Method for isolation and identification of potato virus M, S, X, Y

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Abstract

The common procedure for isolation of potato virus M, S, X, Y, based on precipitation with PEG (final concentration of 6%) and purification by gel filtration on the Sepharose 2B is described. Identification of individual viruses is performed on the basis of electrophoretic analysis of viral coat protein, extracted from purified virus fraction. In electrophoresis coat proteins give single bands with the relative mobility of 1.0, 0.97, 0.94, and 0.80 for PVY, PVS, PVM and PVX respectively.

Determination of protein or RNA content in virus fraction after chromatography allows the quantitative estimation of virus in plant material.

INTRODUCTION

Among potato viruses the rod-shaped PVM, PVS, PVX and PVY are most widely spread in Poland (Świeżyński 1968). The estimation of the extent of infection with these viruses is therefore of great value. For their detection usually immunochemical (Sampson and Taylor, 1968; Purcifull and Gooding, 1970; Shepard, 1970; Shepard et al., 1971) and biological (Sampson and Taylor, 1968; Hinostroza-Orihuela, 1973; Hiruki, 1973) methods are used, which are satisfactory for routine tests. However in some investigations more accurate and detailed characteristic of virus material is necessary. In the present paper the method is described, which is applicable for isolation and identification of four viruses (PVM, PVS, PVX, PVY) studied. The method is based on electrophoretic analysis of viral coat protein extracted from purified virus fraction.

MATERIAL AND METHODS

Material

The following virus isolates were used: M (PVM) from potato cv. Uran, S (PVS) from cv. Leona, X (PVX) from cv. Bintje, Y (PVY) from cv. Lipiński Wczesny. All virus isolates were from the virus collection of Institute for Potato Research, Research Unit Młochów. Viruses were grown on an isolated from the following plants: PVM — tomato (cv. Najwcześniejszy) — 3-8 weeks after inoculation, PVS — potato (cv. Leona) 4-12 weeks after planting of virus-infected tubers, PVX and PVY — tobacco (cv. Samsun) 2-6 weeks after inoculation. Virus-free plants of the same cultivar and in the same stage of development were used as a control. All plants were cultivated in a greenhouse.

Methods

Isolation and purification of viruses

Extraction of viruses was performed as described previously (Czupryn and Błaszczyk, 1974), except that leaves (about 20.0 g) were homogenized immediately after harvesting without freezing and thawing. To the extract solid NaCl was added to the concentration of 0.25 M and then polyethylene glycol (PEG, mol. wt. 6000, Roth) to 6% v/v final concentration. After 30 min the suspension was centrifuged at 10,000 g for 10 min, the supernatant discarded and the pellet extracted three times with cold 0.01 M sodium citrate (3 ml of solvent per 10 g of plant tissue for each extraction). The combined supernatants were applied on the Sepharose 2B (Pharmacia) column, equilibrated with 0.01 M sodium citrate. The volume of the sample applied on the column should not exceed 10% of total bed volume. Elution was performed with 0.01 M citrate at a flow rate 30 ml/hr. Absorbance in eluate was continuously monitored by Uvicord II (LKB Sweden), virus containing fraction (the first peak, eluted at 0.20–0.45 V_c/V_t) was collected and the virus precipitated by polyethylene glycol at final concentration of 6%.

Extraction and identification of viral coat protein

Purified virus preparation was suspended in 0.01 M sodium citrate, using 0.1 ml of solvent per 10 O.D._{260 nm} units and then viral coat protein was extracted as described previously (Czupryn and Błaszczyk, 1974). Extract (20-50 µl/gel) was directly subjected to electrophoresis. 15% polyacrylamide gels, containing 6.25 M urea were prepared

according to Panyim and Chalkley (1969); 75×5 mm tubes were used. Electrophoresis was carried for 4 hr at 150–170 V (about 1 mA/tube). Gels were stained overnight with 0.1% Coomassie Brilliant Blue R-250 (Bio-Rad), dissolved in the mixture: water-methanol-acetic acid (6:3:1) and destained in 7% acetic acid.

Densitometric scans of gels were obtained in ERI-65 densitometer (Carl Zeiss Jena).

Protein was determined by the method of Lowry et al. (1951), using a bovine serum albumin (Sigma) as a standard.

RNA was determined spectrophotometrically after alkaline hydrolysis according to the method of Munro and Fleck (1966), assuming that $E_{260\text{ nm}}^{1\text{ cm}} = 1.00$ is equivalent to $32\text{ }\mu\text{g RNA/ml}$.

Microprecipitin tests with specific antisera were performed at each step of isolation and purification of viruses.

RESULTS AND DISCUSSION

1. Isolation and purification of viruses

The method described allows the isolation and purification of either the individual or a mixture of the viruses studied. Schematic diagram of the procedure used is shown in Fig. 1. For precipitation of viruses poly-

Fig. 1. Scheme of virus isolation procedure

ethylene glycol mol. wt. 6000 in final concentration of 6% was used. This concentration was chosen on the basis of the precipitation analysis of individual viruses and nonvirus cell components at the concentration range of PEG from 2% to 10%. In the precipitated fractions protein and RNA content were measured before and after chromatography on Sepharose 2B. Results are presented in Table 1. As can be seen from these

Table 1
Protein and RNA content in crude and purified virus fraction
at different PEG concentrations

