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Studies on Purification and Serology of Potato Virus X

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감자바이러스X의 純化와 血淸學的 硏究

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ABSTRACT

Potato virus X was purified especially for the preparation of antisera for diagnosis and identification.

Potato virus X was isolated from infected plants by means of indicator plants and identified in electron microscopy. Isolated PVX was multiplied in tomato plants and purified by a modified procedures. The purity of PVX was 0.59mg/ml.

Purified PVX was injected into rabbits once a week for 5 weeks. Antiserum was collected 10 days after the last injection.

Produced antiserum was determined 1/1024 titers by means of microprecipitin tests and showed sharp reactions in agar gel-diffusion tests.

INTRODUCTION

otato virus X(PVX) was initially described by Smith[1] 1931. Bawden and Pirie[5] attempted purification of X, and Chester[4] explained latent mosaic virus group serology in 1936. Cockerkam[8] described the same in between potato virus B and B virus isolated from te of York and Up- to-Date varieties in serological hods in 1943. In Korea, Choi et al[7] reported preparation of PVXantisera by means of using saturating sulphuric ammonium salting-out methods at the Alpine Experiment Station in 1964. However practical organization of production of antisera has not accomplished, in spite of using more effective serological screening tests for potato virus in seed potatoes.

The PVX antisera produced in modified improved methods in this investigation will contribute to screening of virus-free seed potatoes.

MATERIALS AND METHODS

'irus source

otato virus X was obtained from naturally infected to plants in the field. Indicator plants and leaf dip logy[3] with electron microscopy were used to isolate identify the virus. Virus was multiplied in mechanically inoculted tomato plants grown in the greenhouse.

Virus purification:

Tomato plants with marked symptoms were harvested 15 to 20 days after inoculation. Leaf tissue was ground in cold 0.01M(pH 7.0)citrate buffer(2ml/g leaf tissue) for 3 min at high speed in Warning blender with 0.2% thioglycolic acid. Juice was expressed through cheese

cloth and stirred with 30% chloroform for 3 min. The resulting mixture was centrifuged for 15min at 3,000 rpm in a Hitachi RPR 12—157 rotor. The supernatant liquid was recovered, and polyethylene glycol, MW 6,000 (PEG 6,000) was added to make a 6% solution. The solution was stirred for 1 hr at cool temperature, and incubated 1 hr at 4°C, then centrifuged for 15 min at 6,000rpm in a Hitachi RPR 12—157 rotor. The pellet was dissolved in 30ml of 0.01M citrate buffer. A high-speed centrifugation for 90min at 29,000 rpm in a Hitachi RPR 18—243 rotor was sufficient to provide pellets of virus. The clear, transparent to light-green pellets were dissolved in 2ml of 0.01M citrate buffer. Insoluble materials was removed by centrifugation for 15 min at 8,000 rpm in a Hitachi 18—243 rotor. The opalescent supernatant solution was layered on linear 10—40% sucrose density gradients.[6] Gradients were buffered with 0.01M citrate buffer and equilibrated overnight at 4°C before use. Centrifugation was for 150 min at 23,000 rpm in a Hitachi RPS 25—2—245 swing bucket rotor. The bands were removed by syringe and centrifuged for 120 min at 29,000 rpm. Purified virus was determined by spectrophotometric optical density measurements.

Serology:

Two rabbits were injected with virus intravenally once a week for 5 weeks. One to 2ml of purified virus were injected. Antiserum was collected 10 days after the last injection. The titer of antisera produced was determined by microprecipitin tests.[15] Serological reactions were examined using the agar gel-diffusion test[12] Gel plates contained 0.7% agar and 0.02% sodium azide.

RESULTS AND DISCUSSION

Systemic symptoms developed in inoculated plants of *D. stramonium*, *N. debneyii*, *P. floridana* and *L. esculentum*. Local-lesions developed in *C. annuum*, *C. occidentalis*, and *G. globosa*. Tomato plants were used for multiplication of PVX by inoculation from infected *D. stramonium*.

