Indian Journal of Experimental Biology
Vol.21, August 1983, pp. 470-471

Immuno-osmophoresis Technique for Quick Diagnosis of *Oryctes* Virus (*Baculoviridae*) of Rhinoceros Beetle *Oryctes rhinoceros* L.

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Received 20 *August* 1982; *revised* 28 *April* 1983

Immuno-osmophoresis method is suitable for quick screening of field collected rhinoceros beetles and grubs and to assess the intensity of natural incidence of baculovirus disease.

Oryctes rhinoceros L. is one of the major ubiquitous pests of the coconut palm. A virus disease of *O. rhinoceros* caused by a baculovirus, introduced from Malaysia to the South Pacific Islands, has resulted in the successful biological suppression of the pest[1]. The same virus disease has been detected in the natural population of *O. rhinoceros* in Kerala[2]. A rapid serological method for the detection of the virus in grubs and beetles is wanting, more so for the assessment of natural incidence of the disease in the field. Enzyme-linked immunosorbent assay has been used for the detection of *Oryctes* baculovirus[3] in grubs and radioimmunoassay for other baculoviruses[4]. The present report describes the use of immuno-osmophoresis (IOP) for the detection of *Oryctes* baculovirus in *O. rhinoceros* adults and grubs. This technique has already been used for the rapid diagnosis of human[5-8] and plant viral diseases[9-11].

Antiserum to *Oryctes* baculovirus was obtained from Allan Crawford, DSIR, New Zealand and also through Sudha Nagarkatti, NCBC, Bangalore. *O. rhinoceros* beetles were collected from the crowns of infested coconut palms and grubs were obtained from cattle dung pits and dead coconut stumps/logs (breeding sites). A volume of 4.5 ml of 0.8% warm 'Oxoid' agar in 0.045 *M* phosphate buffer, *p*H 7.4, containing 0.1% sodium azide, was pipetted on to a Formvar 15/95 E (0.2% w/v in chloroform) coated microscope glass slide. The solidified agar layer was made firm by overnight refrigeration in a moisture chamber. Pairs of small wells (2 mm diam.) were cut 1 cm apart in 2 or 3 vertical rows, using a gel cutter. The vertical rows of wells on the cathodic end constituted the antigen wells and the ones near the anodic end were the antiserum wells. The apparatus needed for IOP was assembled according to John[9], with the addition of a petri dish containing crushed ice under the slide for cooling the agar gel during electrophoresis.

Field collected *Oryctes* beetles were ether anaesthetised and dissected to expose the alimentary tract. The virus diseased midgut was grossly dilated due to the accumulation of milky fluid of mucoid consistency. This fluid was aspirated out using a 1 ml tuberculin syringe and subsequently the entire gut was excised and homogenised in a tissue grinder in minimum volume of SET buffer (100 m*M*-NaCl, 1 m*M*-EDTA sodium salt, 50 m*M* Tris-HCl, *p*H 8). The aspirate/homogenate was applied directly into the antigen wells for IOP without the need for virus concentration steps. The midguts of healthy beetles were strikingly thin, translucent and contained very little clear brownish fluid. Similarly field collected II and III instar grubs exhibiting symptoms of virus disease[12] as lethargy, soft and supple body consistency, waxy and translucent appearance, in varying degrees were dissected. The midgut fluid was aspirated into a syringe and if enough fluid could not be obtained, the midgut region was rinsed with minimum volume of distilled water and finally aspirated out.

The antigen wells in the agar gel were filled with the midgut aspirates, and antiserum in the opposite wells. A current of 12-15mA and a voltage of 10 V/cm was selected with a running time of 70 min. Further increase in current resulted in cracking of agar gel and migration of cellular debris, from the antigen well, between the interspace of glass and agar gel, due to the intense electric field created. A precipitin line of growing intensity with time could be noticed after the electrophoretic run, but maximum intensity was obtained after 30 min incubation at room temperature. Midgut aspirates of healthy beetles/grubs served as controls, *Oryctes* baculovirus from South Pacific Islands as positive antigen control and normal rabbit serum as antiserum control (Fig. 1).

Detection of baculovirus by IOP in the field collected beetles was also corroborated by other methods of diagnosis such as Giemsa stained midgut smears[13,14], bioassay test[14] and electron microscopy of beetle midgut. Sections of diseased midguts, fixed in

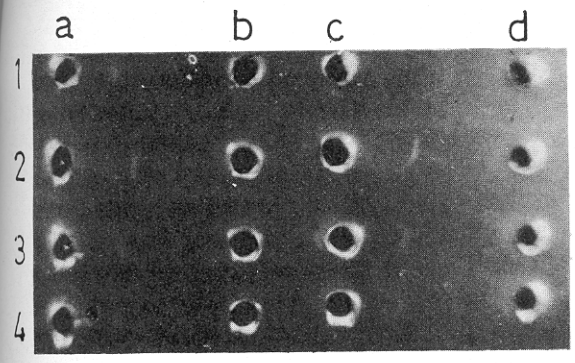

Fig. 1—Immuno-osmophoresis of midgut aspirates of baculovirus diseased *O. rhinoceros* beetles and grubs [Antigen wells: 1a, 2a, 3a—diseased aspirates of beetles; 4a, 3c, 4c—diseased aspirates of grubs; 1c—aspirate of healthy beetle; and 2c—*Oryctes* baculovirus of South Pacific Islands. Antiserum wells: 1b, 2b, 3b, 4b, 1d, 2d, 3d, 4d—antiserum to *Oryctes* baculovirus of South Pacific Islands

2% glutaraldehyde and stained with 1% osmium tetroxide, confirmed the presence of *Oryctes* baculovirus particles. Sometimes the aspirate of healthy beetles containing freshly ingested plant sap gave a broad band of precipitation against antiserum as well as normal serum. This non-specific reaction was presumably due to tannins present in sap and could easily be distinguished from the specific serological reaction by its brown colour and tendency to diffuse in all directions. In majority of the diseased aspirates tested with IOP, the precipitin line was formed near the antigen well. Appropriate dilution of antiserum or slight reduction in agar concentration displaced the line towards the centre to a limited extent. Attempts to improve the migration of viral particles enmeshed in the cellular debris, towards the anode were made by treating the aspirate with dispersing and dissociating agents such as sodium dodecyl sulfate (0.2%), 8 *M* urea, Teepol(0.01%), Triton X-100(0.1%), Tween-20(0.2%), moderate heat at 45°C for 30 min with NaCl, and sonication (43 kc/sec) for 15 min, prior to IOP. Such pretreatments resulted in complete absence of any visible precipitin reaction probably due to total degradation of surface antigens.

The authors thank Dr J J Solomon for guidance and Mr S P Jayapal for assistance. Thanks are also due to Drs M K Arnold and K A Harrap, Institute of Virology, Oxford, for ultrathin sectioning and EM observations.

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