected mesophyll cells contain cylindrical inclusions with pinwheels and bundles or parallel aggregates of virus particles⁴⁾. In oat sap, thermal inactivation point is at 44-46 C for 10 minutes, longevity *in vitro* is between 24-48 hours at 20 C, and the dilution end-point is between 1/100 and 1/1,000 when tested infectivities by the rubbing method⁵⁾. These reported properties of OMV are similar to those of wheat spindle streak mosaic virus (WSSMV)⁶⁾, wheat yellow mosaic virus (WYMV)^{7,8)}, barley yellow mosaic virus (BYMV)⁹⁾ and rice necrosis mosaic virus (RNMV)^{10,11)}. However, purification of OMV and preparation of antiserum against OMV has not been reported. As the details of the properties of OMV are lacking, the relationship between OMV and other viruses with similar properties is still uncertain. This paper describes the results of purification, serology and electron microscopy of this virus.

OMV used in this study was kindly supplied by Dr. T. T. Hebert, North Carolina State University, U. S. A. (import permit number, 49-Y-1191). The virus was maintained, propagated and assayed in the oat variety Taiho by mechanical inoculation. Mechanical inoculations were made by grinding young leaves in 0.1 M phosphate buffer pH 7.0 containing 0.001 M KCN, and rubbing leaves of the test plants at one- to two-leaf stage with the extracts containing carborundum. After inoculation, all the plants were placed in a growth chamber at 10-15 C.

Infected oat plants were ground with a mortar and a pestle in 0.1 M phosphate buffer pH 7.0 containing 0.001 M KCN. The juice was squeezed through one layer of gauze. One volume of organic solvent was added to four volumes of the leaf extract and the mixture was stirred for 15 min. The emulsion was centrifuged at about 10,000 × *g* for 15 min, then the aqueous phase was used for inoculation to oat plants. The infectivity

* Institute for Plant Virus Research, Tsukuba Science City, Ibaraki 305, Japan 農林水産省植物ウイルス研究所

1) Catherell, R. L. and Hay, J. D. (1970). Pl. Path. 19: 78-81. 2) Gold, A. H., McKinney, H. H. and Scott, H. A. (1959). Proc. 4th Int. Congr. Crop Prot., Hamburg, 1959. 1: 351-354. 3) Macfarlane, I., Jenkins, J. E. and Melville, S. C. (1968). Pl. Path. 17: 167-170. 4) Hebert, H. H. and Panizo, C. H. (1975). C. M. I. /A. A. B. Descriptions of Plant Viruses 145, 4pp. 5) Toler, R. W. and Hebert, T. T. (1964). Phytopathology 54: 428-433. 6) Slykhuis, J. T. (1976). C. M. I. /A. A. B. Descriptions of Plant Viruses 167, 4 pp. 7) Saito, Y., Tsuchizaki, T. and Hibino, H. (1968). Ann. Phytopath. Soc. Japan 34: 347. 8) Inouye, T. (1969). Nogaku Kenkyu 53: 61-68. 9) Inouye, T. and Saito, Y. (1975). C. M. I. /A. A. B. Descriptions of Plant Viruses 143, 4 pp. 10) Inouye, T. (1968). Ann. Phytopath. Soc. Japan 34: 301-304. 11) Inouye, T. (1979). *Ibid.* 36: 186.

Table 1. Effects of organic solvents on the infectivity of OMV

Treatments	Infectivity	
	Experiment 1	Experiment 2
Chloroform	3/17 ^{a)}	16/19
Butanol	0/18	— ^{b)}
Carbon tetrachloride	5/21	17/19
Ethanol	2/24	—
Control	5/12	22/23

a) No. of infected plants/No. of inoculated plants

b) Not tested

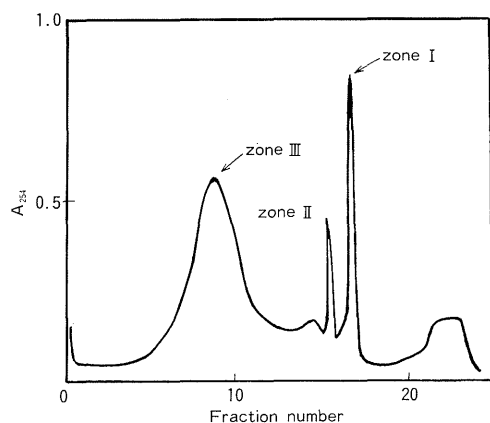


Fig. 2. Scanning profile at 254 nm (ISCO density-gradient fractionator) of an equilibrium density-gradient in cesium chloride

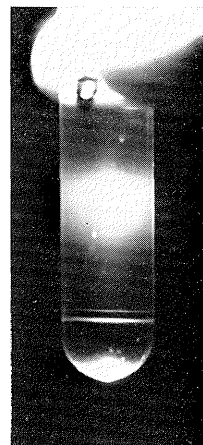


Fig. 1. Photograph of an equilibrium density-gradient in cesium chloride into which a partially purified preparation of OMV has been centrifuged

of the extract was lost after butanol treatment, but after the other solvent treatments the infectivity was preserved. Particularly, in the preparations treated by carbon tetrachloride or chloroform, the infectivity was well preserved and good clarification was obtained (Table 1).

