

MECHANISM OF INFECTION *Spodoptera litura* Multiple Nucleopolyhedrosis Virus (*SpMtMNPV*) ON MIDGUT EPITHELIAL CELL ARMY WORM (*Spodoptera litura*) OBSERVED BY TEM

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ABSTRACT

Spodoptera litura is one of agricultural crop pests. They are resistant to chemical insecticides. One of alternate biological control is *Spodoptera litura* Multiple Nucleopolyhedrosis Virus (*SpMtMNPV*). The research was conducted to determine how *SpMtMNPV* infected midgut epithelial cells of *S. litura* larvae in vitro. The midgut monolayer epithelial cells were infected by *SpMtMNPV*, then incubated for 6 and 24 hours. The mechanism of infection was observed by a transmission electron microscopy (TEM). The result of this observation showed that infecting midgut cell by *SpMtMNPV* involved 5 phases, they are: 1) the attachment of *SpMtMNPV* at the membrane of suitable host, 2) the penetration, formation of tunnels and release of protein envelope, 3) the biosynthesis of virus components in the cell nucleus, 4) the assembling of virus components, and 5) the releasing of MNPV/multiple nucleocapsid through budding.

Keywords: mechanisms of infection, *Spodoptera litura* multiple nucleopolyhedrosis virus, midgut army worm larvae cells

INTRODUCTION

Nuclear Polyhedrosis Virus (NPV) is one of the biological agents to control various insect pests. It can be used to control the larvae of *Spodoptera litura*. Nuclear Polyhedrosis Virus (NPV) is an obligate insect pathogen that is used to control insect populations. In general, NPV/*Spodoptera litura* Multiple Nucleopolyhedrosis Virus (*SpMtMNPV*) infects its host in

larvae phase through the digestive tract. Nuclear Polyhedrosis virus belongs to the genus of Baculovirus, Baculoviridae familia (Gothama *et al.*, 1994). It is a DNA virus. It attacks the *Spodoptera litura* and it is better known as the NPV. This virus has a double stranded DNA wrapped in a protein coat (capsid). It is called nucleocapsid. Nucleocapsids are infective to various insects and wrapped in sheaths/ envelopes called a virion. *SpMtMNPV* has Multiple Nucleocapsid enveloped (MNE) (Gothama *et al.*, 1994). Virions are generally crystalline and covered by matrix protein, multifacet called Polyhedral Inclusion Body (PIB). Hink (1982) has pioneered culture cabbage insects larvae cells (*Trichoplusia ni* TN-368) to produce *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV). Midgley *et al.* (1998) have also succeeded in culturing the cell line (sf9) of *Spodoptera frugiperda* as the host of Baculovirus by monolayer and suspension cultures methods. Based on the research Asri *et al.* (2007), primary cells of the midgut larvae *S. litura* have successfully developed by monolayer method in Grace's medium enriched with Fetal Bovine Serum. The primary cells can be recultured to 27 generations.

Phase of S/NPV infection mechanism in host are: 1) ingesting virus particles by the host (0 h), 2) releasing virus particles into cytoplasm (4-8 hours), 3) undergoing first virus modification in the nucleus of infected cells (16 hours); 4) forming viroplasma (24 hours); 5) replicating of nucleocapsid (36 hours); 6) replicating polyhedra (48 hours); 7) forming complete PIB (72 hours) (Falcon 1975 *cit.* Mangoendiharjo and Pollet, 1991). In addition, based on Lua and Reid

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(2000). *HaSNPV (Helicoverpa armigera* Single Nucleopolyhedrosis virus) replication was observed in the virogenic stroma by appearance of nucleocapsid at 16 h post infection (p.i), Polyhedron formation was detected by 24 h p.i, and the polyhedron envelope (PE) was completely formed by 72 h. p.i.

The research was aimed to observe the behavior of *SpltMNPV* through the completion of virus particles replication using TEM (transmission electron microscope).

