IN VIVO STUDY ON A GRANULOVIRUS IN ARMYWORM, *SPODOPTERA LITURA*

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Introduction

Granulovirus (GV) and nucleopolyhedrovirus (NPV) are the most common and widely studied insect viruses in invertebrate. GV comprises of capsules containing a single virus particle while NPV has polyhedra containing many occluded virus particles, either one nucleocapsid per envelope (SNPV) or several nucleocapsids per envelope (MNPV). The potential of NPVs as biological control agents has been well documented for over a decade. However, not much work being reported for GVs. *Spodoptera litura* (Lepidoptera: Noctudae) , armyworm, is a polyphagous pest feeding on various agricultural crops. It occurs only sporadically, but often causing great devastation. Larvae of *S. litura* infected by nucleopolyherovirus has been reported by Maeda et al. (1990). Malaysian isolates of nucleopolyhrovirus that infect *S. litura* have been reported for years. This is the first report of a granulovirus that infects *S.litura* in Malaysia.

Materials and Methods

Purification of virus capsules. The diseased larvae were triturated in 0.1% SDS before filtering through four layers of muslin cloth (Harrap et al. 1977) . The filtrate was centrifuged at low speed to discard the debris. Supernatant was collected and spun at 7000 g for 10 min (Singaravelu and Ramakrishnan, 1998). The pellet containing capsules was resuspended in distilled water and then layered onto a discontinuous sucrose gradient of 40-80% (v/v). After centrifugation at 87,000g for 120 min, the capsules were collected, diluted in distilled water, and pelleted at 87,000 g for 60 min. The capsules were washed twice and the concentration of capsules was determined under phase contrast microscopy by using a counting chamber. Viral inoculation. Late 2^{nd} –instar larvae were starved for 24 hr and then infected orally with diet containing 4.0 x 10^8 capsules. Controls were fed with diet that were inoculated with water only. Samples from the abdomen were taken at 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, 96 and 120-hr post inoculation (p..i.) for electron microscopy. Electron microscopy. Specimens were fixed in 4% buffered glutaraldehyde, post-fixed in 1% buffered osmium tetroxide, and followed by dehydration in a series of acetone, infiltration, embedding and sectioning in a standard manner. Specimens were stained in 2% methylamine tungstate.

Results and Discussion

The infection began in the midgut cells. Capsules of GV were dissolved in the midgut lumen deliberating the virus particles. The peritrophic membrane disintegrated and the virus particles attached to the microvili releasing the nucleocapsids into the cytoplasm after 12-hr p.i. After 24-hr p.i., a large number of naked nucleocapsids were observed in the vicinity of virogenic stroma. Virogenesis continued in the cytoplasm. The outer layer of the nucleocapsid was formed from the endoplasmic reticulum after 48-hr p.i. Enveloped nucleocapsids occurred singly or in aggregates in vesicles. After 72-hr p.i., the formation of capsules was observed. The deposition of crystalline protein began from one end of the virus particle and extended towards the other end until the complete capsule was formed. Many capsules were observed in the cytoplasm after 72-hr p.i. Each capsule showed only one virus particle that contained only a single nucleocapsid. The virus particle was rod-shaped, occluded by the proteinaceous matrix. After 120-hr p.i., matured capsules were seen to have an outer covering over the surface of the occlusion body. This envelope is also known as calyx by other researcher. The above observation was mainly based on fat bodies. No sign of infection was detected in the trachea matrix. The formation of GV in the larvae was confirmed to have the same sequence of infection as reported by Begon et al. (1993). At the final stage of infection, external symptoms were yellowish or whitish covering the whole dorsal and ventral areas of the larvae. They became fragile and burst releasing the milky haemolymph containing the capsules. Infected larvae were bigger in size than the control larvae.

Conclusions

From the electron microscopy investigation, the granulovirus produced the characteristic pathological changes in infected cells of *Spodoptera litura.*

References

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