

PHYTOPATHOLOGICAL NOTES

Immunodiffusion Assay for Potato Virus M Infection

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ABSTRACT

Ouchterlony double-immunodiffusion and single radial-diffusion procedures were successfully applied to the serodiagnosis of potato virus M (PVM). The addition of pyrrolidine to a final concentration of 2.5% to infective potato leaf extracts provided for the production of diffusible test antigen. The procedure was dependent upon the use of antibody specific for PVM pyrrolidine-degraded protein. Antiserum production was more successful in a goat than in rabbits. Goat antiserum exhibited much higher homologous antibody levels and was obtained in greater amounts. *Phytopathology* 61:873-874.

Additional key words: serology, goat immunization.

Diagnosis of latent infections by potato virus X (PVX) and potato virus S (PVS) was recently made possible through immunodiffusion procedures (1, 3). Ouchterlony double-diffusion tests were useful for limited numbers of samples, whereas radial-diffusion systems were more appropriate for mass diagnosis. In each instance, antiserum to the degraded viral protein was prepared, then used to test plant extracts to which a suitable virus-degrading compound had been added. Potato virus M (PVM) is yet another virus which in certain potato varieties causes mild or barely discernible symptoms. Hence, a procedure for its serological detection may have important applications in programs concerned with production of virus-free seed potatoes. The present report describes a procedure by which PVM infections may be diagnosed by immunodiffusion tests in agar.

The isolate of PVM used was kindly provided by N. S. Wright, Canada Department of Agriculture, Vancouver, B.C. Virus was increased in potato (*Solanum tuberosum* L. 'USDA 41956') from infected tubers, and purified in the manner previously described for PVS (1).

Initially, attempts were made to produce PVM degraded-protein (D-protein) antiserum in five outcrossed rabbits according to the protocol established for PVS (1). These efforts, however, met with little success. After an immunization period of 4 weeks, only two rabbits produced reactive antibody. Furthermore, the dilution end points of these two antisera (1:2 and 1:4) were too low to be useful. Additional attempts that involved injecting pyrrolidine-degraded PVM protein at higher concentrations or at more frequent intervals into five additional rabbits were similarly unfruitful.

These results suggested that the rabbits at our disposal possibly were genetically poor responders to the PVM D-protein antigen. If this were indeed the case, either an inbred line of suitably responding rabbits or perhaps another animal species would be necessary for PVM D-protein antiserum production.

Unpublished data from our laboratory indicated that goats may respond better to certain antigens than do normal outcrossed rabbits. Antisera were produced against the PVX D-protein antigen in two goats, and ranged in potency from 4 to 8 times that achieved in rabbits. Precipitin titers to PVX D-protein were normally 1:128, and reached 1:256 during hyperimmunization in goats, but did not exceed 1:32 in rabbits. This suggested that perhaps a goat also may respond better to the PVM D-protein antigen.

A 4-month-old goat weighing 21 lb. was injected intramuscularly weekly for 4 weeks with a 1:1 mixture of antigen and Freund's incomplete adjuvant. Approximately 1 mg of PVM D-protein prepared by pyrrolidine degradation and formaldehyde fixation (1) was injected/kg of body wt per week. Bleedings were begun 4 weeks after the initial administration of antigen, and 500 ml of blood were collected from the jugular vein at each drawing. Antiserum was separated and titered for activity against pyrrolidine-degraded PVM as described previously for PVS (1). The titer of goat antiserum to PVM D-protein was 1:16 at week 4, and held at this level for the next 2 weeks before dropping to 1:8 on the 7th week after the first injection. Based on these results and on those obtained previously with PVX, it appeared that the use of goats should make possible production of large amounts of PVM D-protein antiserum of suitable potency.

Expressed sap from infected plants was tested as follows. The goat PVM D-protein antiserum was first fractionated with 24% ammonium sulfate and the gamma globulin component dialyzed against 0.05 M Tris [tris(hydroxymethyl) aminomethane] -HCl buffer at pH 7.2 and containing 0.85% NaCl (2). The antibody was then used to verify the presence of PVM in pyrrolidine (2.5% final concentration) -treated potato leaf

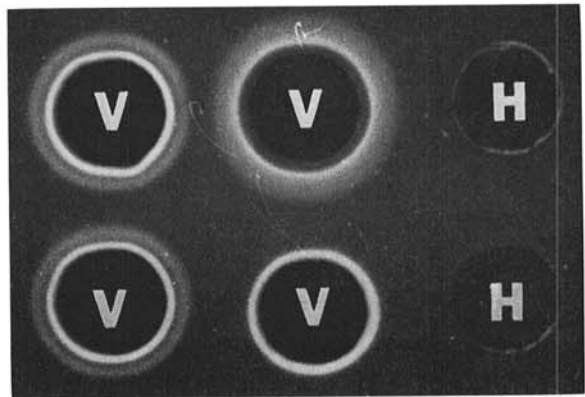


Fig. 1. A radial-immunodiffusion plate containing pyrrolidine-treated extracts from potato virus M-infected potatoes (wells labeled V) and from uninfected potatoes (wells labeled H).

extracts by Ouchterlony double-immunodiffusion tests. A prominent straight band of precipitation typical of low molecular-wt antigen was observed midway between central serum wells and depots which contained infective sap. No reaction occurred with control sap from uninfected potatoes or from PVS-infected potatoes. When 2.5 ml of fractionated antiserum (titer of 1:16) were used/10 ml of antibody-agar in a radial-immunodiffusion plate, pyrrolidine-treated extracts from PVM-infected plants produced characteristic precipitin rings around charged depots (Fig. 1). Similar reactions were not observed in pyrrolidine-treated sap from uninfected potatoes.

The results show that if suitable antibody to PVM D-protein can be prepared, either in goats or perhaps in better-responding rabbits, immuno-diffusion procedures may be used for detecting PVM in pyrrolidine-treated plant extracts. Comparable to results previously reported for PVS (1), pyrrolidine was an effective degrading compound for use in PVM serodiagnosis, whereas pyridine was not. The addition of 30% pyridine to PVM preparations eliminated stream birefringence, and although the degraded protein reacted

with PVM D-protein antiserum in tube precipitin tests, diffusible antigen fragments were not produced. Pyrrolidine also may prove useful for other viruses, especially if antiserum to the pyrrolidine-degraded viral protein is used. The usefulness of this procedure, however, may depend on the nature of the oligomers produced if denaturation accompanies subunit depolymerization, and on the extent of cross-reactivity between viral antiserum and those oligomers. In any case, it is necessary to select a denaturant for serodiagnosis which produces nonaggregated structure unit protein if diffusion in gel analysis is to be conducted.

LITERATURE CITED

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