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Purification by molecular sieving of a leek virus related to onion yellow dwarf virus

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Onion yellow dwarf virus (OYDV) (*/*:*/*:E/E:S/Ap) is one of those viruses of the potyvirus group of which little is known because it is difficult to purify. There have been some futile attempts to purify OYDV by Špánik et al. (1961), Jermoljev et al. (1962), and Głowinkowska (1973). When working on a virus isolate from leek (*Allium porrum*) (Bos, 1972), resembling but differing in host range from OYDV (Bos, personal communication) we found that the main reason for the poor results in purifying the leek isolate was the high content of mucilage in leek. The mucilage easily sediments in high-speed centrifuging, even at the relatively low centrifugal forces applied in purifying viruses of the potyvirus group (Huttinga, 1973). Several attempts were made to break the mucilage down by enzymes, or to get rid of it by freezing the sap, by salting-out procedures with various salts and at different pH values, and by filtration. All the attempts failed.

In the end we had good results with the following method including molecular sieving. One hundred grams of infected leaves of *A. porrum* 'Goliath' grown in the open, were ground in a Waring blendor in a mixture of 500 ml of 0.1 M tris buffer adjusted to pH 9 with thioglycolic acid, 20 ml of chloroform, 20 ml of carbon tetrachloride, and 10 ml of diethyl ether. The homogenate was centrifuged for 10 min at 4,000 *g*. The supernatant fluid was decanted and centrifuged for 1.5 h at 26,500 *g*. The pellets were resuspended in 50 ml of 0.1 M tris-HCl buffer pH 9. After 2 h standing at 4°C, the suspension was filtered through filter paper using a Büchner funnel to remove large lumps of mucilage. The filtrate was then loaded on top of a Sephadex G-200 column and this eluted with 0.1 M tris-HCl buffer pH 9, containing 4×10^{-4} M NaN_3. The Sephadex G-200 column had a diameter of 3 cm and was 59 cm high. The flow rate was kept at 4.6 ml/h/cm^2 with a peristaltic pump. The effluent was led through a UV absorption meter and then fractionated using a fraction collector. A typical recording of a column effluent is presented in Fig. 1. The large peak on the left represents the material which was excluded from the pores in the Sephadex G-200 particles. In the material from this peak which was free from mucilage the virus was present. The appropriate fractions were combined and the virus was further purified and concentrated by centrifuging $1^3/_4$ h at 47,000 *g*. After resuspending the pellets a greenish virus suspension was obtained that contained no mucilage. One of the electron micrographs of such a suspension after an additional purification by density-gradient centrifuging in a 10–40% sucrose gradient for 2 h at 25,000 rpm (Beckman SW 27 rotor), is presented in Fig. 2. It shows that the prepara-

Fig. 1. UV absorption pattern of the effluent of a Sephadex G-200 column, loaded with partially purified leek virus and eluted with 0.1 M tris-HCl buffer pH 9, containing 4×10^{-4} M NaN_3. Flow rate during elution 4.6 ml/h/cm^2. The peak on the left, which left the column first, contained the virus.

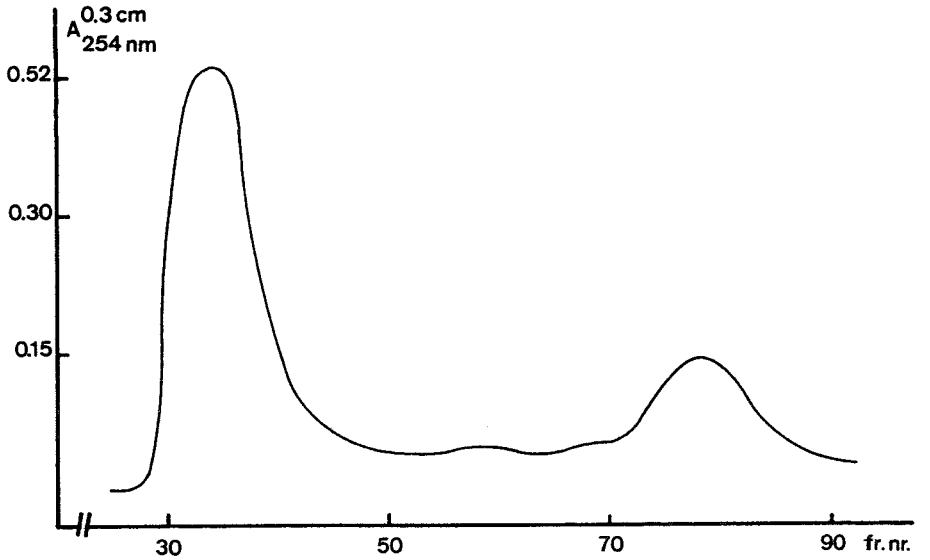

Fig. 1. UV-absorptiepatroon van het effluent van een kolom met Sephadex G-200, die werd geladen met gedeeltelijk gezuiverd preivirus en geëlueerd met een 0,1 M tris-HCl buffer pH 9, die 4×10^{-4} M NaN_3 bevatte. De elutiesnelheid bedroeg 4,6 ml/uur/cm^2. De piek aan de linkerkant, die het eerst uit de kolom kwam, bevatte het virus.

tions were highly homogeneous. The Sephadex G-200 column can be used for a limited number of separations only. The pores of the gel become clogged by the mucilage, resulting in poor separation, as indicated by a severe tailing of the peaks in the UV-recording.

