Granulovirus Isolated from *Spodoptera litura*

Norani Abdul Samad

Faculty of Science and Environmental Studies
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

Telephone Number of Corresponding Author: 03-89466709
E-mail of Corresponding Authornoraini@fsas.upm.edu.my

Key words: *Spodoptera litura*, granulovirus, capsules, baculovirus

Introduction

Spodoptera litura Fabricus commonly known as armyworm, riceworm or tobacco caterpillar, is a species from the order Lepidoptera. It is a polyphagous pest of various agricultural crops, and caused serious damage to a 14-hectare vegetable plot in Banggol Katang that was first reported in 1989.

Naturally field infected *S. litura* larvae were collected from MARDI Research Station in Kelantan in 1993. Diseased larvae were pink yellowish and turned dark brownish when dead. Fluid liberating from the integument was negatively stained with 2% methylamine tungstate (MAT). A mixture of baculoviruses, nucleopolyhedrovirus (NPV) and granulovirus (GV), was found in the diseased larvae. In the present paper, we have isolated the GV and studied its morphological characteristics and infection process *in vivo*.This is the first report of a granulovirus that infects on *S. litura* in Malaysia.

Materials and Methods

Isolated GV was propagated and repurified for a few generations in insect larvae. Purification of GV was carried out by modification of method described by Singaravelu and Ramakrishnan in 1998. Diseased larvae were homogenized in a 10-ml Wheaton USA homogenizer and incubated in 2 volume of 0.1% SDS for 45 min at room temperature. The homogenates were then filtered through four layers of muslin cloth and centrifuged at 1000 g for 10 min in order to discard the larval cells and tissues. Supernatant was spun at 12000 g for 10 min and the pellet containing occlusion bodies was resuspended in distilled water and purified by centrifugation in 40-80% (w/w) sucrose gradient at 28000 rpm for 2 hours in a Beckman SW 41Ti rotor. A band was collected at 55-60% sucrose and washed twice with distilled water by centrifugation at 30000 rpm for 15 min in a Beckman Type 30 rotor in order to remove the sucrose. Pellet was then collected and resuspended in distilled water.

Virus particles were released from the capsules after 30-min treatment with equal volume of 0.1 M Na_2CO_3 at 37°C. Distilled water was added and the mixture was spun at 30000 rpm for 1 hour. Virus particles were resuspended in distilled water and layered onto 40-80% (w/w) sucrose gradient and centrifuged at 30000 rpm for 1 hour. Two white bluish bands were harvested at 50-55% sucrose, diluted and pelleted again at 30000 rpm for 1 hour. Both bands contained single rod-shaped virus particles.

Purified virus particles were incubated in a final concentration of 2% ISEPA CA-630 for 30 min at 37°C. After centrifugation at 30000 rpm for 1 hour, pellet were resuspended in distilled water and layered onto 40-80% sucrose gradient for 1-hour centrifugation at 30000 rpm. Purified nucleocapsids were recovered at 50-55% sucrose and pelleted again at 30000 rpm for 1 hour.

Purified nucleocapsids were incubated in final concentration of 1 M NaCl and 2% ISEPA CA-630 for 13 hour at 37°C. Centrifugation was carried out at 30000 rpm for 1 hour. Pellet containing capsids was resuspended in distilled water. A small amount of purified capsules, virus particles, nucleocapsids and capsids was stained in a grid for 5 min with 2% MAT.

Results and Discussion

Capsules were ovocylindrical in outline. They were 0.2 to 0.3 μm in width and 0.45 to 0.55 μm in length. However, some abnormal capsules were having v-shaped or elongated shaped as reported for other granuloviruses (Huger, 1963). The abnormal elongated capsule was 5 to 6 times the length of a normal capsule. It might be the result of the fusion of several capsules.

A single enveloped virus particle could be observed within the proteinaceous capsule. The rod-shaped virus particle was about 280 ± 8 x 85 ± 7 nm. The size of the nucleocapsid was 303 ± 11 x 60 ± 7 nm . It is longer than the enveloped nucleocapsid. The capsid was having pore at both ends and measured to have 263 ± 9 x 50 ± 8 nm in size. This granulovirus possesed certain features in common with other granuloviruses (Ackermann, 1983; Im, 1986; Singaravelu and Ramakrishnan, 1998).

