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# A METHOD FOR PURIFICATION OF POTATO VIRUS M

Potato virus M (PVM) is widely spread both in production fields and in breeding material (seedlings) in the Estonian SSR (Randalu, 1968, Нурмисте, 1969).

Despite the great distribution of PVM and the marked decreasing effect upon potato yields, our knowledge of its physico-chemical data is rather limited. Apparently no suitable virus-host combination has been found so far in which the virus is present in sufficiently high concentration, and no appropriate method for the purification of PVM has been developed either.

The present paper deals with a possibility of PVM purification by zone electrophoresis in sucrose density gradient.

## Materials and methods

One of the most stable virus strains of our potato virus M collection — strain $M_{11}$ — was used in this work. The virus strain was isolated from a rugose seedling of a 1961-cross (382/48×*Prieculu visagrie*, No. 11) at the Jõgeva Plant Breeding Station. This strain occurs in relatively high concentration in susceptible plant species. *Solanum demissum* Lindl. appeared to be the most suitable host.

The specimens of finally purified virus preparations as well as partially purified ones at different stages of purification were examined in an EM-7 electron microscope.

For shadowing, the virus samples were applied to Formvar film-coated grids, dried and covered with chromium-nickel at an angle of 15°. For negative staining, the samples were placed on Formvar film-coated grids, excess fluid was removed with a piece of filter paper after 30 sec.; after that, a drop of 2% phosphotungstate, pH 6.8, was applied to sample, and excess solution was removed after 30 sec. Specimens were allowed to dry in air.

Ten plants of *Solanum demissum* were infected to determine the changes in virus concentration at various stages of disease. The average samples from the top leaves were taken on every third day after inoculation, ground in a mortar and diluted with distilled water in the proportion of 1 : 10,000. The preparations were shadowed with metal as described above, and the number of particles in twenty holes of the grid was counted in an electron microscope. The mean values were calculated, and a graph was drawn on the basis of these data (Fig. 1).

Purification Procedure. The infected leaves were harvested three weeks after inoculation and stored at about —10 °C overnight. On

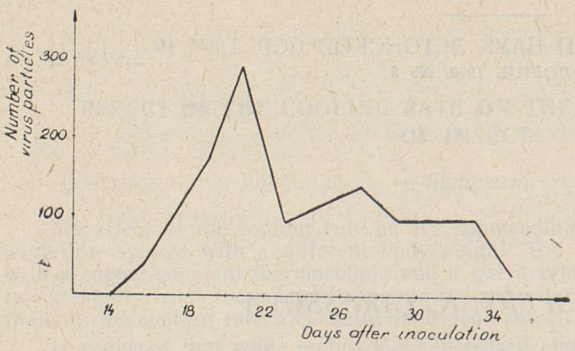


Fig. 1. Virus particles concentration changes in *Solanum demissum* Lindl. plants infected with PVM.

the next day, the frozen leaves were crushed in a meat-chopper. 0.05 M citrate buffer, pH 6.7 (containing 0.01 M $Na_2SO_3$ and 0.02 M ascorbic acid), one millilitre per gram of leaves, was added to the macerate. The pulp was homogenized in a blendor with one-third volume of chloroform at 8,000 r. p. m. for 30 sec. and centrifuged at 5,400 g for 15 min. The supernatant (Fig. 2) was ultracentrifuged at 90,000 g for 60 min. The pellet was resuspended in 0.005 M borate buffer, pH 9.0, and centrifuged at 17,000 g for 30 min. The supernatant (Fig. 3) was dialysed against the borate buffer for zone electrophoresis, pH 8.6 (van Regenmortel, 1964; Polson and Russell, 1967). The centrifugation at 17,000 g was repeated after dialysis, if necessary. Sucrose was added to the supernatant to obtain a 37% final concentration. A few drops of phenol red were also added to the virus preparation.

A sample of virus was introduced into the column for zone electrophoresis (type AEV) containing sucrose gradient (0 . . . 40%). The column was filled as described earlier (Hödrejärv et al., 1970). Electrophoresis was allowed to proceed until sufficient separation had been obtained. With a current of 30 ma and a potential difference of 170 v., the time needed varied from 28 to 35 h.

After the end of electrophoresis, the distances of visible bands from the starting-point were measured, the fractions of 2 ml were collected, and the optical densities at 260 and 245 nm. were determined. The fractions with $D_{260}/D_{245} \geqslant 1.02$ were joined and centrifuged at 90,000 g for 60 min. The pellet was resuspended in 0.005 M borate buffer, pH 9.0, and centrifuged at 17,000 g for 15 min. The residue was discarded, and the supernatant (Figs 4 and 5) collected.

## Results

As shown in Fig. 1, virus particles concentration reached its maximum in PVM-infected *Solanum demissum* plants on the 21st day after inoculation. Then a drop followed, and virion concentration decreased to about ten per cent only (from maximal concentration) on the 35th day.

Three bands were visible in the electrophoresis column after the end of zone electrophoresis of PVM. They were located from the top to the bottom of the column in the following way: phenol red, a green band of plant material, and an opalescent virus-containing zone.