MATERIALS AND METHODS

SpltMNPV In Vivo Propagation and Virus Purification

Larvae of *S. litura* were maintained using artificial feed until the third instar. Then they were infected by *SpltMNPV* using the feed contamination method. Infected larvae were maintained until their death. The typical death symptoms, that is, and easily broken when it is touched and releases liquid contains viruses. Viruses were isolated from the dead larvae using centrifugation at 3500 rpm for 15 minutes (Arifin, 2006).

SpltMNPV's Infection on Midgut Cells of Larvae

The *SpltMNPV* concentration in the culture was 7.6×10^7 PIBs/ml. The volume of *SpltMNPV* infected to 4 ml media contained epithelial midgut cells was as much as 0.2 ml. Infection process of cell line was done after *SpltMNPV* had purified and its concentration was calculated using a *haemocytometer*. Polyhedra of *SpltMNPV* at first was broken using Na_2HCO_3 0.5 M before being infected in the cultured cells (in preliminary research). The infected cells were incubated in 30°C for 6 and 24 hours.

Observation by TEM (transmission electron microscope)

The infected and incubated cells were fixated and then processed for TEM microscopic slides.

RESULTS AND DISCUSSION

RESULTS

Phases of the *SpltMNPV* infection mechanism was observed 2 times at 6 and 24 hours. Phase one and two of the infection mechanism were got within 6 hours incubation while phase three, four and five were got within 24-hour incubation.

The infection cycle of *SpltMNPV* in *Spodoptera litura* epithelial midgut cells were divided in 5 phase, they are, 1) the attachment of *SpltMNPV* at the membrane of suitable host, 2) the penetration, formation of tunnels and release of protein envelope, 3) the biosynthesis of virus components in the cell nucleus, 4) the assembling of virus components, and 5) the releasing of MNPV/multiplenucleocapsid through budding. (Figure 1-5).

The attachment phase of *SpltMNPV* on the cell membrane of suitable host (Figure 1). In this phase was occurred the attachment of some MNPVs (multiple nucleopolyhedrosis viruses) on the host cell membrane. There was changes of compounds in the cell membrane so that the cell membrane changed to be brighter than previous condition (Phase 1A). Invagination was formed after Phase 1A (described in Phase 1B).

The phase 2 of mechanism of infection *Spodoptera litura* multiple nucleopolyhedrosis virus (*SpltMNPV*) is the penetration and then releasing of nucleocapsid out of the envelope to the cytoplasm (Figure 2). In this phase occurs penetration and releasing of MNPV from envelope to be single nucleocapsid. The MNPV entered to the cytoplasm through the tunnel (Figure 2a), which was formed during the invagination. The envelope of MNPV opened and its contents (single nucleocapsid) was released into the cytoplasm. After this phase, cell showed empty and remained only envelope and a single nucleocapsid was released (Figure 2B).

