

MOLECULAR BIOLOGY STUDIES



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5.1. Preparation of JE virus antigen in cell culture

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Starting Date : October 2004.

Duration : Three years

Funding agency : Intramural, ICMR.

Objective

To prepare Japanese encephalitis virus (JEV) antigen in Vero cell lines.

Cell culture derived antigen (CCDA) JEV antigen was developed and was partially purified. The effect of BPL treatment on the virus antigen titer was studied. Results have show that there was a slight reduction in the CCDA titer was observed

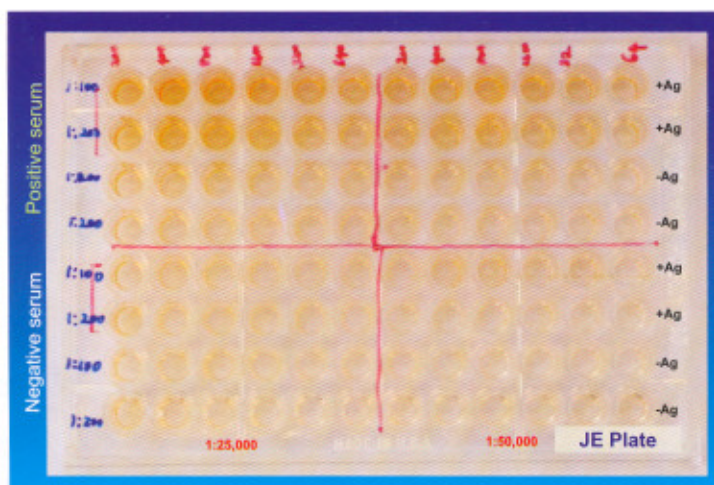


Fig. 16. IgG ELISA using tissue culture JEV antigen

when compared to the control antigen (*ie.*, BPL untreated). The BPL inactivated CCDA was further exploited in the serological assays. In order to determine the optimum dilution of the various components used in the test, such as CCDA, sera and the HRP-conjugate, checker-board titration was carried out. An experiment was also carried out to find out the possible non-specific reaction of the CCDA with the HI positive and negative human sera samples. The results have shown that no nonspecific reaction was observed between CCDA and the human sera tested (Fig. 16). From this test the optimum dilution of the CCDA, sera and the HRP-conjugate was determined. Further work is under progress.

5.2. Identification of flavivirus receptor present in adult *Toxorhynchites splendens* mosquito

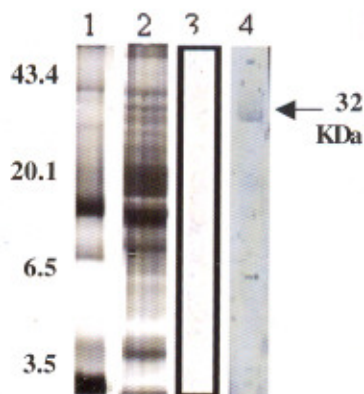
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Starting Date : January 2005.

Duration : One year

Funding agency : Intramural, ICMR.

In order to find out the flavivirus receptors present in the adult *Toxorhynchites splendens*, attempts were undertaken to standardize the SDS-PAGE gel electrophoresis technique. After resolving the proteins in SDS-PAGE, the proteins were



- 1- Molecular weight Marker (3.5-43.4 KDa)
- 2- Protein pattern of *Tx. splendens*
- 3- Negative Control
- 4 - Virus Overlay protein blot assay on *Tx. splendens*

Fig. 17. Detection of JE virus binding protein of *Toxorhynchites splendens*

transferred to PVDF membrane by Western blot method. Initially, the successful transfer of protein from gel to membrane was confirmed by staining the piece of PVDF membrane by coomassie blue. The protein transferred membrane was post coated with bovine serum albumin (BSA). Tissue culture derived JEV antigen was allowed to react with the proteins (Virus Overlay Protein Blot Assay [VOPBA]). After washing steps, JEV monoclonal antibody was used to trace the virus antigens. Anti mouse HRP-conjugate was allowed to react with the antibodies after necessary washings. Addition of substrate showed a band in the PVDF membrane. Throughout the experiment, a negative control was used by adding all the components, except in the place of JEV infected cell culture antigen; the uninfected JEV cell culture was used. The results have detected a protein, which could act as a receptor for the binding of JEV (Fig. 17). Further work is under progress.

