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HOST RANGE OF THE AUTOGRAPHA CALIFORNICA MULTICAPSID NUCLEAR POLYHEDROSIS VIRUS (ACMNPV) IN RELATION TO GRANULOSIS VIRUSES SUSCEPTIBLE LEPIDOPTERAN SPECIES IN EGYPT

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ABSTRACT

The host range of Autographa californica multicapsid nuclear polyhedrosis virus (AcMNPV) was studied in Egypt on 3 pest species susceptible to granulosis viruses. Among the cotton leafworm, Spodoptera littoralis, the maize corn borer, Sesamia creatica and the potato tuber moth Plutella xylostella, only S. creatica and P. operculata were susceptible to the AcMNPV. Ultrastructural studies and virus detection by nucleic probe showed that S. creatica was highly susceptible, while only 5% infection was obtained in P. operculata.

Key Words: AcMNPV, host range, GV, Spodoptera littoralis, Sesamia creatica, Plutella xylostella.

INTRODUCTION

The Autographa californica multicapsid polyhedrosis virus (AcMNPV) became a reference model for the viral genetic researches, biotechnology and biological control of insects. Its wide range of interest was due to several reasons. A large number of lepidopteran species belonging to twelve families, often representing important pests with high geographical distribution, were susceptible to AcMNPV (Albrecht, 1986). The AcMNPV could be mass produced and several of its hosts could be reared industrially on artificial diets (Vail et al., 1972). The AcMNPV could be multiplied in vitro on a certain number of insect cell lines with large scale of origin (Granados and Hashimoto, 1989). The AcMNPV could be used as expression vector for broad range of genes with different origins (Eldridge et al., 1992) and its great capacity of recombination could be used to modify its pathogenicity or host range by introducing foreign genes (Müller et al., 1983).

As in our laboratory, the use of granulosis viruses (GVs) as biological control agents and as models for molecular studies represented a great importance, AcMNPV affinity of certain hosts which are susceptible to GV in Egypt was examined. (Abol-Ela et al., 1994; Matthiessen et al., 1978; Fédière et al., 1992). The objective of this study is to obtain models which will permit the analysis of viruses produced from genetic recombinations between GV and NPVs.

MATERIALS AND METHODS

Virus

AcMNPV strain was obtained from Dr. Croizier (INRA, France). For conserving the initial viral properties, the virus strain was multiplied in SF9 cell line grown in Grace's modified medium (Léry and Fédière, 1990). The AcMNPV was also multiplied on Pectinophora gossypiella (Vail et al., 1972) and used as a source of infectious haemolymph.

Insect Rearing

Both P. pectinophora and S. littoralis were reared under laboratory conditions on semiartificial diets (Shorey and Hale, 1963; Couilloud and Giret, 1980). For P. operculata, small potato tubers were used as natural feeding source, while S. creatica was reared on a new semiartificial diet (unpublished data).

Larval Infection

AcMNPV produced on P. gossypiella larvae was purified and concentrated up to 60 x 10^3 PBs per millilitre. AcMNPV produced on the SF9 cell line was purified as follows: infected cells were scraped from the flask walls and centrifuged at 13000 g for 15 min. The pellets were suspended in 1 ml tris (10 mM pH 7.8). The polyhedra were mixed with Triton-X at the final concentration of 0.1%, then used as infectious suspension on the artificial diet or potato tubers.

Newly hatched larvae of S. littoralis and P. operculata were exposed to contaminated diets while 2nd and 3rd instars of S. creatica and P. gossypiella were used for experimental infections.

Extraction and Analysis of Viral DNA

Infected larvae were homogenized in 50 mM Tris, 2 mM SDS pH 7.8, then filtered through cheesecloth. The suspension was purified by centrifugation on sucrose gradient 30-70% (W/V). Virions were liberated by alkaline treatment with Na2CO3 (60 mM final, pH 11). DNA was extracted by addition of 2% sodium lauryl sarcosine and 200 μg/ml of protease K for 2 hrs at 50°C, followed by two extractions with phenol-chloroform-isooamyl alcohol (25/24/1), two phenol and two chloroform isooamyl (24/1) extractions. Finally, the DNA was precipitated with 2 volumes of glacial ethanol in presence of 1/10 volume of sodium acetate (3M) at -20°C overnight. Electrophoretic mobilities of different DNAs, digested with Eco R and Hind II restriction endonucleases and the migrations through 1% agarose gel were realized according to Maniatis et al. (1989).

Ultrastructural Studies

Larval tissues were prepared for electron microscope observations as follows: small pieces of fat body were fixed in 2.5% glutaraldehyde prepared in 0.01 M sodium cacodylate, then post-fixed in osmium tetroxide 1% in distilled water and embedded in Epon Araldite. Finally, ultrathin
sections were stained with uranylacetate saturated in 50% ethanol, then by lead citrate (Reynolds, 1963). Grids were examined through transmission electron microscope ZEISS EM10.

**Preparation of Non Radioactive Nucleic Probe and Hybridization**

Synthesis of non radioactive and dot blot hybridization for both AcMNPV and GVs were performed according to the supplier's protocol (Boehringer Mannheim).