Purification of OMV was performed according to the method of Usugi and Saito (1976)¹²⁾. Inocu-

lated oat plants were harvested 5–7 days after the first symptoms appeared. The plants were ground in 3 ml of 0.1 M citrate buffer pH 7.0 per gram of leaves with a small meat grinder. The sap was expressed through one layer of gauze to remove the fibers. The preparation was kept cold during this procedure and the subsequent steps. One volume of carbon tetrachloride was added to four volumes of the extract and stirred vigorously for 15 min. The emulsion was centrifuged at about $3,000 \times g$ for 15 min. The top aqueous phase was centrifuged at about $10,000 \times g$ for 15 min. The supernatant was centrifuged at $70,000$ or $100,000 \times g$ for 60 min. The precipitate was resuspended in the same buffer, then centrifuged at about $10,000 \times g$ for 15 min. The supernatant was subjected to 3–4 cycles of differential centrifugation. The final supernatant was designated as the partially purified preparation.

Equilibrium density-gradient centrifugations were made in cesium chloride in a Hitachi SW 40 rotor as follows: 1.33 g of CsCl was added to 4 ml of the partially purified preparation and dissolved. The tubes were centrifuged for 20 hours at $36,000$ rpm at 4°C .

12) Usugi, T. and Saito, Y. (1976). Ann. Phytopath. Soc. Japan 42: 12–20.

After centrifugation, the partially purified preparations formed three bands in CsCl gradients (Fig. 1). These bands were designated as zone I, zone II and zone III. Zone I was a prominent opalescent band, zone II was a faint opalescent one at 1–2 mm above zone I and zone III was a broad and faintly brownish band. Zone II and zone III were observed when preparations from both healthy and diseased plants has been subjected to centrifugation. However, zone I was observed in preparations from diseased plants but not from healthy ones. The buoyant density for zone I was 1.30 g/ml (in cesium chloride in 0.1 M citrate buffer). The absorbancy profile obtained with an ISCO density-gradient fractionator showed three peaks (Fig. 2). Zone I was removed from the density-gradient tubes by withdrawing samples with hypodermic syringes. But it was difficult to remove zone I without bringing along contamination from zone II because of the close buoyant densities of the two zones. Zone I was diluted with the buffer and then centrifuged at $135,000\times g$ for 60 min. Virus pellets were resuspended in citrate buffer or phosphate buffered saline. These virus preparations were designated as the purified preparation.

A small amount of the purified preparation suspended in citrate buffer was inoculated to oat plants. Ten or fourteen days after inoculation, 25 of the 45 plants inoculated showed the typical symptoms of OMV.

The purified preparations were shadowed with chromium, and examined with a Hitachi H-500 electron microscope. The electron micrographs showed that the virus particles were markedly flexuous rods and the particle length was quite variable (Fig. 3). The histogram of particle length distribution of a partially purified preparation showed that the majority of the particles were 250–550 nm long and a few particles were 550–1,000 nm long (Fig. 4). The virus particles were degraded in 1 % sodium phosphotungstate at pH 7.0 unless the preparations were previously fixed with 3.5 % formaldehyde. However, in the preparations stained with 2 % ammonium molybdate or 1 % uranyl acetate, intact virus particles could be observed without fixation by

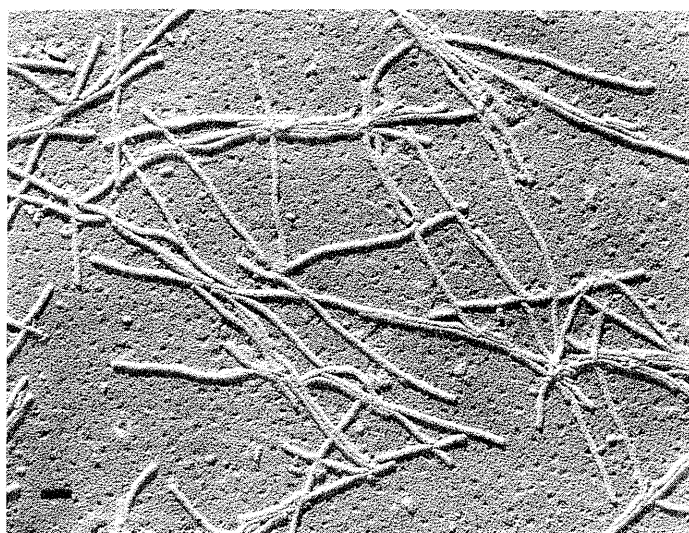


Fig. 3. Electron micrograph of purified preparation of OMV. Bar represents 100 nm.