5.3. Mosquito pupal exuvium – an alternative source of genomic DNA

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Period : November 2005 - January 2006

Funding : Intramural

Genomic DNA isolation is very much essential for the molecular studies. Mosquito whole body or part of body segment is being used for isolation of genomic DNA. For the first time the usefulness of pupal exuviae for genomic DNA isolation has been tried and proved as a good source of genomic DNA.

Genomic DNA extraction

The pupal exuviae of *Aedes aegypti* and *Anopheles stephensi* were homogenized separately in 200 µl of "DNA Extraction Solution" kit (Genei, Bangalore) and the homogenate was centrifuged at 10,000 rpm for 15 minutes. The supernatant was transferred to a fresh microcentrifuge tube and the DNA was precipitated with equal volume of 100% ethanol. Precipitated DNA was sedimented by centrifuging at 10,000 rpm for 5 minutes. The precipitated DNA was washed twice with same volume of 95% ethanol with spin at 5000 rpm for 5 minutes. A final wash was given with same volume of 70% ethanol and sedimented the DNA by centrifuging at 5000 rpm for 5 minutes. The DNA pellet was allowed to dry retaining some moisture (as per manufacturer's instruction). The pellet, after removal of excess alcohol, was dissolved in DNase free deionized water.

Randomly Amplified Polymorphic DNA (RAPD) assay

Randomly Amplified Polymorphic DNA technique is used to analyze genetic variants in a given population. It is basically a Polymerase Chain Reaction (PCR) in which the target DNA is amplified with an arbitrary primer.

To check whether the genomic DNA isolated from exuviae could further be used in other molecular applications it was subjected to a random amplification with a commercially available arbitrary primer. The randomly amplified DNA fragments were visualized in a 1.2% agarose gel (Fig. 18). The results proved that the genomic DNA isolated from exuviae could be amplified in a polymerase chain reaction. Hence the study proves the suitability of exuviae as a good source of genomic DNA, useful in molecular applications.

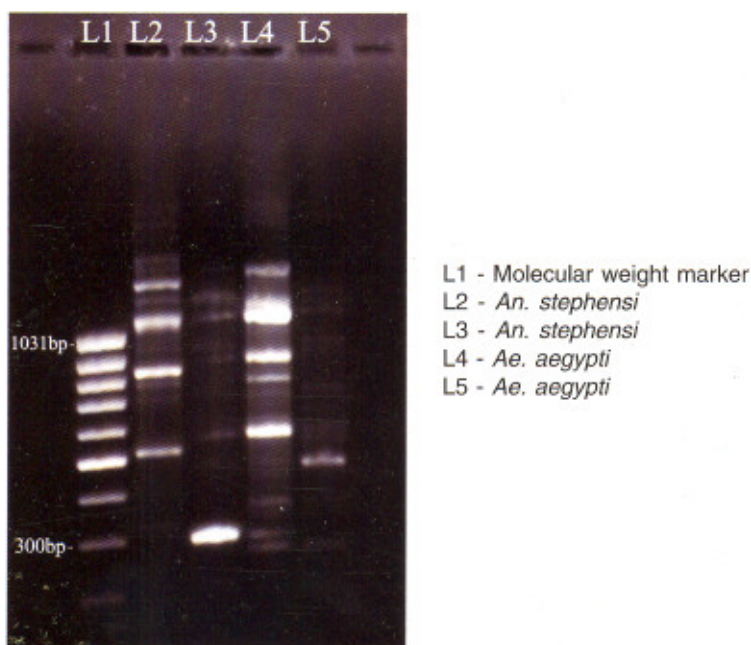


Fig. 18. RAPD amplification of genomic DNA isolated from exuviae (pupal skin) of *An. stephensi* and *Ae. aegypti*