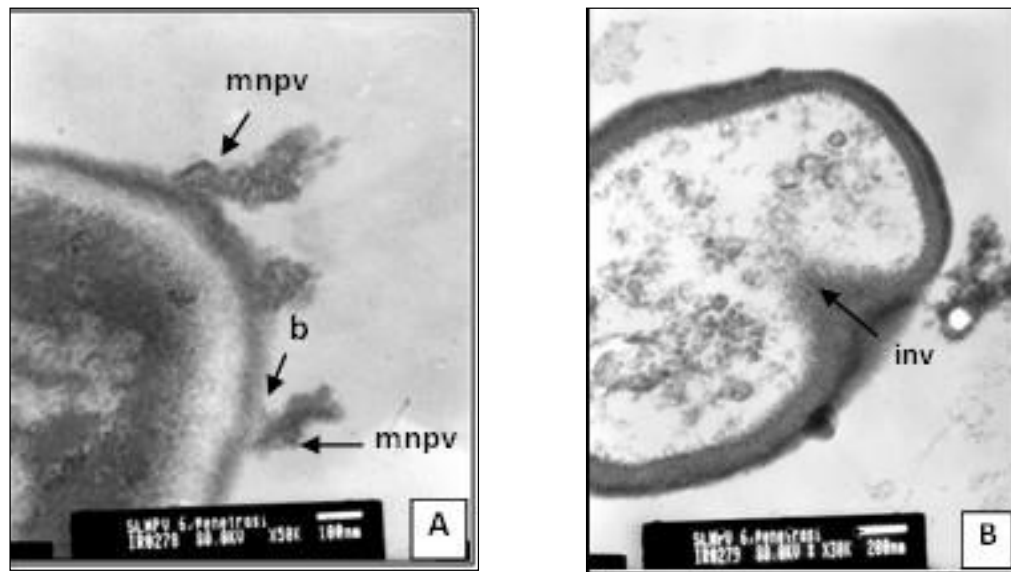


Figure 1. The first phase, the attachment of viruses in the host cell

- A. The attachment of *SpltMNPV* at the membrane of suitable host. mnpv= multiple nucleopolyhedrosis virus, b= brightener (magnification x 50,000, bar = 100 nm)
- B. The invagination of cell membrane. Inv = invagination (magnification x 30,000 bar = 200 nm)

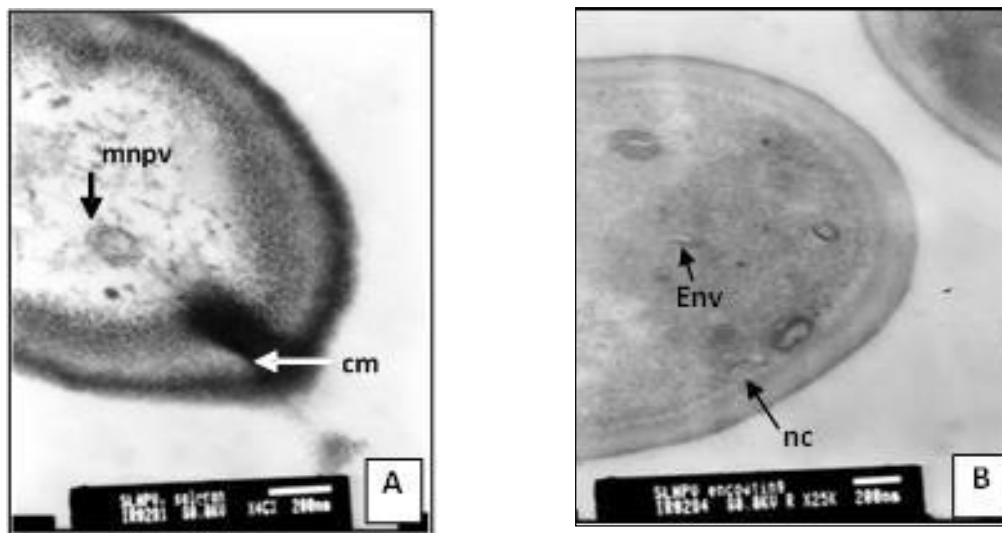


Figure 2. Phase 2, penetration and releasing nucleocapsid in the envelope to the cytoplasm

- A. Tunnel form by host cell membran. tn= tunnel membrane (Magnification x 40,000, bar=200 nm)
- B. The releasing of nucleocapsid from MNPV's envelope. nc=nucleocapsid, Env=envelope (magnification x 25,000, bar = 200 nm).

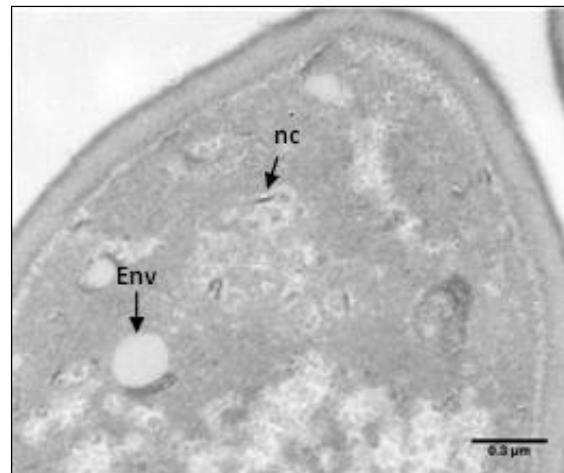


Figure 3. Phase 3, biosynthesis of the virus components (magnification x 5000, bar = 0.3 μm)
Remarks: nc: nucleocapsid, Env: envelope

