VIROBACTERIAL AGGLUTINATION (VBA) FOR DETECTION OF PLANT VIRUSES

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ABSTRACT

A rapid slide agglutination test using polyclonal antisera conjugated to protein A-rich *Staphylococcus aureus* cells was used for the detection and identification of viruses from known infected and healthy plants. The specificity and sensitivity of the technique was evaluated in 11 antibody/antigen combinations representing 8 virus groups.

The advantages of the *Staphylococcus aureus* agglutination technique include speed, simplicity and the ability to identify viruses directly from infected plant tissues. It was applied to the detection of the following viruses: arabis mosaic, barley stripe, bean common mosaic, soybean mosaic, cowpea mosaic, squash mosaic, soybean stunt, tobacco mosaic, tomato mosaic, cowpea mild mottle and southern bean mosaic.

INTRODUCTION

The use of serological techniques for the detection and identification of viruses is well established. Direct agglutination of particulate antigens with homologous antiserum has been used for many years. Greater sensitivity can be achieved by the attachment of the immunoglobulin to an inert carrier, including latex beads, but wide variation in the conditions for asorption is often encountered and antiserum of high titre is necessary (Maat, 1970).

The requirement for such high titre antiserum was overcome by sensitizing the latex with protein A derived from *Staphylococcus aureus* (Querfurth & Paul, 1979; Torrance, 1980). An alternative method is to conjugate the antiserum directly to protein A on the surface of *S. aureus* cells, thus using the bacteria as a carrier. This technique was utilized by Chirkov *et al.* (1984) for the immunodiagnosis of plant viruses and showed that protein A, *in situ* on the staphylococcal cell, absorbs immunoglobulins without affinity for other antiserum proteins. They also showed that the technique eliminated non-specific agglutination encountered with undiluted plant sap when using latex beads or bentonite.

The paper reports the use of the virobacterial agglutination method to polyclonal antisera to detect a number of important seedborne plant viruses in infected plant saps.

MATERIALS AND METHODS

The method adopted was from Walkey (1991). The 11 antisera used, together with their homologous viral isolates are listed in Table 1.

Reagents used

Basic fuchsin stain: 25 mg/ml in 96% EtOH.

Virus antigen was diluted with phosphate buffered saline (PBS) pH 7.2, containing 2 mg/ml sodium azide.

Stock solution (0.5 M)

PBS/pH 7.2 - Na_2HP0_4 = 90.0 g
KH_2P0_4 = 9.86 g
NaCl = 8.5 g

PBS/pH 7.4 - (0.05 M) NaCl = 8.0 g
KH_2P0_4 = 0.2 g
$Na_2HP0_4.12H_20$ = 2.9 g
KCl = 0.2 g

Make up to 1 litre with distilled water.

Preparation of antigen

Plant saps were extracted into Phosphate buffered saline (PBS), pH 7.2.

Preparation of *Staphylococcus aureus* working reagent

The immunodiagnosticum was prepared *ex tempore* by mixing 1 volume of 10% *S. aureus* cells suspension with 5 volumes of the antiserum taken.

Conjugation of working reagent

Each batch of fresh antiserum was already titred for optimal dilution using ELISA. For most purposes the following quantities were used. Antiserum (1:1 mixture with glycerol) 20.8 µl, PBS 500 µl, *S. aureus* working reagent 104 µl and filtered saturated alcoholic basic fuchsin (2 drops). The preparation was stored at 4°C.

Test procedure

The *S. aureus* conjugated working reagent and the antigens from infected plant tissues were mixed together for several seconds on an ELISA plate cover, using a sterile glass stirrer. Volumes used were:70 µl conjugated *S. aureus* working reagent or 50 µl conjugated working reagent and 50 µl viral extract obtained from a macerated leaf.

The specificity of the test was checked using the following controls: (i) *S. aureus* cells sensitized by antiserum but no virus added, (ii) non-sensitized cells treated with appropriately diluted virus, (iii) healthy plant sap added to sensitized cells. No agglutination was observed in controls within the time required for the specific agglutination reaction.

The capillary tube method for microprecipitin test was also used to compare the results against using the wells of the titre plate cover.

In an attempt to determine the sensitivity of the method in terms of detection of 3 viruses, Soybean mosaic virus (SMV), Southern bean mosaic virus (SBMV) and Cowpea mosaic virus (CPMV) suspensions were prepared in PBS buffer and a series of dilutions made from a mixture of infected and healthy sap. Each dilution was tested with its homologous conjugated antiserum by mixing 5 µl of the test dilution with 5 µl of the conjugate, briskly stirring with a glass stirrer for 10 secs and then rocking the slide for a further 50 secs. Results were taken 5 minutes later for the full reaction to finish. Agglutination scores were then carried out.

RESULTS AND DISCUSSION

The results of the experiment were given in Table 2.

There is a need for a simplified serological test for the identification of plant viruses, both from seeds and plant tissues. The normal slide or capillary tube agglutination method involves the preparation of a suspension of the antibody/ antigen reaction for flocculation to occur.

The use of antisera conjugated to *S. aureus* eliminates many of the restrictions imposed by the normal agglutination method. The turbidity of the suspension is determined by