

Microslide Ouchterlony Technique for Serological Detection of Potato Virus X

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Accepted for publication 23 September 1970.

ABSTRACT

Microslide Ouchterlony methods employing plastic templates enabled the detection and comparison of strains of intact, native, potato virus X. Crude juice-saline (1:1) extracts of potato and *Nicotiana glutinosa* as well as purified virus from *Nicotiana*

tubacum and *Nicotiana glutinosa* were used as antigen sources in obtaining positive reactions in gel double-diffusion tests against a commercially available source of antiserum. *Phytopathology* 61:290-292.

The inability of long, flexuous rod-shaped viruses to diffuse readily in gel double-diffusion plates has hampered attempts to classify and identify them by this serological method (8). This has been particularly true when the antigen consisted of crude plant extracts or juices expressed from infected tissues. Ball et al. (1) detected a high mol wt component occurring as a sharp line near the antigen well when using purified preparations of potato virus X (PVX) in gel double-diffusion plates. Wright & Stace-Smith (12) also obtained positive results with purified preparations of PVX and TMV in an agar gel test that required 3-7 days, but reported that other serological methods were more sensitive. Shepard (9) and Shepard & Secor (10) detected pyridine-degraded PVX in crude sap extracts against antiserum prepared from degraded PVX in single radial-diffusion and double-diffusion gel methods. When infected sap was used without the addition of pyridine, no lines occurred in their double-diffusion tests. Purified preparations of intact PVX, however, did produce a precipitin line close to the antigen depot after an incubation period of 18 hr. Tomlinson & Walkey (11) also reported positive results with purified PVX, but obtained faster diffusion rates with physically disrupted PVX. Francki & McLean (5) also were successful with double-diffusion tests utilizing a purified PVX preparation. Koenig (6) obtained positive Ouchterlony results with fragmented PVX, and also pointed out the existing difficulties of using intact virus as an antigen.

Crude extracts of other viruses having particle lengths similar to PVX have been used successfully in immunodiffusion tests. A precipitin band close to the antigen well was reported by deBokx (3) in Ouchterlony tests with papaya mosaic virus in expressed sap from infected papaya (*Carica papaya*) and snapdragon plants (*Antirrhinum majus*). Ford (4) reported successful agar diffusion tests with clover yellow mosaic virus (CYMV) in crude, clarified sap extracted from CYMV-infected peas, but reported negative results with PVX, apparently due to aggregation.

This paper reports the use of a micro-Ouchterlony test to detect and compare strains of PVX in crude sap extracts of potato (*Solanum tuberosum* L.) and *Nicotiana glutinosa* L. This test was also used with purified preparations of PVX.

The use of plastic templates in gel double-diffusion adapted to microslides and described by Crowle (2) was employed previously in our laboratory in research with the potato scab pathogen. This technique was adapted with little change for the tests with PVX. Standard 3- \times 1-inch microslides treated with Siliclad (Clay Adams, Inc., N.Y.) were used. Two layers of plastic electrical tape (Fedtro, Inc.) $\frac{1}{8}$ inch wide were placed across the slide parallel to each other and 2.5 cm apart. Two layers gave a thickness of 0.4 mm. A clear piece of $\frac{1}{8}$ -inch Siliclad-treated Plexiglas 2.5 \times 3 cm was placed over the tape, and the space between filled with a gel solution. The gel was applied with a small capillary pipette and rubber bulb while holding the glass and Plexiglas between the fingers. A stock solution of 0.4% NaN₃ and 1.7% NaCl in distilled water was prepared in advance. Equal portions of this stock and a 1.7% Ionagar No. 2 (Oxo Limited, London) distilled water solution, previously heated in an Arnold sterilizer to dissolve the agar, were combined and used as soon as possible. A precipitate forms if the melted agar is not used promptly. Immediately after the agar cooled, the Plexiglas was carefully slipped off the gel, the tape removed, and a $\frac{1}{8}$ -inch Siliclad treated Plexiglas template with appropriate funnel-shaped holes for the reactants was placed on the edge of the gel layer. A drop of saline was then added to the front edge of the template to prevent the formation of air bubbles and to facilitate pushing the template over the thin gel surface. Once in place, the agar beneath the hole was removed with a flat-tipped syringe needle and slight vacuum. The size of the holes in the templates tapered from 3.5 mm at the top to 1 mm at the bottom. Space between holes varied from 7 mm in some templates (Fig. 1-A, C, D) to 5 mm in others (Fig. 1-B). Care was required when filling the tapered holes in the templates to avoid trapping a bubble of air at the bottom of the hole which would prevent the diffusion of reactants.