The virus is now available for further physico-chemical characterization. The buoyant density of the virus particles and the molecular weight of the coat protein subunit were determined as described before (Huttinga and Mosch, 1974). The values found were 1.326 g/cm^3 and 34,000 daltons, respectively.

Samenvatting

De zuivering door moleculair zeven van een aan uiegeelstreepvirus verwant preivirus

De zuivering van het uiegeelstreepvirus uit *Allium*-soorten wordt in sterke mate belemmerd door het voorkomen van grote hoeveelheden slijm in deze planten. Bij pogingen tot het afzonderen van een aan het uiegeelstreepvirus verwant virus uit prei bleek dit slijm niet door differentiëel centrifugeren van het virus te scheiden. Wel was het mogelijk het planteslijm uit gedeeltelijk gezuiverde viruspreparaten te verwijderen door moleculair zeven op een kolom van Sephadex G-200 (Fig. 1 en 2).

Het gezuiverde virus had een soortelijk gewicht van 1,326 g/cm^3 en het molecuulgewicht van de manteleiwiteenheid bedroeg 34.000 daltons.

Fig. 2. Electron micrograph of the leek virus after negative staining with 1% potassium phosphotungstate pH 6.5 in water. The bar represents 1 μm.

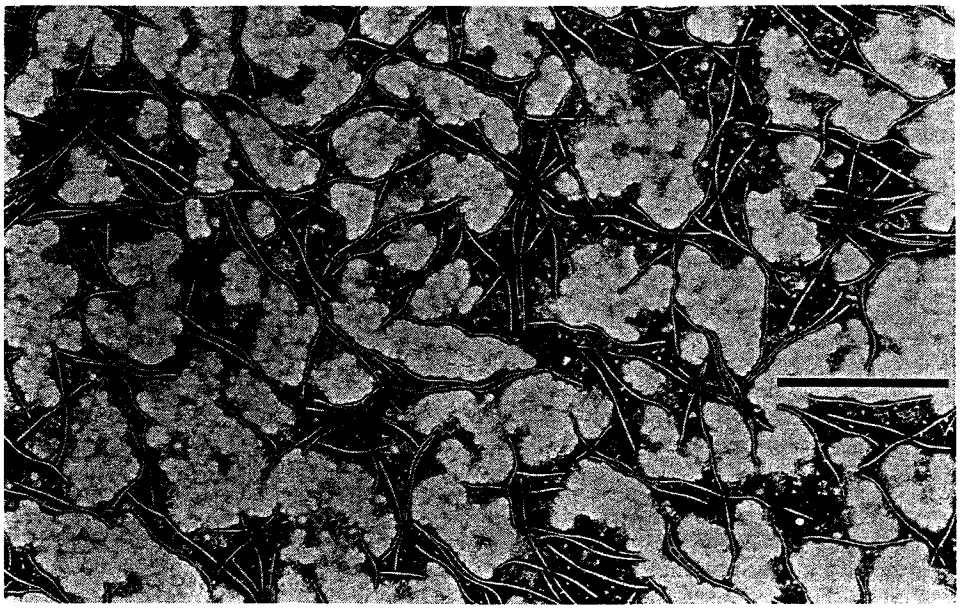

Fig. 2. Elektronenmicroscopische foto van het preivirus na negatieve kleuring met 1% kaliumfosforwolframaat pH 6,5 in water. De vergrotingsstreep geeft 1 μm weer.

References

Bos, L., 1972. Ernstige uitbreiding van uiegeelstreepvirus in prei. Gewasbescherming 3: 81–87.

Głowinkowska, A., 1973. Oczyszczanie wirusów roślinnych metodą chromatografii kolumnowej oraz ultrawirowania. Zesz. probl. Postęp. Nauk roln. 142: 29–36.

Huttinga, H., 1973. Properties of viruses of the potyvirus group. 1. A simple method to purify bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus and potato virus Y^N. Neth.J.Pl.Path. 79: 125–129.

Huttinga, H. & Mosch, W. H. M., 1974. Properties of viruses of the potyvirus group. 2. Buoyant density, S value, particle morphology, and molecular weight of the coat protein subunit of bean yellow mosaic virus, pea mosaic virus, lettuce mosaic virus, and potato virus Y^N. Neth.J.Pl. Path. 80: 19–27.

Jermoljev, E., Čech, M., Pozděna, J. & Chod, J., 1962. Serodiagnostika proužkovitosti cibule. Rostlinná výroba 8: 551–556.

Špánik, V., Valenta, V. & Bystrický, V., 1961. Pokus o purifikáciu a elektrónmikroskopické znázornenie vírusu žltej prúžkovitosti cibule. Biológia, Bratisl. 16: 615–618.

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