Multiplication of *Sesamia cretica* granulovirus in two homogeneous cell lines

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**ABSTRACT**

A complete replication of the *Sesamia cretica* Granulovirus (ScGV) was obtained in vitro by viral infection, as a first record, in two *Spodoptera* cell lines, S152 and Sf 9. ScGV was multiplied in the two cell lines cultivated and established at 27°C after infection with concentrated purified virions used as inoculum. The presence of virus was detected by a DNA nucleic probe, confirming previous results detected by ELISA test. The observation of ultrathin sections of infected cells indicated that complete granules were present in the cell cytoplasm in a significant amount. Purified suspension of granules was obtained after ultracentrifugation on sucrose gradient. The biological characterization of ScGV was completed by in vitro replication. This important Granulovirus was already used by the authors for microbial control of *S. cretica* larvae on maize.

**Key words:** Granulovirus, *Sesamia cretica*, viral replication in vitro, *Spodoptera* cell lines, maize.

**INTRODUCTION**

Granulovirus (GV) was isolated from the maize pink borer *Sesamia cretica* Lederer (Lepidoptera, Noctuidae) in Egypt (Fediere et al., 1993). This Granulovirus was already used for microbial control of *S. cretica* larvae in maize fields (Fediere et al., 1997). Establishing an in vitro permissive system is desirable to progress in the biological characterization of the ScGV.

**MATERIALS AND METHODS**

**Cell lines**

The two homogeneous cell lines S152 and Sf 9 derived from *Spodoptera littoralis* Saunders and *Spodoptera frugiperda* J.E.Smith, respectively and established at 27°C were a gift from Dr.J.M. Quiot, Saint-Christol-Les-Ales, France.

**Virus stain**

The *S. cretica* Granulovirus (ScGV) strain used to test the susceptibility of the different cell lines was the original strain propagated in the maize pink borer larvae for several years in our laboratory. The suspension of granules was digested by Sodium carbonate solution (0.1 M, pH 10.2) for 30 min. After dissolution in the alkali, the solution containing the virions was centrifuged at 5,000 g for 10 min to remove undissolved granules and was purified on sucrose gradient (30-70%), centrifuged at 80,000 g during 75 min, then the band of virions was collected and washed using Tris-Buffer (0.05M, pH 7.8). The virions were pelleted by centrifugation at 80,000 g during 75 min, and then, the virus was resuspended in 2 ml of Tris-Buffer, and...
the concentration of the final suspension was measured on the spectrophotometer at 260 nm.

Infection

The cell cultures, seeded at 2x10^6 cells in 25 cm² tissue culture flasks were infected after 24 hr, only with virions. The cells were infected with 1.5ml of media without FBS, containing virions at 1 DO260, filtered on 0.45 um. 12 hr of contact between the virus and the cells were used, then the supernatant was removed and replaced by 4ml of fresh media containing 10% and 15% FBS for S9 and S152 respectively. The cells were incubated at 27°C during 2 weeks.

Tests of virus susceptibility

ELISA tests: For detecting the viral proteins, ELISA was made using the direct method. Specific conjugate was elaborate by extracting globuline from a total rabbit immunoserum anti-ScGV. IgG-enzyme conjugate was prepared and used at a concentration of 1/1000. The same dilution was used for the globuline. For the samples, either the healthy cells, as for control, or the infected cells, several dilutions of 1/10, 1/100 and 1/1000 were used.

DNA probe tests: A DNA probe prepared from the total extract DNA of the ScGV was used. The digoxygenin-labelled DNA probe was applied according to the protocol recommended by the supplier (Boehringer). The same protocol was applied for the dot-blot hybridization technique.

Purification of the virus from the cells

The ScGV infected cells and their supernatants were collected 15 days post infection scrapped from the flasks and centrifuged for 5 min at 400 g. The pellet was treated in STE buffer (NaCl 0.15 M, Tris 0.02 M, EDTA 1mM, Aprotinine 95% and NP40 0.5 %) during 10 min at 0°C. 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-Buffer, deposited on a 30 % to 70 % (w / w) sucrose gradient and centrifuged for 30 min at 30,000 g. The band containing the granules was collected as above and stored at 20°C.

Electron microscopy

Purified viral suspension was negatively stained in 2 % Uranyl acetate, pH 7.4 and examined through an electron microscope. For the ultrastructural studies, the infected cells were prefixed in Glutaraldehyde-Buffer (2.5% in 0.1 M Cacodylate-Buffer, pH 7.4) for 1 hr at 4°C. The cells were pelleted 5 min at 400 g and dispersed in 2 % low melting agarose before post-fixation in 1% Osmium tetroxide, dehydrated and embedded in pure epon. Stained ultrathin sections were examined through an electron microscope.

RESULTS AND DISCUSSION

Fifteen days after infection with virions originating from infected larvae, positive reactions using ELISA tests were noted in both cell lines, cultivated at 27°C. These results were confirmed by DNA probe tests which indicated the presence of the S. cretica Granulovirus in the cells.

After purification of infected cells, complete granules were identified under an electron microscope in the viral suspension (Fig.1). For the first time, complete replication of ScGV was obtained in vitro. Ultrastructural studies of ultrathin sections of infected cells, showed the presence of Granulovirus usually are called capsules or granules. They measure 200 to 300 by 400 to 500 nm. The virus appears to be confined to the cytoplasm, free as well as included in vacuoles (Fig. 2). In the infected cells, it was noticed that the membrane of the nucleus remains without changes and had been not disrupted. No
virions were observed inside intact nuclei. The same cytopathology characteristics of Granulovirus infection were observed \textit{in vivo} in the larval fatbody tissue of \textit{S. cretica} infected with ScGV (Fediere, 1996). The shape of the granule does not seem to conform to any precise geometric pattern. Longitudinal sections of capsules showed structural differentiations. The ovocylindrical complete granules. Occlusion bodies of shape sometimes appears angular or even hexagonal. Around the enveloped nucleocapside (virion) the matrix of the GV consists of a paracrystalline protein lattice called granulin. The arrangement of the granulin appears to be random. Cross sections of the granules showed in the center the electron dense nucleocapsid, a surrounding unit membrane and the capsule matrix of granulin (Fig. 3).

The \textit{in vitro} multiplication of this important Granulovirus completed its biological characterization. This would provide investigators a more completely known pathogen for use as microbial agent against \textit{S. cretica} larvae on maize.

\textbf{Fig. (1)}: Electron micrograph of a purified suspension of ScGV multiplied in Sf9 cell line. Bar represents 300 nm.

\textbf{Fig. (2)}: Electron micrograph of SIS2 cell infected with ScGV. In the cytoplasm, a large number of complete granules, free, as well as included in vacuole (V). Bar represents 250 nm.
Fig. (3): Electron micrograph of S152 cell infected with ScGV. In the cytoplasm, a number of granules were sectioned at different angles. Longitudinal and cross sections showed the crystalline lattice of the granulin (G), the unit membrane (M) and the electron dense nucleocapsid (N). Bar represents 100 nm.

REFERENCES


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