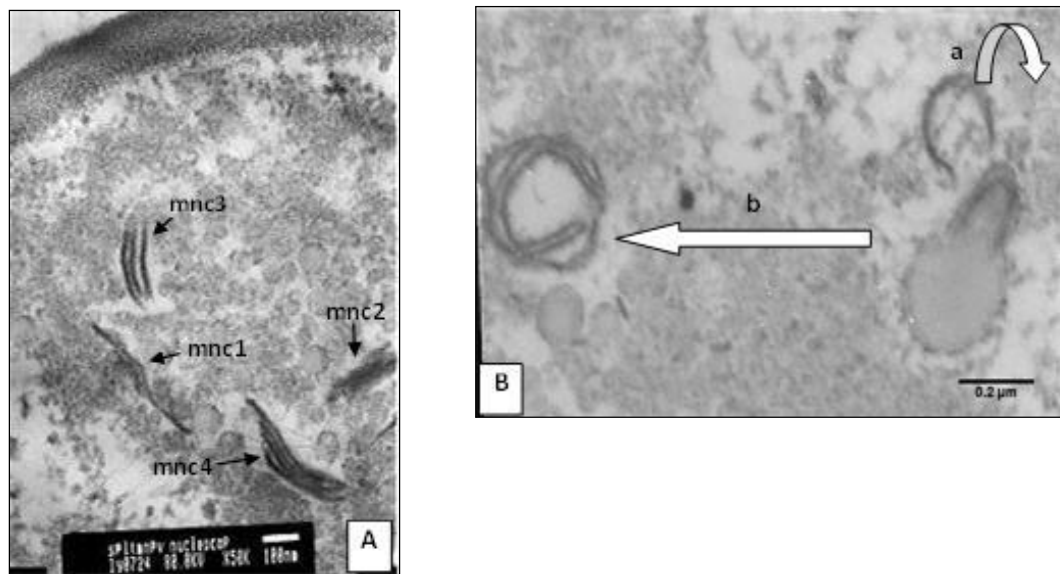


Figure 4. Phase 4, the assembling of the virus components
A. The assembling of the nucleocapsid to multiple nucleocapsid such as mnc1, 2, 3 and 4 = multiple nucleocapsids (there were several arranged nucleocapsids i.e. four nucleocapsids arranged into in one cluster, and etc.) (magnification x 50,000, bar = 100 nm)
B. The mechanism of nucleocapsid entering the envelope. a. Nucleocapsid go to envelope. b. Envelope contains some nucleocapsid (magnification x 8000, bar = 1 μm)

Biosynthesis of the virus components was the third phase of *SpMNPV* infection mechanism (Figure 3). This phase was the formation stage of virogenic stroma which contains genetic material and envelope. Figure 3 showed the nucleic acid

was surrounded by capsid (called nucleocapsid). Nucleocapsid in a rod shape was distributed in the cell nucleus, and there were some empty envelopes and envelopes contained nucleocapsid.