Granulovirus is very productive for controlling the outbreak of *S. litura*. Infected larvae were bigger in size and majority was 2 to 3 times larger than the non-infected larvae, especially when very low doses of granulovirus were given (Huger, 1963). Dead larvae were observed during 5 to 15 days p.i. when 3rd –instar larvae were infected with $3.92x10^{11}$ capsules/ml. In the final stage of infection, yellowish color covered the whole dorsal and ventral of the larvae. The larvae became fragile and burst out releasing the milky haemolymph containing the capsules . A study of disease progression was carried out by using late 2nd-instar larvae. Larvae were starved for 24 hours and then orally infected with leaf disc containing $4.0x10^{8}$ capsules. Samples for electron microscopy were taken at 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 48, 72, 96 and 120-hour p.i. A lot of naked nucleocapsids were observed in the vicinity off virogenic stroma after 24-hour p.i. The outer layer of the nucleocapsid was formed from the endoplasmic reticulum after 48-hour p.i.. Enveloped nucleocapsids may occur singly or in aggregates in vesicles. After 72-hour p.i., the formation of capsules was observed. The deposition of crystalline protein began from one end of the virus particle and extended toward the other end until the complete capsule was formed. Many capsules were observed in the cytoplasm after 72-hour p.i. The capsules were clearly shown to have only one virus particle and each virus particle contained only single nucleocapsid. After 120-hour p.i., matured capsules were seen to have an outer, known as calyx, covering over the surface of the occlusion body . This is the stage where larvae start having diarrhea and vomiting

Conclusions

This virus was identified as granulovirus. A comparative study on the pathogenesis of NPV and GV isolated from *S. litura* larvae will be carried out.

Benefits from the study

Use of GV as biopesticide for the control of a serious pest *Spodoptera litura* in vegetables

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

1. Lau, W.H., N.W. Nekmat and N. Abdul Samad. 2002. In vivo production of nucleopolyhedrovirus in Spodoptera litura larvae. In: Biopesticides: Positioning biopesticides in pest management systems. Ed. M.S. Mulla, Univ. of California, USA.

2. Lau, W.H., H.H. Haris and N. Abdul Samad. 2002. In vivo production of in Spodoptera litura granulovirus. In: Biopesticides: Positioning biopesticides in pest management systems. Ed. M.S. Mulla, Univ. of California, USA.Pp. 129-134.

3. MAT AMIN, N., W.H. LAU and N. ABDUL-SAMAD. 2002. Infectivity of Malaysian granulovirus isolate to various instars of Spodoptera litura. In: Biopesticides: Positioning biopesticides in pest management systems. Ed. M.S. Mulla, Univ. of California, USA. Pp. 124-128.

4. A.S. SAJAP , J.R. KOTULAI, W.H. LAU, M.A. BAKIR, , N. ABDUL SAMAD and A.K. HUSSAN. 2000. Pathogenecity and characteristics of Spodoptera litura nucleopolyhedrovirus from Peninsular Malaysia. J. Trop. Agric. 23, 23-28.

Project Publications in Conference Proceedings

1. LAU, W.H., H.H. HARIS and N. ABDUL-SAMAD. 2002.In vivo production of Spodoptera litura granulovirus. 3rd International Conference on Biopesticides, Kuala Lumpur.

2. LAU, W.H., N.W. NEKMAT and N. ABDUL-SAMAD. 2002. In vivo production of nucleopolyhedrovirus in Spodoptera litura larvae. 3rd International Conference on Biopesticides, Kuala Lumpur.

3.SAJAP, A.S., M.A. BAKIR, H.A. KADIR and N. ABDUL-SAMAD.2002. Effect of temperature on mortality of Spodoptera litura larvae infected with nucleopolyhedrovirus. 3rd International Conference on Biopesticides, Kuala Lumpur.

4. LAU, W.H., A.S. SAJAP, K. YUSOFF , A.M. ALI and N. ABDUL-SAMAD.2002. Characterization of the genome of Spodoptera litura granulovirus. 3rd International Conference on Biopesticides, Kuala Lumpur.

5. LAU, W.H., A.S. SAJAP, K. YUSOFF , A.M. ALI and N. ABDUL-SAMAD.2002. Molecular characterization of granulovirus and nucleopolyhedrovirus isolated from Spodoptera litura. 3rd International Conference on Biopesticides, Kuala Lumpur.