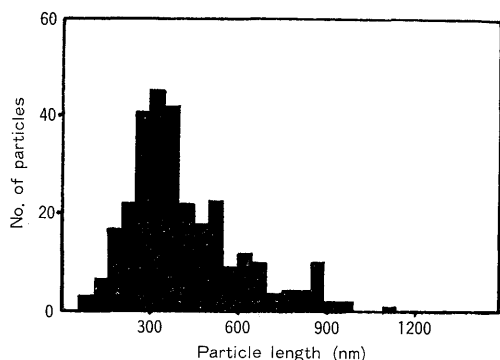


Fig. 4. Particle length distribution of OMV in partially purified preparation

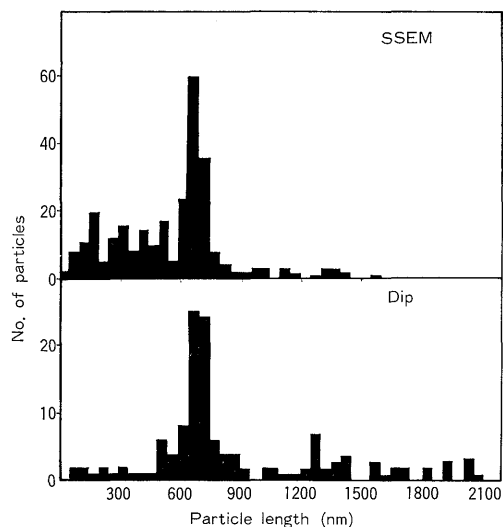


Fig. 5. Particle length distribution of OMV in leaf-dip and SSEM preparations

formaldehyde.

Normal length determinations were made in samples prepared by the dip method and serologically specific electron microscopy (SSEM), using TMV as a standard of 300 nm. The SSEM method was applied according to Derrick and Bransky (1976)¹³⁾. Anti-OMV serum prepared in this study was diluted 1:1,000 with tris buffer (0.05 M, pH 7.2), and the virus extract was diluted 1:50 with tris buffer (0.05 M, pH 7.2) containing 0.15 M NaCl. After allowing the reaction to stand for 1 hour, the grids were shadowed with chromium. The distribution of the particle length is shown in Fig. 5. These results indicate that the normal length of OMV ranges between 650 and 750 nm which differs from that of WYMV, WSSMV, BYMV and RNMV.

An antiserum was prepared by injecting purified preparations into a rabbit. The rabbit was injected intramuscularly twice with the purified preparations emulsified with Freund's complete adjuvant and then intravenously once with the purified preparation. The antiserum had an antibody titer of 1/320 against OMV and 1/160 against the host constituents in the complement-fixation test. Serological relationships among

Table 2. Serological relationship among OMV, WYMV, WSSMV and BYMV

Virus	Titer ^{a)} obtained with antisera for the virus indicated			
	OMV	WYMV	WSSMV	BYMV
OMV	320	20	10	80
WYMV	80	1280	320	80
WSSMV	80	640	320	80
BYMV	80	320	80	320
Healthy component	0	0	0	0

a) Figures are reciprocals of the dilution end points of the antisera in complement-fixation tests.

13) Derrick, K. S. and Bransky, R. H. (1976). *Phytopathology* 66:815-820.

OMV, WYMV, WSSMV and BYMV were investigated using these viruses and the antisera against the viruses. WSSMV was kindly supplied by Dr. J. T. Slykhuis (import permit number, 49-Y-1211). To absorb host constituent antigenic components, all the antisera used were mixed with healthy wheat sap concentrated by 1 cycle of differential centrifugation before use. All the virus antigens were partially purified according to the same method described this paper. Serological tests were performed by complement-fixation test. As a result, in all kinds of the combinations of viruses and antisera, positive reactions were observed. Homologous reactions had higher dilution end points than heterologous reactions (Table 2). These results suggest that the viruses are serologically related but distinct from each other.

In view of the results so far obtained, it appears that the soil-borne filamentous viruses belong to the same group. However, OMV is slightly different from the other viruses especially in its particle morphology.

(Received April 20, 1981)