The glass slides with templates were placed in a moist chamber to develop and were stored at either 4 C or room temp for up to 72 hr. If slides with templates are prepared in advance, it is best to charge the wells with saline to counter the loss of liquid and stabilize the gels before adding the reactants. Any saline left in the holes is removed before addition of

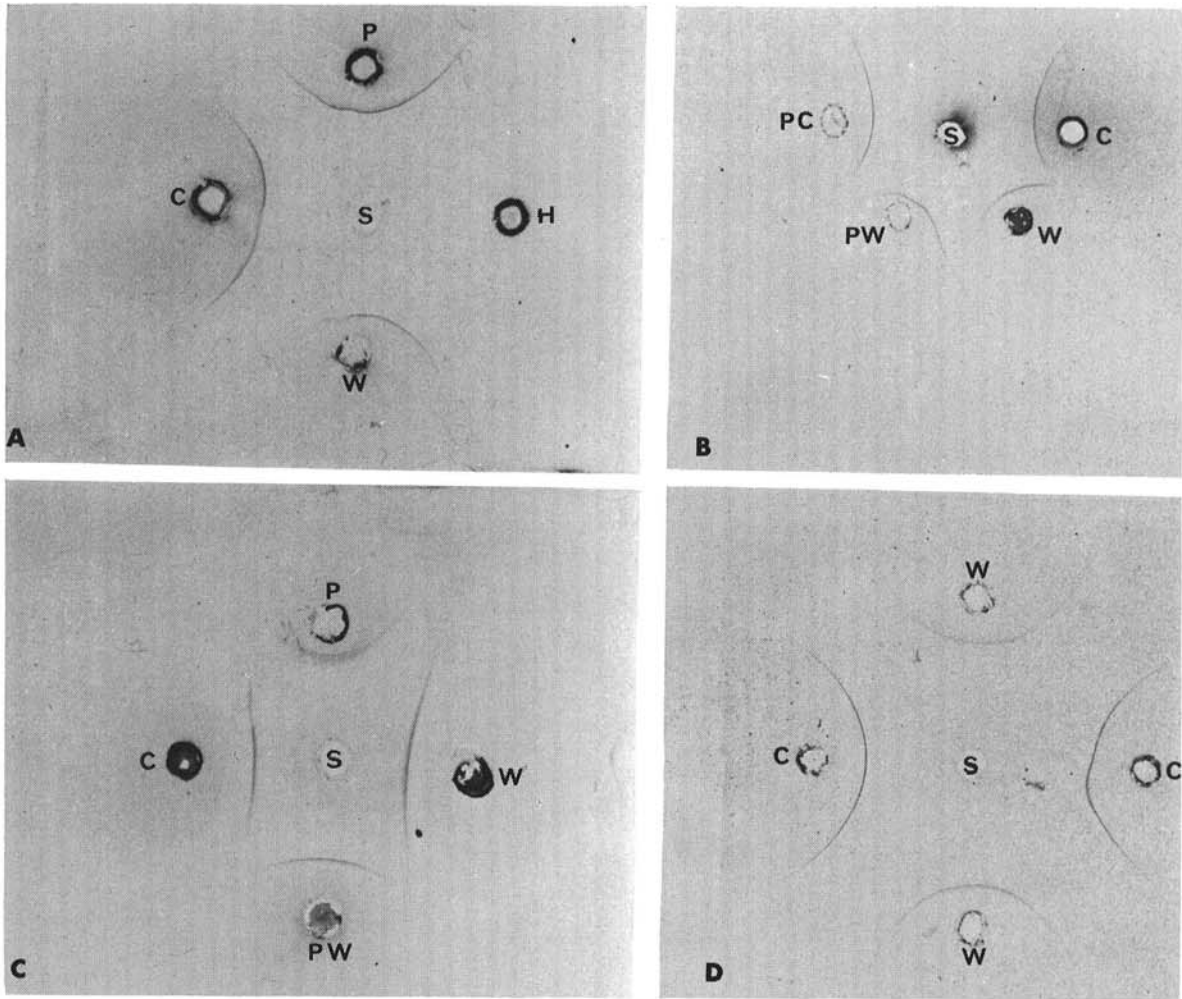


Fig. 1. Stained microslide immunodiffusion patterns of saline-diluted expressed plant juices and purified preparations of potato virus X (PVX) and PVX antiserum (Lisse) ($\times 3$). Wells contained antiserum to PVX (S), *Nicotiana glutinosa* infected with C strain of PVX (C), Russet Burbank potato infected with PVX (P), PVX-free Russet Burbank (H), *N. glutinosa* infected with W strain of PVX (W), purified PVX from *N. glutinosa* infected with W strain (PW) and with C strain (PC). Distance between wells **B**) 5 mm; **A, C, D**) 7 mm.

the reactants. After development, the plastic templates can be carefully removed in a saline bath and the slides photographed. The precipitin lines, visible to the naked eye with diffused lighting, do not necessarily need to be stained before photographing. Staining does, however, enhance the lines and increase the usefulness of the slides as a permanent record. For staining, two rinses of 0.85% saline in a 24-hr period were used to remove the excess proteins. The slides were rinsed in two changes of distilled water over a 6-hr period, then stained in a 1% Buffalo Black NBR (or 1% Thiazine Red), 7.5% acetic acid, distilled water stain for about 5 min, destained in 7.5% acetic acid, and air-dried.

The antiserum for PVX is one used routinely in the Maine seed potato indexing program (7) and is available from The Netherlands (The Laboratorium Voor Bloembollenonderzoek, Lisse). For the crude plant antigens, fresh leaves from PVX-infected plants were either ground in a mortar or pressed in a handpress

(Salm & Kipp, Amsterdam), and equal portions of 0.85% saline were added to the extracted juice. This preparation was then centrifuged at 2,200 g for 20 min and the clear supernate added to the slides.