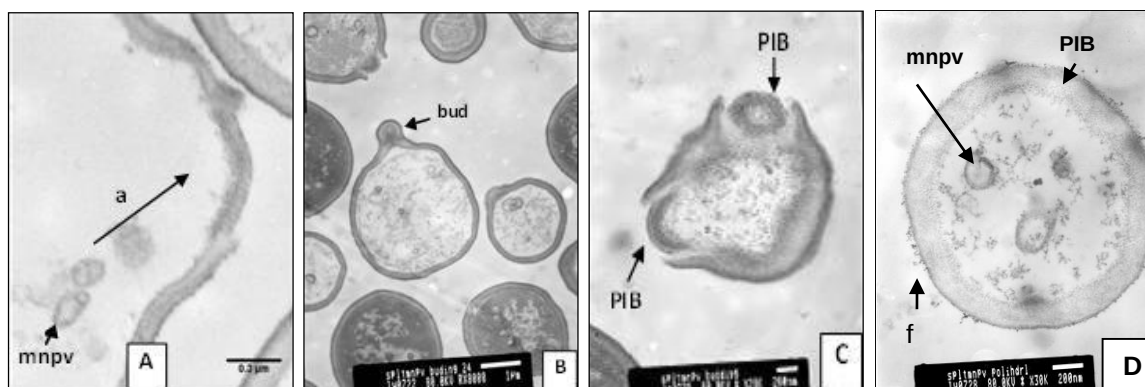


Figure 5. Phase 5, the releasing of *SpltMNPV* (*Spodoptera litura* Multiple Nucleopolyhedrosis Virus)
 A. Polyhedra was formed from host's cell membrane (a) (magnification x 8000, bar = 1 µm).
 B. Budding of midgut cell membrane contained multi-nucleocapsid. Bud = budding (magnification x 8000, bar = 1 µm).
 C. Two *SpltMNPV* Polyhedra were released from the host cell (magnification x 28,000 bar = 200 nm)
 D. Polyhedra inclusion bodies of *SpltMNPV* after released from the host cell. f= filli of polyhedra; PIB = Polyhedra inclusion bodies (magnification 30,000x, bar = 200 nm).

The assembling of the virus components was the fourth phase (Figure 4). In this phase occurred a single rod-shaped nucleocapsid assembling into multiple nucleocapsid (1, 2, 3 and 4 line respectively) which have not been wrapped by the second protein envelope (Figure 4A). Single nucleocapsid entered into the envelope by curved structure to form multiple nucleopolyhedrosis virus one by one (Figure 4B).

The last phase of mechanism of infection *SpltMNPV* was releasing of *SpltMNPV* (Figure 5). In this phase occurs polyhedra formation from host's cell membrane, budding and releasing polyhedra of *SpltMNPV*. Polyhedra which had filis released from the host cell wasn't symmetric

DISCUSSION

The *SpltMNPV* infection mechanism phases were observed 2 times at 6 and 24 hours. Phase one and two of the infection mechanism were found in the 6 hours incubation, while phase three, four and five were found at 24-hour incubation. This fact is different from the previous theory that the phase of *SINPV* infection mechanism in host cell (in vivo) have 72 hours (Falcon 1972 cit. Mangoendiharjo and Pollet, 1991). The infection cycle of *SpltMNPV* in *Spodoptera litura* epithelial midgut cell was divided in 5 phase.

The Attachment of Viruses in The Host Cell

The attachment of viruses in the host cell is the first phase of *SpltMNPV* invasion to host cell. (Figure 1). Some multi-nucleocapsid begin to recognize the host cell membrane by attaching to a specific place (receptor site) and release a particular compound that can change the cell membrane so that the cell membrane appears brighter (Figure 1A). Because of the conformation changing of the compounds. Such phase is continued in Figure 1B, some multi-nucleocapsid (*MNPV*) recognize the host cell membrane and attach to a specific place (receptor site) and release a compound that can cause particular changes in cell membrane so that the membrane invaginates, brings the *MNPV*. According to Rohrmann (2008) at phase 1 multi-nucleocapsid attach larva midgut cells by interacting between vp91 (virus protein 91) and midgut cells. *MNPV* envelope has sensitive receptor sites so that it can bind proteinases and change the conformation of the compounds in the epithelial cell membrane. Therefore the cell membrane appears brighter (Figure 1A).

Penetration and Releasing Nucleocapsid in The Envelope to The Cytoplasm

Penetration and releasing nucleocapsid in the envelope to the cytoplasm is the second phase after the attachment of virus in the host cell. The second phase shows that *MNPV* has

already attached to the host and it causes the invagination to form a tunnel into the cell (Figure 2A). After the MNPV penetrates into the cell (Figure 2B), the envelope of MNPV will open and its contents are released, therefore the envelope becomes empty (Figure 2B). This fact is different from the previous theory. General viruses will penetrate and release the envelope to the cytoplasm by creating pores/holes in membran cell or by endocytosis and vesicles formation (Tortora *et al.*, 1995; Rohrmann, 2008).

