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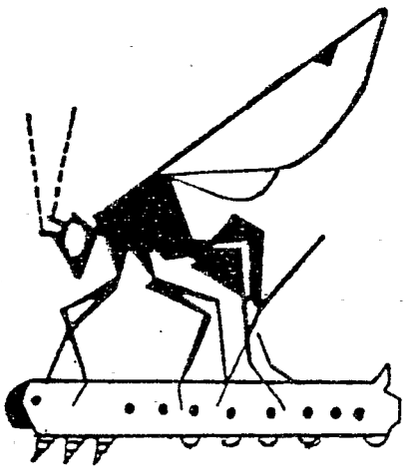

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MULTIPLICATION OF THE POTATO TUBER MOTH GRANULOSIS VIRUS IN *PHTHORIMAEA OPERCULELLA* CELL CULTURES

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ABSTRACT

Using cell cultures, deriving from embryonic cells of *Phthorimaea operculella* which were cultivated at 19°C, the complete replication of the potato tuber moth granulosis virus (PTM GV) is obtained *in vitro* as a first record.

Key Words: *Phthorimaea operculella*, cell line, granulosis virus, replication.

INTRODUCTION

The potato tuber moth (PTM) *Phthorimaea operculella* Zeller (Lepidoptera:Gelechiidae), a cosmopolitan dominant pest in sub- tropical and tropical areas, especially in Egypt, is responsible for very important losses in potato production (Das *et al.*, 1982). The use of chemical insecticides in PTM control was dangerous for humans and generates potatoes unfit for consumption (Von Arx *et al.*, 1987). Among the possible alternatives to chemicals, one of the most credible is the granulosis virus (Baculoviridae, Eubaculoviridae) isolated from this insect and already applied in several countries (Raman *et al.*, 1987).

At this step, it will be important to select viral clones to be used as biological agent against this pest, to investigate their molecular biology and to start genetic manipulations on these viruses. Until recently, this possibility was only existing on the other group of the baculoviruses, the nuclear polyhedrosis viruses (NPV) for which virus susceptible cell lines have been selected (Goodwin *et al.*, 1970). The first report of GV replication in a cell line was by Naser *et al.* (1984) who demonstrated complete replication of *Cydia pomonella* GV (CpGV) in a cell line originating from *C. pomonella* embryonic cells. Other attempts have also been made with other species, but with negative or slight multiplication of the virus (Granados *et al.*, 1986; Dwyer *et al.*, 1988). All of aforementioned cell lines, lost their susceptibility to the GV after a few months in culture.

On the PTM, no indication concerning GV multiplication was reported (Pant *et al.*, 1977; Grace, 1980). Until 1991, a number of cell cultures from *P. operculella* was obtained in our laboratory by different methods of culturing, by cloning and by selecting factors as media or temperature. Firstly, negative results were noticed (Lery *et al.*, in press), but recently, a DNA multiplication was obtained in cloned cell lines of PTM, from our first established cell line ORS-Pop-93 multiplied at 27°C (unpublished data).

As no complete virions and granules were obtained from the PTM GV *in vitro*, the susceptibility of all new cell cultures, established from *P. operculella* in our laboratory were tested against the PTM GV.

MATERIALS AND METHODS

Cell Lines

ORS-Pop-93 cell line obtained from heterogeneous embryonic cultures (Lery *et al.*, in press) and five cell lines from homogeneous cell cultures, obtained by selecting (Pop2, Pop3, Pop4) or cloning (c11A, c12B) (publication in process) were cultivated in Grace's modified medium containing 10% fetal bovine serum (FBS) and incubated at 27°C (Lery and Fédière, 1990). Twenty five new selected cell cultures, deriving from a new established cell line of *P. operculella* (ORS-Pop-95), always multiplied at 19°C, were cultivated under the same conditions and incubated at 19°C. The cells were routinely subcultured every 5 to 7 days.

Virus

A granulosis virus isolated from the potato tuber moth, *P. operculella* (PTM GV), Tunisia isolate, gift from Dr. El-Bedewi (International Potato Center, Egypt) was used to test the infectivity of the cell lines of *P. operculella*.

Infection and Transfection of the Cells

Infections: Cell cultures, seeded at $2x10^6$ cells into 25 cm^2 tissue culture flasks were infected after 24 hours with virions prepared from infected larvae. They were homogenized and ultrasonicated 1 min in 2 ml Grace's modified medium without FBS. After 2 centrifugations at 5000 g for 20 min, the supernatant diluted in Grace's modified medium, was filtered on 0.45 µ. After 2-hour contact, the 1.5 ml of viral suspension was removed and replaced by 4 ml of fresh medium containing 10% FBS and the cells were incubated at 27°C or 19°C, depending on the cell lines used.

Transfection: The cell cultures in the same conditions as above, were rinsed with Grace's medium then inoculated with 2 µg viral DNA, mixed with 20 µl DOTAP (Boehringer) and diluted in 1.5 ml of Grace's modified medium. After 4-hour contact, the DNA was removed, the cells were then rinsed and 4 ml of Grace's modified medium containing 10% FBS were added. The cells were incubated at 27°C or 19°C, depending on the cell lines used.