Extracts from Russet Burbank potato and *N. glutinosa* gave clear lines in the microslide Ouchterlony tests (Fig. 1-A, C). Similar extracts from PVX-free plants produced no lines in the saline-cleared slides (Fig. 1-A). Preparations of purified PVX (5) made from infected frozen leaves of *N. glutinosa* (Fig. 1-B, C) and *Nicotiana tabacum* L. also gave good precipitin patterns. In routine inoculation tests conducted at Presque Isle, Maine, the strain designated as "common" (C) exhibits symptoms on *Gomphrena globosa* L. and *Datura stramonium* L., while the strain designated as "weak" (W) produces symptoms only on *G. globosa*.

The microtest offers a reliable and convenient method of testing for intact, native PVX in crude plant sap

extracts. Positive results have on occasion been obtained in our lab using identical crude extracts with the Ouchterlony petri dish method, but this technique is generally unreliable and slow. In addition to being faster and more reliable, the microtest requires smaller quantities of reactants. The agar film of the microslide system is much thinner than the layer of agar in an Ouchterlony petri dish, and thus tends to facilitate rapid diffusion of reactants; moreover, the diffusion rate may be further enhanced by the hydrostatic pressure of the reactants intrinsic in the design of the Plexiglas template. These factors of greater speed and conditions more conducive to diffusion may also act to avoid aggregation of virus particles, a problem frequently encountered and reported by other workers. The slow diffusion rate inherent in the petri dish system results in precipitin bands immediately adjacent to the individual antigen wells, and serological relationships with antigens in neighboring wells cannot be determined. The microtest can be used for identification and comparison studies with strains of PVX without the need for purifying the virus or degrading virus protein. It may also serve as a useful technique with other rod-shaped viruses.

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