6.BAKIR, M.A., A.S. SAJAP, A.K. HUSSAN, N. ABDUL SAMAD and M.Y. HUSSEIN. 2001. Field trials with single application of Spodoptera litura nucleopolyhedrovirus (SpltNPV) for controlling Spodoptera litura on Brassica rapa. 4th Asai-Pacific Conference of Entomology, Kuala Lumpur.

7. BAKIR, M.A., A.S. SAJAP, A.K. HUSSAN, N. ABDUL SAMAD and M.Y. HUSSEIN. 2000. Evaluation of materials as UV protectants of nucleopolyhedrovirus of armyworm, Spodoptera litura Fab. (Lepidoptera: Noctuidae), 5th Graduate Congress, Dept. of Biological Science, NUS Singapore.

8. BAKIR, M.A., A.S. SAJAP, A.K. HUSSAN, N. ABDUL SAMAD and M.Y. HUSSEIN. 2000.Effect of sunlight on nucleopolyhedrovirus of Spodoptera litura (Fab.) (Lepidoptera: Noctuidae). 4th UNESCO National Workshop on Promotion of Microbiology in Malaysia, Kuala Lumpur.

9. LAU, W.H., A.S. SAJAP, K. YUSOFF , A.M. ALI and N. ABDUL-SAMAD.2000. Morphology studies of Granulovirus and Nucleopolyhedrovirus in larvae of Spodoptera litura. Proc: 9th Scientific Conference EMSM, pp. 201203.

10. SAJAP, A.S., M.A. BAKIR, N. ABDUL-SAMAD, M.Y. HUSSEIN and H.A. KADIR. 1999. Effect of ultraviolet light on efficacy of *Spodoptera litura* nuclear polyhedrosis virus. Fifth International Conference on Plant Protection in the Tropics, Kuala Lumpur.

11. SAJAP, A.S., M.A. BAKIR, H.A. KADIR, N. ABDUL-SAMAD and M.Y. HUSSEIN. 1999. Infectivity of nuclearpolyhedrovirus (NPV) to Spodoptera litura Fab. (Noctuidae: Lepidoptera) at different temperatures. 22nd Microbiology Symposium and JSPS Seminar, Penang.

12. SAJAP, A.S., M.A. BAKIR, H.A. KADIR, N. ABDUL-SAMAD and M.Y. HUSSEIN. 1999. Infectivity of nuclearpolyhedrovirus (NPV) to Spodoptera litura Fab. (Noctuidae: Lepidoptera). 11th National Biotechnology Seminar, Melaka.

13.LAU, W.H., A.S. SAJAP, K. YUSOFF and N. ABDUL-SAMAD. 1999. In vivo study on a *Granulovirus* in armyworm, *Spodoptera litura*. Fifth International Conference on Plant Protection in the Tropics, Kuala Lumpur.

14. LAU, W.H., A.S. SAJAP, K. YUSOFF and N. ABDUL-SAMAD. 1999. A granulovirus from armyworm, Spodoptera litura. Symposium Biologi in the Next Millennium, Kuala Lumpur .

15. LAU, W.H., A.S. SAJAP and N. ABDUL-SAMAD. 1997. Restriction endonuclease analysis of the DNA from *Spodoptera litura* nucleoplyhedrosisvirus (SpltNPV). Twenty-second Konferensi Persatuan Biokimia Malaysia, Bangi, Selangor.

16. LAU, W.H., A.S. SAJAP and N. ABDUL-SAMAD. 1997. Histology of nucleopolyhedrosis infected *Spodoptera litura*. Twentieth Symposium of Microbiology Society, Kuala Lumpur.

17. SAJAP, A.S., J.R. KOTULAI, N. ABDUL-SAMAD, M.Y. HUSSEIN and H.A. KADIR. 1996. Pathogenecity and characteristics of *Spodoptera litura* nuclear polyhedrosis virus from Peninsular Malaysia. XX International Congress of Entomology, Firenze, Italy.

18.ABDUL-SAMAD, N., N. M. NOOR and A.S. SAJAP. 1996. Electron microscopy of a Malaysian isolate of baculovirus from *Spodoptera litura* Fab. XX International Congress of Entomology , Firenze, Italy.

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Lau Wei Hong	Characterization of NPV and GV	Virology/Molecular Biology	PhD	2002

IRPA Project number01-02-04-0398
UPM Research Cluster:AFF