Viral serial infection: Infectious supernatants were used to infect new cell cultures to test the efficiency of the virus

among the passages. 0.75 ml of each supernatant was diluted with the same volume of Grace's modified medium without FBS to infect a 25 cm^2 flask. The infection was performed according to the above mentioned procedure.

ELISA Test

For detecting the viral proteins, a specific mouse serum was prepared by injecting the total proteins of granules. The indirect method of ELISA test (enzyme linked immunosorbent assay) using alkaline phosphatase, was conducted according to Kelly *et al.* (1978).

DNA Probe

The dioxygenin labelled SIGV DNA probe was applied according to the protocol recommended by the supplier (Boehringer). The same protocol was applied for the hybridization "dot blot" technique.

Purification of the Virus

The PTM GV infected or transfected cells were collected 15 to 20 days post-infection or transfection, scrapped from the flasks and centrifuged 5 min at 400 g. The pellet was treated in STE buffer (NaCl 0.15M.; Tris 0.02M; EDTA 1 mM; Aprotinine 0.5% and NP40 0.5%) during 10 min at 0°C under agitation. The treated pellet and supernatant were homogenized with Potter and ultrasonicated. After centrifugation for 30 min at 15,000 g, the pellet was resuspended in Tris 0.0M (pH 7.5), deposited on a 30% to 70% (W/W) sucrose gradient and centrifuged 20 min at 30,000 g. The band containing granules was collected and concentrated as above and stored at -20°C. The supernatant was ultracentrifuged 30 min at 100,000 g. The pellet resuspended in Tris 0.01M pH 7.5 overnight, was deposited on a 20% to 50% (W/W) sucrose gradient and centrifuged 1 hour at 100,000 g. The band containing virions was collected and the particles were concentrated as above and stored at -20°C.

Electron Microscopy

Purified virus preparations were negatively stained in 2% (W/W) uranyl acetate, pH 7.4 and examined through a Zeiss electron microscope.

RESULTS AND DISCUSSION

The susceptibility of the 25 new cell cultures originated from embryonic cells of *P. operculella*, cultivated at 19°C and the 6 cell lines cultivated at 27°C, were tested against the PTM GV. Positive reactions were noted on 5 cell cultures cultivated at 19°C using DNA probe. As previously noted, the clone 2B showed also positive reaction with DNA probe. ELISa test gave no significant results.

After purification, complete granules were identified at a low concentration in the five cell cultures incubated at 19°C.

Three successive passages of the virus on the same culture, produced the same result without notable decrease in the virus production.

This result represent the first report of the replication of the PTM GV *in vitro*. The fact that complete granules were obtained after infection of 19°C selected cell cultures, confirmed the results obtained by Winstanley and Crook (1993) on the *C. pomonella* model. Comparing with their results, the rate of multiplication seems to be relatively low with the PTM GV, indicating that a few population of the cells are susceptible to the virus. The occurrence of incomplete multiplication in cloned cells, even if they were multiplied at 27°C, permits us to suppose that the cloning of the cell cultures grown at 19°C, could easily increase the multiplication of the PTM GV by selecting the susceptible cells.

These results open a way to the cloning of the PTM GV and its complete characterization, to study its mode of replication and compare it to other baculoviruses and finally to manipulate them to increase their potentiality for biological control application. In this study, we have only tested the Tunisia isolate of PTM GV, but different strains of PTM GV were described (Vickers *et al.*, 1991). The susceptibility of our cell cultures could be tested against all the isolates to compare their pathogenicity *in vitro*.

Finally, our previously described results indicated the multiplication of the *Autographa californica* MNPV (Ac MNPV) on the same cell lines (Lery *et al.*, in press). The study of the possible ogenetic recombinations between the Ac MNPV and the PTM GV, in order to obtain more performant viruses is highly required.

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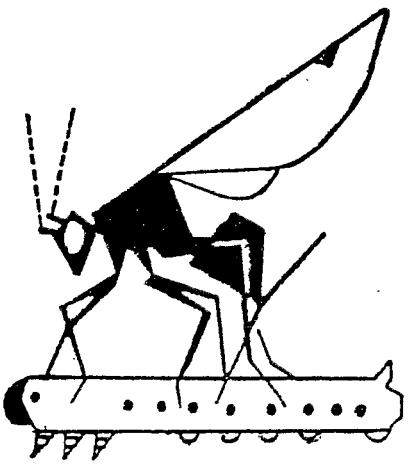

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Titre original : Multiplication of the Potato tuber moth granulosis virus in *Phthorimaea operculella* cell cultures.

Titre en Français (si le document est en langue étrangère) : Multiplication de la granulose de la teigne de la pomme de terre en cultures cellulaires de *Phthorimaea operculella*.

Mots-clés matières (10 au plus) : *Phthorimaea operculella*, lignée cellulaire, granulose, Baculovirus, teigne de la pomme de terre.

Résumé en Français (150 mots maximum) : Grace à l'obtention de cultures cellulaires de *Phthorimaea operculella* dérivant de cellules embryonnaire cultivées à 19°, une réplication complète du virus de granulose de la teigne de la pomme de terre (PTM GV) a été obtenue in vitro pour la première fois

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