Type of virus	PEG concn. range (%)	Protein content (mg/100 g fresh wt.)		RNA content (mg/100 g fresh wt.)		Serological test	
		crude fraction	virus fraction*	crude fraction	virus fraction*	crude fraction	virus fraction*
PVM	0—2	31.0	17.3	2.95	1.64	++	+++
	2—4	15.5	4.0	1.54	0.50	+	+
	4—6	17.0	4.9	1.63	0.51	+	+
	6—8	62.0	2.9	2.81	0.22	±	±
	8—10	70.0	0.7	2.12	0.06	—	—
PVS	0—2	20.0	7.6	3.34	1.03	++	++
	2—4	13.1	4.2	2.48	0.45	+	+
	4—6	15.3	3.2	2.43	0.36	+	+
	6—8	72.0	1.6	3.88	0.15	—	—
	8—10	74.4	1.4	2.91	0.12	—	—
PVX	0—2	15.0	5.6	0.82	0.22	+	+
	2—4	137.2	110.3	8.15	7.27	+++	+++
	4—6	28.9	11.0	0.25	0.13	+	+
	6—8	64.0	0.4	0.81	0.10	—	—
	8—10	66.9	0.2	1.00	0.07	—	—
PVY	0—2	9.9	3.2	0.35	0.13	++	++
	2—4	12.0	3.6	0.36	0.32	+	+
	4—6	15.8	2.8	0.68	0.25	±	±
	6—8	95.5	1.5	0.83	0.11	—	—
	8—10	97.8	0.2	0.81	0.08	—	—

* Peak No. 1 after chromatography on the Sepharose 2B.

data each of the studied viruses was almost completely precipitated with PEG concentration up to 6%. At the same time relatively low coprecipitation of nonvirus material was observed.

The separation of viruses from host cell contaminants was performed by gel filtration on the Sepharose 2B column (Fridborg et al., 1965;

Haman and Pett, 1971; Skrzeczowski, 1973). Elution profiles for individual viruses precipitated with PEG in concentration of 6% are shown in Fig. 2. Viruses (peak no. 1) were distinctly separated from other components (peak no. 2).

Fig. 2. Elution profiles on Sepharose 2B for virus fraction precipitated at 6% of PEG. Peak no. 1 — purified virus, peak no. 2 — contaminants. Solid line — $E_{280\text{ nm}}$, broken line — $E_{260\text{ nm}}$

The same procedure applied for noninfected plants yielded only negligible amount of material in the first peak. It provided the basis for quantitative estimation of virus content by determination of either protein or RNA in virus containing peak after gel chromatography.

The results of Table 1 show that PEG concentrations higher than 6%, which were used by the other authors (Albrechtova and Klir, 1970; Skrzeczowski, 1974) yielded the virus preparation highly contaminated by nonvirus material.

2. Extraction and identification of viral coat protein

Identification of all examined viruses was based on electrophoretic analysis of their coat protein. Protein monomers were obtained by slightly modified method of Fraenkel-Conrat (1957), described in previous paper (Czupryn and Błaszczuk 1974). Among several examined methods, applied by the other authors (Reichmann 1960; Shepard 1970; Lesnaw and Reichmann 1970) for the extraction of viral protein only this one was found to be applicable for all studied viruses.

Fig. 3. Densitometric scans of electrophoretograms of viral coat proteins from A -PVM, B -PVS, C -PVX, D -PVY, E -mixture. For details see methods

For electrophoretic separation of proteins the method of Panyim and Chalkley (1969) was adapted because of its best resolution capability. Typical electrophoretic pattern of viral coat proteins is illustrated in Fig. 3. Each viral coat protein was visible as a single band with different electrophoretic mobility. Assuming the mobility of PVY coat protein as 1.0 corresponding relative mobilities for other viral proteins were 0.97 for PVS, 0.94 for PVM and 0.80 for PVX. None of these bands was observed on the electrophorograms of preparations obtained from control plants.

Different electrophoretic mobilities of the coat proteins allow the detection of individual viruses in their mixture (Fig. 3 E). Thus taking a coat protein from one of these viruses as a marker it is possible to identify the other ones by estimation of their relative mobilities.

It should be noted that the material infected with PVM or PVS isolates often gives on electrophorograms additional protein band with relative mobility of 0.87 (Fig. 4). This additional component probably corresponds to coat protein of non-identified rod-shaped plant virus.

Thus the method described above allows not only the detection and identification of each of the studied viruses, but also reveals the existence of the other rod-shaped viruses when present in the studied material.

Fig. 4. Localization of coat protein of not-identified virus, accompanying virus M and S. 1 -not-identified, 2 -PVM, 3 -PVS

Moreover estimation of protein or RNA content in virus fraction after chromatography on the Sepharose 2B makes possible the quantitative estimation of virus.

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Metoda wydzielenia i identyfikacji wirusów M, S, X, Z ziemniaka

Streszczenie

Metoda wydzielenia polega na wytrącaniu wirusów glikolem polietylenowym (M. cz. 6000) w stężeniu 6%, a następnie na chromatografii na Sepharose 2B. Identyfikację poszczególnych wirusów przeprowadza się na podstawie analizy elektroforetycznej białek kapsydu, ekstrahowanych z oczyszczonej frakcji wirusowej. W opracowanych warunkach białka kapsydu poszczególnych wirusów dają pojedyncze pasma o względnej ruchliwości elektroforetycznej: 1.0 — wirus Y, 0.97 — S, 0.94 — M, 0.80 — X.

Oznaczenie zawartości białka lub RNA we frakcji wirusowej po chromatografii może stanowić miarę wirusa w materiale.