Biosynthesis of the Virus Components

Biosynthesis of the virus components is the third phase of *SpMNPV* after penetration and releasing envelope in cytoplasm. The third phase, shows the biosynthesis of the virus components. This phase is obtained from a virus infection in incubated cultured cells for 24 hours. The figure shows that virogenic stroma containing genetic materials and capsid protein (nucleocapsid) has been formed. The nucleocapsid resembles rod-shaped threads scattered in the cell nucleus. The nucleus membrane of stretches so that it lied closer to the cell membrane. Virus genetic materials have been compacted and shortened. Some of them are short rod-shaped, some are longer and some are curved. Virus components are visible in an empty cavity but the DNA strands are not visible because it is too small or too thin so that only nucleocapsids are visible (Figure 3). Figure 3 shows that some of the MNPV envelopes are empty and some others still the envelope that contains genetic material.

Virogenic stroma is formed because some nucleocapsid can succeed in penetrating genetic material (DNA) into the cell nucleus. Nucleocapsid penetrates into the cell nucleus through the pores which are the size approximately 38 nm. Virogenic stroma formation is regulated by a virus protein called PP31 (Rohrmann, 2008).

The Assembling of the Virus Components

The assembling of the virus components is the fourth phase after biosynthesizing the virus components. Figure 4 shows that the new nucleocapsids are formed in the incubation period of 24 hours. Beginning the nucleocapsid formation in the nucleus is separated (Figure 4A) then they nucleocapsids get closer each other and form a group of nucleocapsid which is called

multiplenucleocapsid. Figure 4A show that nucleocapsid in two line, 3 line and 4 line. The nucleocapsids have not been surrounded by envelope. The next mechanism can be observed in Figure 4B. It shows the mechanism of nucleocapsid entering the envelope. The longer rod-shaped nucleocapsid is curved to adjust the envelope shape and then enters the envelope. Furthermore nucleocapsids follow the previous step, that is, it is curved then enters the envelope. This mechanism enables the efficiency space can be achieved. The small envelope can be infiltrated by more than single nucleocapsid. This fact is different from the previous theory that nucleocapsids have straight form (Adam, 1977 *cit* O'Reilly. *et al.*, 1992). The nucleocapsids of *SpMNPV* infecting larvae of *S. litura* epithelial midgut cells of have curved shape and they pile up each other in the envelope.

The releasing of *SpMNPV* is the last phase after the assembling of virus components. In this phase the *SpMNPV* are released from epithelial cells of larvae that is shown in Figure 5. Figure 5A shows that the cells begin to form a budd' midgut, and MNPV move to either nucleus membrane or cell membrane to get polyhedral. Two MNPVs are going to the membrane of nucleus and membrane cell and then they buds (Figure 5B). Figure 5B shows that the cell membrane contains a budd of multiplenucleocapsid and whereas Figure 5C, Shows the cell membrane opens and releases *SpMNPV* polyhedra. Figure 5C, shows that budding's direction is not symmetrical and is not patterned. It is caused by viruses gathering as in a group in the *in vitro* medium and all cells will be the next target of infection. The location of Polyhedra *SpMNPV* budd in epithelial cell membran is defferent with general NPV in host cell (*in vivo*). The budding of general NPV *in vivo* cell exist in basal and lateral tip. The budding location will be away from the lumen but closer to the cell trachea as next cell target of infection (Rohrmann, 2008).

Polyhedra Inclusion Bodies (PIB) of the mature *SpMNPV* are shown in Figure 5D. The PIB *SpMNPV* is round in form double membranes. Inner membrane is taken from the membrane of nucleus, while the outer membrane is taken from the cell membrane. The outer membrane has fili as found in of epithelial midgut cells of *S. litura* larvae.

CONCLUSIONS

The infection mechanism of *Spodoptera litura* multiple nucleopolyhedrosis virus in midgut epithelial cells *Spodoptera litura* larvae were observed using a transmission electron microscope appear at 6-hour incubation. The six hour incubation has the following phases: 1) the attachment of MNPV (Multiple Nucleopolyhedrosis virus) on the host cell; 2) the penetration of MNPV into the cytoplasm. Beside of that, in the 24 hour incubation we can look the following phases: 3) the biosynthesis of virus components, 4) the assembling of virus components; and 5) the releasing of *Splt*MNPV in the form of polyhedral inclusion bodies.

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