The results of the electrophoresis are presented in Table. According to the data, as regards its migration rate in borate buffer, pH 8.6, PVM (approximate α* value 5.6) is situated between tobacco necrosis virus and potato virus Y, quite near to the latter. Approximate α value for tobacco

* α — the relative number showing how many times phenol red migrates faster than virus particles.

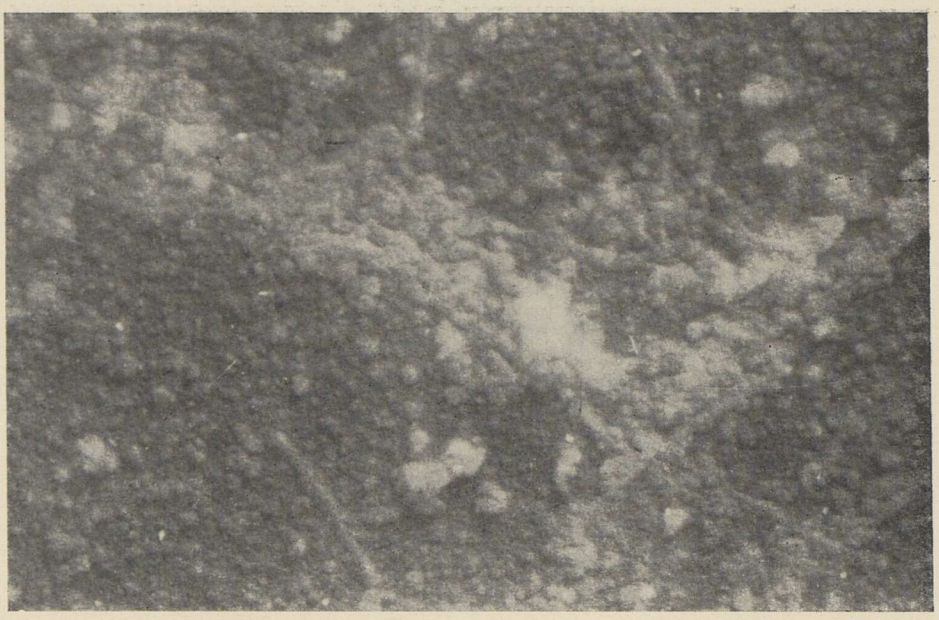

Fig. 2. Partially purified PVM preparation after clarification with chloroform. (The first stage of purification.) Magnification 16,200 × 3. The preparation is shadowed with chromium-nickel.

Fig. 3. The same, after a single cycle of differential centrifugation. (The second stage of purification.) Magnification 16,200 × 3. The preparation is shadowed with chromium-nickel.

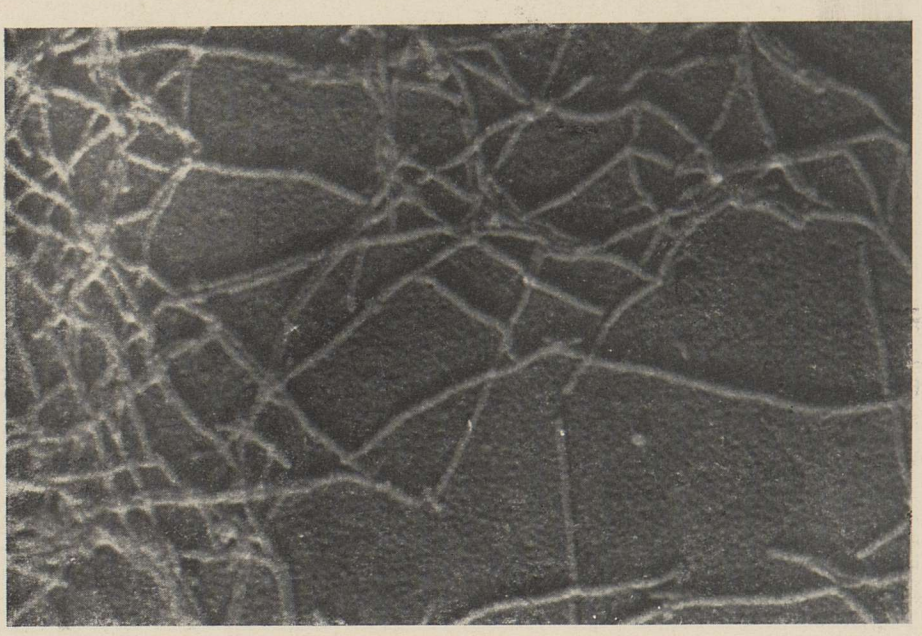

Fig. 4. Finally purified PVM preparation after electrophoresis and differential centrifugation. (The third stage of purification.) Magnification 16,200 × 3. The preparation is shadowed with chromium-nickel.