**RESULTS AND DISCUSSION**

Four trials with AcMNPV (10x10^3 PIBs/ml) were carried out to infect *P. opercula*. No viral infection was observed. No late symptoms could be seen. Of the second, infection of *P. opercula* with AcMNPV polyhedra, purified from infected cells of the SF9 cell line, caused 4% death and 13% abnormalities in larvae. Examination through optical microscope demonstrated the existence of inclusion bodies with cubic-shape and a significant number of granules (PTMGV) distributed in tested tissues were observed as well. Nucleic probes originated from both AcMNPV and PTMGV, separately applied, revealed that only 5% of larvae mixed infected with AcMNPV and PTMGV, while 75% of total insects were only infected with GV (results not shown). No AcMNPV infection alone was found. The presence of both GVs and AcMNPV could lead to the role, not yet precised, of PTMGV as an enhancing factor like other GVs previously studied (Yafeng et al., 1989; Hashimoto et al., 1991).

Concerning the trials for AcMNPV multiplication in PTM healthy larvae, only 3 larvae died at the 2nd instar 9 days post-infection showing high infection by AcMNPV (Fig. 1), while 40 larvae seemed to be normal and active at 4th to 5th instar and completed their life cycle. These low levels of AcMNPV multiplication in *P. opercula* per os was not correlated with the successful multiplication in vitro of the same virus on *P. opercula* cell lines (Léry et al., 1995, in press). This contradiction could be due to one or more of the following reasons. Different levels of susceptibility to the viral infection within the insect populations could exist. The same probability could be expected in the viral population, especially because the detection of AcMNPV by the nucleic probe in the slightly abnormal alive larvae revealed its multiplication. Finally, this phenomenon could be simply due to the viral doses employed.

As previously reported by Schepens and Wysoki (1989), no multiplication of AcMNPV was obtained in *S. littoralis* larvae.

Between 4 to 5 days post-infection of *S. cretica* by AcMNPV, the larval activity was remarkably reduced. Seven days post-infection, larvae seemed to be inactive and death began to occur. Examination of fat body tissue revealed the presence of polyhedra in the nuclei. Electrophoretic profiles of viral DNAs obtained from three infection replicates of *S. cretica*, analyzed with restriction endonucleases *Eco* RI and *Hind* II, compared to AcMNPV, indicated the identical DNA fragments sequences (Fig. 2). The examination of fat body tissues by electron microscopy revealed the presence of both viral particles of nuclear polyhedrosis type and ScGV in the cytoplasm (Fig. 3).

The different results obtained on the 3 tested insects, regarding their susceptibility to AcMNPV could be considered according to two points of view. Firstly, if the tested host is not susceptible to AcMNPV (*S. littoralis*), it can play the important role of filter for the selection of genetically modified AcMNPV recombined with GV, in order to enlarge its host range. Secondly, if the insect is susceptible to AcMNPV (*S. cretica*), the tested insects will be added to the host list of AcMNPV, which could represent a possible alternative baculovirus for biological control after the achievement of necessary studies of the viral pathogenicity and its persistence, particularly at the Delta and Nile valley.

In case of *P. opercula*, the fact that a little response to AcMNPV was observed, could permit us to consider the concept of filter.

In conclusion, using these different models, the potentialities of AcMNPV could be enhanced to play a new role in biological control programs, where the GV was the only possibility.

**REFERENCES**


Fig. (1) Intranuclear polyhedral inclusion bodies (P) in *P. operculella* fat body cells.

Fig. (2) Electrophoretic profiles of AcMNPV DNAs.

1. AcMNPV multiplied in SF9.
2. AcMNPV multiplied in *S. cretica*.
(E) AcMNPV digested with Eco RI endonuclease.
(H) AcMNPV digested with Hind III endonuclease.
(M) DNA marker III (Boehringer).
Fig. (3) Electron micrograph of *S. cretica* fat body infected with AcMNPV (bar represents 2μ).

(P) Polyhedral inclusion body.


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Titre original : Host range of the Autographa californica multiple nucleopolyhedrovirus (AcMNPV) in relation to granulosis viruses susceptible lepidopteran species in Egypt

Titre en Français : Spectre d'hôte du virus de polyéthylene nucléaire de Autographa californica (VPNAc) envers les lepidoptères sensibles à d'autres granuloses en Égypte.

Mots-clés matières : VPNAc, spectre d'hôte, granulose, Autographa, Spodoptera littoralis, Sesamia nonella, Pheosinoptera specularia.

Résumé en Français : Le spectre d'hôte du virus de polyéthylene nucléaire de Autographa californica a été étudié en Égypte sur 3 ravageurs susceptibles à la granulose. Parmi les espèces testées, Spodoptera littoralis ravageur du cotonier, Sesamia nonella, et Pheosinoptera specularia, larvaires de la pomme de terre, seul S. nonella est susceptible. L'étude ultrastructurale et la détection du virus par sonde nucléique résulèrent que S. nonella est très sensible alors que 5% seulement d'infestation a été obtenu avec P. specularia.

Les titres, mots-clés matières et résumés en Anglais sont indispensables pour les documents destinés à entrer dans les Bases AGRIS et ASFA (Aquatic Sciences and Fisheries Abstracts).