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Purification and Serology of Potato Virus S

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감자 바이러스 S의 純化와 抗血淸製造

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ABSTRACT

The study was conducted to produce an antiserum of potato virus S for identification and screening of seed-potatoes.

Potato virus S was isolated from infected plants and identified by means of indicator plants and electron microscopy. Isolated potato virus S was multiplied in *Nicotiana deebneyii* and the virus was purified by a modified method that was developed through this study. The purity of potato virus S was 1.18mg/ml.

Purified potato virus S was injected into rabbit intravenously once a week for 5 weeks. Antiserum was collected 10 days after the last injection. The produced antiserum was determined to have a titer of 1/2048 by means of microprecipitin tests.

Introduction

Potato virus S was first described by Bruyn Ouboter and Rozendal(1952)[1] in the Netherlands. Potato virus S, a member of the carla virus group[7] is one of the most common viruses of potato, *Solanum tuberosum* L. A typical symptom is deepening of veins on the upper side of leaves, which may become rugose[9]. Infected potato plants yield an abnormally high production of small tubers in some cultivars[3], thus presenting serious problems in seed potato programs[11].

Wetter[15] attempted purification of potato virus S. He accomplished it by sedimentation and clarification of extracted sap from infected leaves with two cycles of high and low centrifugation. Also he attempted antiserum preparation by means of injecting partiall purified virus into rabbits with complete or incomplet adjuvant.

In recent years, the serological test is considered t be the best way of diagnosing the virus in seed potat screening. In Korea, however, there are no reports cor cerning purification of virus or the production of an tiserum of potato virus S. In this study, we tried t improve of purification of potato virus S and the prq cedure for the production of antiserum.

Materials and Methods

Virus isolation

Potato virus S was isolated from naturally infecte potato plants in the field. Electron microscopy wit

dip or leaf dip serology[2,12] and indicator plants were used to isolate and identify the virus. Virus was multiplied in *Nicotiana debneyii* by mechanically inoculation in the greenhouse.

Virus purification:

Infected *Nicotiana debneyii* leaves were harvested 3 to 4 weeks after inoculation, and were usally chilled for 20 hrs at 4°C as whole leaves in sealed polyethylene bags. The leaves were homogenized in 1.5 to 2.0 ml/g tissue of a cold, freshly prepared 0.05M (pH 8.2) borate buffer with 1% sodium sulphite. The extract was expressed through two layers of cheesecloth, incubated in room temperature for 60 min with stirring occasionally, and emulsified with 30% cold chloroform by stirring in a warming blender for 1 min. The emulsion was centrifuged for 15 min at 4,000 rpm in a Hitachi RPR 12-137 rotor. The clear supernatant liquid was collected, and polyethylene glycol, MW 6,000 (PEG 6,000) was added to make a 5% solution. The solution was stirred for 1 hr at cool temperature, and incubated for 1 hr at 4°C, then centrifuged for 20 min at 7,000 rpm in a Hitachi RPR 12-137 rotor. After removal the supernatant, the pellet was dissolved in 10ml of 0.05M (pH 7.5) citrate buffer..

The suspenions were clarified by centrifuging at 8,000 rpm in a Hitachi RPR 18-243 rotor. The clear supernatant was collected and centrifuged for 90 min at 29,000 rpm in a Hitachi RP 30-535 rotor. Pellets were resuspended in 3 ml of 0.05M (pH 7.5) citrate buffer. The suspensions were clarified by centrifuging for 15 min at 8,000 rpm in a Hitachi RPR 18-243 rotor. The supernatant were retained. The partially purified preparations were futher purified by rate-zonal (10-40% sucrose), density-gradient centrifugation[4]. Gradients were buffered with 0.05M (PH 7.5) citrate buffer and equilibrated overnight at 4°C before use; then centrifuged for 150 min at 23,000 rpm in a Hitachi RPS 25-2-245 swing bucket rotor. The virus containing zones were collected by syringe and centrifuged for 120 min at 29,000 rpm. Purified virus was determined by spectrophotometric optical density measurements.

Serology:

One rabbit was bled for normal serum, then injected intravenously with virus purified as described. The rabbit recieved five injections of 1.5 ml once a week. Antiserum was collected 10 days after the last injection. The titer of antiserum produced was determined by micro-precipitin tests[14].