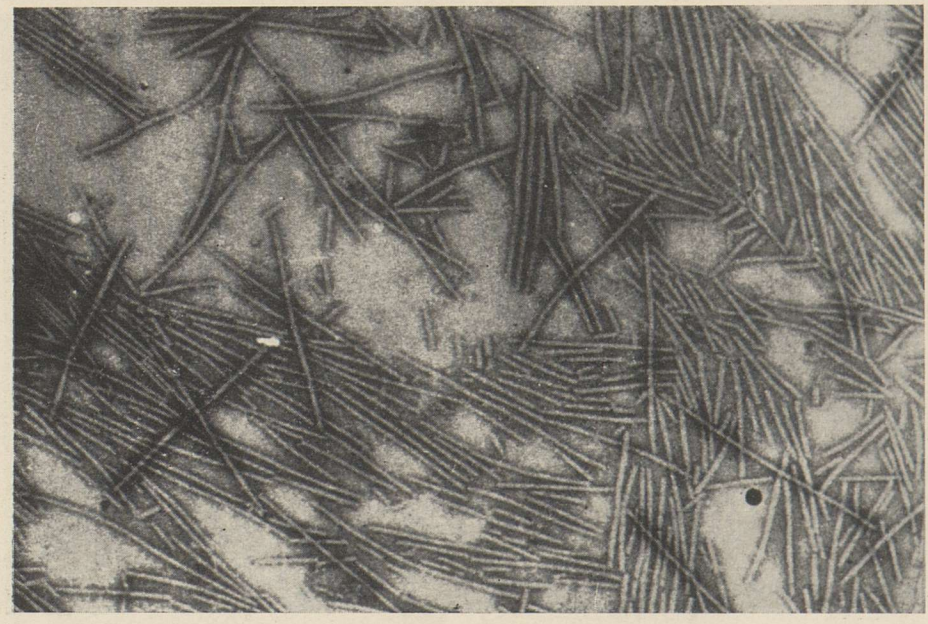

Fig. 5. The same, stained with 2% phosphotungstate. Magnification 16,200×3.

**Positions of initial virus preparations and fractions after electrophoresis in sucrose density gradient column**

| Virus strain | Starting point of virus preparation (cm) | Virus particles containing fraction (cm) | Plant material containing fraction (cm) | Phenol red (cm) | α* |
|---|---|---|---|---|---|
| Potato virus $M_{II}$: | | | | | |
| experiment 1 | 0—0.7 | 1.4—2.2 | 3.1—4.3 | 8.1— 9.2 | 5.8 |
| „ 2 | 0—1.4 | 1.9—3.3 | 3.4—5.1 | 10.8—12.4 | 5.7 |
| „ 3 | 0—1.0 | 1.6—2.7 | 2.8—4.5 | 8.6— 9.2 | 5.4 |

necrosis virus is 9.5 and for potato virus Y — 4.9, as calculated according to data in literature (van Regenmortel, 1964; Polson and Russell, 1967).

The purification of potato virus M proceeds in three stages. After the so-called preliminary purification (clarification with chloroform), the preparation still contains plenty of different plant contaminations (Fig. 2). The following single cycle of differential centrifugation diminishes the amount of contaminants markedly (Fig. 3). Further purification by differential centrifugation does not improve the purity of the obtained preparations essentially. At the same time, there are substantial losses in virus yields because of an aggregation of virus particles. Due to this, the final purification of virus preparations by electrophoresis in sucrose density gradient was undertaken. This method makes it possible to obtain PVM preparations with sufficient purity for further physico-chemical investigations, as stated on the basis of electron micrographs (Figs 4 and 5). Virus yields in preparations purified by the method presented here, are approximately from 10 to 15 mg per kilogram of leaf material.

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## KARTULI-M-VIIRUSE ERALDAMISE METOODIKA

*Resümee*

Esitatakse kartuli-M-viiruse eraldamise metoodika, mille kohaselt kogu menetluse võib jaotada kolmeks etapiks: eelpuhastus kloroformiga, järgnev eraldamine diferentsiaalse tsentrifuugimise abil ja lõplik puhastamine tsonaalsel elektroforeesil sahharoosi tihedusgradiendis.

Elektronmikroskoopiliste andmete põhjal on sel teel saadud preparaatide puhtus küllaldane nende edasisteks füüsikalis-keemilisteks uurimisteks.

Kilogrammist *Solanum demissum* Lindl. lehtedest on ligikaudsete arvestuste põhjal võimalik sel meetodil eraldada 10 ... 15 mg kartuli-M-viirust.

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Saabus toimetusse
20. X 1969

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## МЕТОДИКА ОЧИСТКИ ВИРУСА М КАРТОФЕЛЯ

*Резюме*

Приводится методика очистки вируса М картофеля. Очистку можно разделить на три этапа: предварительная очистка хлороформом, очистка дифференциальным центрифугированием и окончательная очистка методом зонального электрофореза в градиенте плотности сахарозы.

По электронно-микроскопическим данным полученные препараты являются достаточно чистыми для дальнейших физико-химических исследований.

Выход вируса из растений *Solanum demissum* Lindl. по приблизительным расчетам составляет 10—15 *мг* на килограмм листьев.

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