Purification of Arabis Mosaic Virus Isolated from a Jasmine Plant Introduction

H. E. Waterworth

Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, Plant Introduction Station, Glenn Dale, Maryland 20769.

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ABSTRACT

A virus serologically identified as arabis mosaic (ArMV) was isolated from a symptomless *Jasminum mesnyi* (*J. primulinum*) introduction. Its experimental host range and physical properties were similar to that of previously described ArMV. The best of several comparative purification procedures, in terms of virus yield, involved blending tissue in phosphate buffer and chloroform and concentrating the virus by ultracentrifugation. A rabbit injected with purified virus produced antisera with a titer of 1:1,024. About 60% of the RNA was recovered when extracted using both sodium dodecylsulfate and phenol. Base ratio of the RNA was A = 24.9%, C = 25.6%, G = 21.6%, and U = 27.9%.

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Viruses were isolated from petals and immature leaves of *Jasminum mesnyi* Hance (*Jasminum imulinum* Hemsl.) being introduced into the U.S. from Scotland. Studies showed that the jasmine plants were infected with two viruses serologically identified as tobacco ringspot and arabis mosaic (ArMV-J). This report includes new data on purification methods (1) and on properties of ArMV-J ribonucleic acid (RNA).

MATERIALS AND METHODS.—Virus antisera were supplied to ArMV from celery (4) by D. Walkey, to ArMV from Sambucus by M. Hollings, and to ArMV from rhubarb by R. Stace-Smith. Sources of anion exchange resin for base ratio studies, other specialized chemicals, and descriptions of equipment used in this study are the same as reported earlier (5).

Purification.—Tissue of *Chenopodium quinoa* Willd. inoculated 2 weeks earlier with ArMV-J was blended in 0.025 M to 0.1 M potassium phosphate buffer (pH 7.0-8.0), with 0.02 M 2-mercaptoethanol and cold chloroform in the ratio of 1:3:0.2 (v/v). The slurry was centrifuged at 10,000 *g* for 10 minutes. Virus in the clear amber supernatant was then concentrated by: (i) centrifugation at 105,000 *g* for 75 minutes, (ii) precipitation with 10% (v/w) polyethylene glycol (PEG) M. wt. 6,000, or (iii) precipitation with 50% saturated ammonium sulfate. In the PEG procedure, the preparation was then centrifuged at 12,000 *g* for 10 minutes, usually 30 minutes after the PEG had been dissolved by stirring at 4 C. Concentrated virus pellets were resuspended in 0.04 M potassium phosphate, combined, and after 4-16 hours, centrifuged at 8,000 *g* to remove insoluble plant materials.

Various combinations of extraction-clarification-concentration procedures were compared, in terms of virus yield and amount of host plant material, using density-gradient centrifugation. Equal volumes of buffer containing the concentrated virus and plant proteins variously prepared were layered onto adjacent sucrose gradients and centrifuged 2 hours at 95,000 *g*. Virus in gradient fractions was bioassayed and then reconcentrated by ultracentrifugation after the sucrose was removed by dialysis. Pellets were resuspended in 0.04 M sodium phosphate buffer, checked for purity and particle types with the electron microscope, and for amount of virus with the spectrophotometer using a specific extinction coefficient of $E_{260}^{0.01} = 8.45$ mg/ml (3).

Ribonucleic acid.—RNA was extracted from ArMV-J by the phenol method. Purified virus was mixed with a final concentration of 0.1 M sodium phosphate buffer pH 7.0 and 3% sodium dodecylsulfate (SDS) containing a trace of bentonite to remove RNase, and then extracted with phenol as described earlier (5). The final RNA precipitate was dissolved in water, diluted, and the concentration of RNA and its freedom from phenol were determined with a spectrophotometer.

To determine base ratio, 2 mg of RNA was digested in a final concentration of 0.3 M KOH for 18 hours at 37 C in a water bath. The nucleotide mixture was then layered onto a column, 1.5 cm deep by 1 cm in diameter, of anion exchange resin formate form (5) and separated by

stepwise elution with formic acid and ammonium formate. The eluent was monitored with an ISCO system (5). The bases were collected separately, and the concentration of each determined with the spectrophotometer. The RNA of turnip yellow mosaic virus was used as a control.

RESULTS AND DISCUSSION.—I saw no viruslike symptoms in the source plant of *J. mesnyi* during 3 years of observation. Its experimental host range on 45 species was like that of previous reports on ArMV, except that ArMV-J did not incite systemic symptoms in *Cucumis sativus* L. 'Improved Long Green' nor *Gomphrena globosa* L. The most diagnostic symptoms were the chlorotic vein net and apical necrosis incited in *Chenopodium quinoa* Willd. and the 1- to 2-mm diameter chlorotic ringspots in inoculated leaves of *Datura stramonium* L.

Purification and electron microscopy.—Two distinct opalescent bands were observed in density gradients. Upon fractionation, a center component was also detected as a distinct peak or as a shoulder on the trailing edge of the bottom component. Yields of virus ranged from 0.22 to 1.05 mg/g of fresh tissue depending on the procedure followed. In general, (i) a single high-speed centrifugation resulted in higher yields with less nonvirus material than concentration by precipitation with PEG or ammonium sulfate without high-speed centrifugation, (ii) tris buffer was as suitable as phosphate as an extraction buffer, and (iii) yields were higher from tissue blended in 0.04 M (pH 7.0) buffer, or when blended in 0.01 M buffer at pH 8.0.

Particles of purified virus on Formvar-coated grids averaged 25 nm in diameter. Intact, RNA-devoid, and partially RNA-devoid particles were observed like those shown earlier (3).

Serology and relationship to other viruses.—Antiserum produced to ArMV-J rose to an antibody titer of 1:1,024 in successive bleedings. In comparative tests with other ArMV antisera, maximum antisera dilutions that produced a visible band in 0.75% Ionagar with a 5-mm well spacing and with concentrated ArMV-J were Walkey's celery isolate, 1:512; Stace-Smith's rhubarb isolate, 1:64; and Holling's Sambucus isolate, 1:256. Homologous antisera titers were: 1:1,024, 1:320, 1:512 and to ArMV-J antiserum 1:1,024. Since three of the ArMV antigens were unavailable, reciprocal tests could not be made. ArMV-J did not react with any of 32 other antisera tested.

Ribonucleic acid.—RNA from ArMV-J produced a typical ultraviolet absorption curve with a maximum/minimum ratio of 2.3. Yields ranged from 50 to 70% of the total calculated nucleic acid in the samples when both SDS and phenol were used during extraction. Without SDS, yields were much lower and the resultant preparations contained protein. Average base composition of the digested RNA from four replicates was A = 24.9%, C = 25.6%, G = 21.6%, and U = 27.9%.

ArMV has been identified from and causes diseases in a wide range of naturally infected species in Europe (2, 3, 4). Imported jasmine, as well as most woody ornamental introductions, are not required by quarantine regulations to be indexed for viruses. However, various viruses have been isolated from this species, and in this instance a virus not known to occur in the U.S. was detected by chance indexing. Antiserum to ArMV-J is available.

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