Results and Discussion

Systemic symptom developed in inoculated plants of *Nicotiana debneyii*, and *Nicotiana clevelandii*. Local-leisons were produced on inoculated leaves of *Chenopodium amaranticolar*, *Chenopodium quinoa*, *Gomphrena globosa*, and *Vigna sinensis*. A.J. Gibbs et al.[1] reported that potato virus S was propagated in *Nicotiana clevelandii*. But according to this investigation, *Nicotiana debneyii* was better host for propagation of potato virus S than *Nicotiana clevelandii* because of its rapid growth.

Particles of isolated potato virus S are straight, sometimes curved, filaments with modal legth of 650 ×12nm[6].

After sucrose density-gradient centrifugation, the virus containing zone was 30 mm below the meniscus. As shown in Fig. 1, purified preparations produced a typical nucleoprotein spectrum with a minimum absorption at 245nm and maximum absorption at 260 nm. Each had a 260:245 ratio of 1.18 and 260:280 ratio of 1.56. Yields of purified virus (Fig. 2) were 1.18 mg/ml by spectrophotometric optical density measurements[5].

In purification procedures, Wetter[15] added ascorbic acid to 0.2% (w/v) and sodium sulphite to 0.2% (w/v) to extracted sap from infected leaves. In this investigation, 1% sodium sulphite was used as the reducing agent. Potato virus S could be precipitated from sap clarified with diethyl ether and carbon tetrachloride by adding saturated ammonium sulphite (at room temperature) to sap[8]. However, we used chloroform and polyethylene glycol 6,000 (PEG 6,000) for precipitation of virus. In this way much less of the virus precipitates completly. And high-speed pellets were routinely resuspended in 0.0M (pH 7.5) citrate buffer instead of 0.01M phosphate buffer used by Wetter[15].

As indicated in the microprecipitin tests (Table 1), positive antisera titers were indicated with a 1/2048 dilution with the homologous antigen. The methods and procedures employed for producing antisera of viruses are quite different in each laboratry. However,

the basic idea of methods for producing antigens, and of injection of animals, and of preparing and using antisera are similiar accrding to Mattews(1957), Smitg (1960), Kabat & Mayer(1961).[10, 13, 14] As recent advances, the improved methods are introduced for creasing titer or improving antiserum specifity.

Table 1. The titer of potato virus S 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
+	+	+	+	+	+	+	+	+	+	−

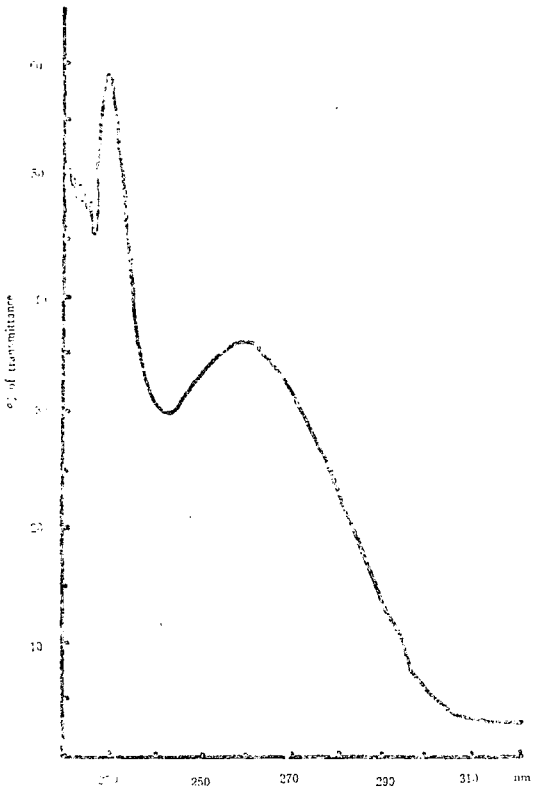

Fig. 1. *Ultraviolet absorption spectra of purified potato virus S (16 fold diluted) in 0.05M (pH 7.5) citrate buffer.*

Fig. 2. Purified potato virus S negatively stained w phosphotungstate (S fold in citrate buffer).

摘　　要

감자 바이러스 S (PVS)의 진단, 동정 및 씨감자의 검정에 이용할 항혈청을 만들기 위하여 이병주로 부터 PVS를 순수분리 순화하여 항혈청을 제조하였다.

PVS는 저료식물과 전자 현미경으로 순수 분리하여 *Nicotiana debneyii*에서 증식하여 순화하였다.

순화된 PVS의 순화도는 1.18mg/ml이 였으며 이것을 1.5ml씩 7일 간격으로 5회 토끼에 주사 하였으며 마지막 주사후 10일에 채혈하여 항혈청을 분리하였다.

제조된 PVS 항혈청의 역가는 미량침강법에 의하여 1/2048로 나타났다.

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