After sucrose density gradient centrifugation, the virus containing zone was 3cm below the meniscus. As shown in Fig. 1, purifed preparations produced a typica

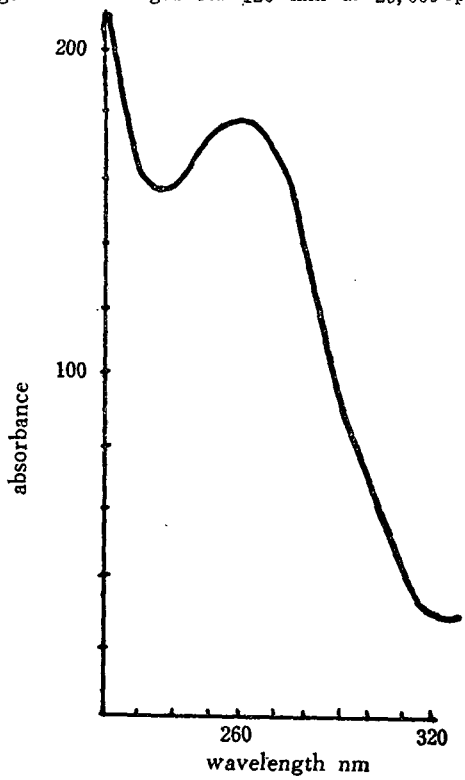

Fig. 1. Ultraviolet absorption spectra of purified PVX in 0.01M citrate buffer (pH 7.0)

Fig. 2. Purified preparation negatively stained with phosphotungstate. Bar showed 100nm. (8 fold diluted purified PVX in PBS)

nucleoprotein spectrum with a minimum absorption at 245nm and maximum absorption at 260 nm, Each had a 260:245 ratio of 1.13 and 260:280 ratio of 1.40. Yields of purified virus were 0.59mg/ml by spectrophotometric optical density measurements.[9]

A variety of methods were tried in attempts to purify PVX. In density gradient centrifugation methods, the virus obtained was not sufficiently pure. This was perhaps due to the fact it was not subjected to treatment in an ISCO density-gradient fractionator.

In Japan. mercaptoethanol is added to buffers employed in maceration. In this investigation, however, thioglycolic acid was used as the reducing agent. In purification of the virus, polyethyleneglycol 6, 000 (MW) was used instead of the acetic acid and saturated sulphuric ammonium salting-out procedure previously described by Choi et al.[7] Precipitation with PEG is dependent on the specific character of the virus, specific ally the concentration of virus and salts present in solution.[13,10]

As indicated in the microprecipitin tests in Table 1, positive antisera titers were indicated with a 1/1024 dilution with the homologous antigen. In the agar-gel-diffusion experiment illustrated in Fig. 3, there was a positive reaction with 1/128 dilution against a 4-fold diluted antigen with juice extracted from infected tomato leaves.

The slide precipitin test method was described by Wetter.[17] Less satisfactory results with flexible elongated viruses may be attributed to a low diffusion coefficient, and to their tendency to form aggregates.[16,14] Ball et al.[2] also obtained precipitation lines with purified preparations of PVX. However, weare able to show sharp precipitation lines with crude infected plant sap in our experiment.

Table 1. The titer of PVX antiserum determined by microprecipitin test

Antiserum										
1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
+	+	+	+	+	+	+	+	+	−	−

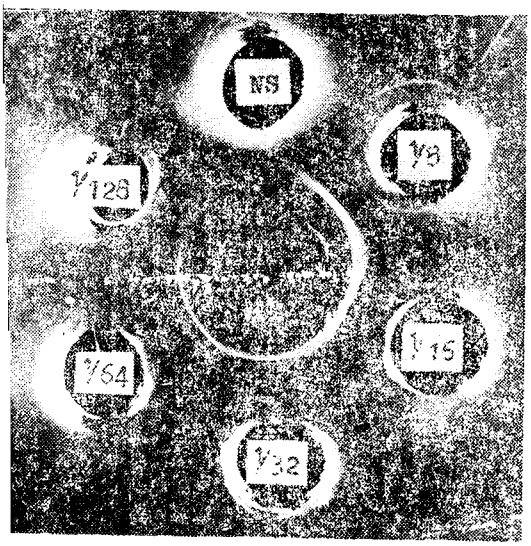

Fig. 3. Agar gel-diffusion plate with precipitin reaction between isolated PVX and PVX antiserum. The center well was filled with 4 fold diluted crude juice of PVX. Peripheral wells (clockwise starting from the top well) were filled with 1/8 1/16, 1/32. 1/64, and 1/128, PVX antiserum. The top well was filled with normal serum.

摘 要

감자바이러스X(PVX)의 診斷과 同定을 爲하여 PVX를 純粹分離 純化하여 抗血淸을 製造하였다.

PVX는 指標植物과 電子顯微鏡으로 純粹分離하여 도마도에서 增殖한後 改良된 方法으로 純化하였다.

純化된 PVX의 純化度는 0.59mg/ml이었으며, 이를 1~2ml씩 7일 간격으로 5회 토끼에 注射하였다. 마지막 注射後 10일에 採血하여 抗血淸을 分離하였다.

製造된 抗血淸의 力價는 微量沈降法에서 1/1024이었고, 寒天內擴散法에서도 鮮明한 反應을 觀察할수 있었다.

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