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Review

THE CURRENT STATUS OF BACULOVIRUS AND THEIR IMPLICATION FOR INSECT PEST CONTROL

STATUS BACULOVIRUS DAN IMPLIKASINYA DALAM PENGENDALIAN SERANGGA HAMA

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INTISARI

*Selama lebih dari setengah abad baculovirus telah dipromosikan sebagai agens pengendali hayati yang menjanjikan. Akan tetapi hanya sedikit saja yang terbukti sukses dan hampir tidak ada satupun yang berhasil secara ekonomi, atau dipergunakan dalam pengendalian hama skala besar. Bioinsektisida saat ini hanya mempunyai segmen yang sangat kecil dalam pasar pestisida. Keberhasilan tanaman rekayasa genetik (*Bt-crop*) merupakan prestasi tersendiri di dalam pasar bioinsektisida. Kendala utama bagi baculovirus untuk berkembang sebagai pemain utama dalam pasar insektisida adalah kecepatan membunuh yang lambat, dan sempitnya kisaran inang, dibandingkan dengan insektisida kimiawi. Apa dan bagaimana baculovirus penting untuk diketahui, dan berangkat dari situ dicari terobosan baru untuk mewujudkan baculovirus sebagai insektisida yang handal dan bersaing.*

Kata kunci: status, baculovirus, pengendalian serangga hama

ABSTRACT

Baculoviruses have been promoted as the promising bioinsecticides for their pest control potential for more than half a century. But only a few have been successful as biological control agent, and almost none has been proven as commercial success, or widely used for large-scale insect pest control. The bioinsecticides currently represent only a small fraction of the world pesticide market. The successful of the Bt crop marked a special achievement in the bioinsecticide market growth. How about the baculoviruses? The main hurdle for baculovirus to be developed as bioinsecticide is its poor performance compare to synthetic chemical ones, include the speed of kill, and host range. It is important to understand the nature of baculovirus, and explore the possibilities to develop new way in applying the baculovirus as bioinsecticides.

Key words: current status, baculoviruses, insect control

INTRODUCTION

Insect pest problems remain as one of the most important problems facing the 21st century. Considering the pressure of a rapidly expanding human population, the contamination of the environment with chemical pesticides, and the decline of biodiversity, the development of biological pesticides is become increasingly important (Hukuhara *et al.*, 1999). During the 1970s, the first baculoviral product was introduced into the commercial arena, Elcar (*Helicoverpa zea* NPV). This product was accompanied by three non-commercial preparations produced by the US Forestry Service, namely Gypheck (*Lymantria dispar* NPV), TM BioControl-1 (*Orgyia* *pseudotsugata* NPV), and Neocheck-S (*Neodiprion sertifer* NPV) (Miller, 1997).

Problems related with developing baculovirus insecticides are high production costs, poor formulation, and generally inferior field performance like narrow host range, low-speed action when compared to chemical alternatives. These problems were eventually lead to the cease of those viral insecticides with the exception of Gypheck (Black *et al.*, 1997).

The other problem related with developing baculovirus insecticides is that the insects are commonly become increasingly resistant to baculovirus infections as they age (Engelhard & Volkman, 1995). These phenomena will give great impacts on the effectiveness of baculoviruses in pest control program because it is necessary to adjust application levels in response to the demographic condition of the target insect population. Exact evidence of the mechanism responsible for this developmental resistance is not known, but indirect evidence indicates that the mechanism of resistance may involve events that take place during infection of the midgut (Teakle, 1986).

In order to develop more effective baculovirus to meet the market demand, it is important to take a look back at the baculovirus structure, pathogenesis, and also its ecology.

BACULOVIRUS STRUCTURE AND PATHOGENESIS

The *Baculoviridae* is a large family of occluded viruses that is composed of two genera that are differentiated by the size of their occlusion bodies. The nucleopolyhedroviruses (NPVs) produced large polyhedron-shaped structure called *polyhedra*, which contain many virions, while the granuloviruses (GVs) have smaller occlusion bodies called *granules*, which normally contain a single virion. Critical to the ability of baculoviruses to efficiently replicate within insects and then to spread the infection throughout population is the structure of their virions, which are present in two forms during a single infection cycle. The occlusion-derived virus (ODV) is encapsulated in a protein matrix composed predominantly single protein called polyhedrin (or granulin in GVs), whereas the budded virus (BV) is not included (see Figure 1).

Although occluded virions are highly efficient infecting insects, they do not spread the infection within insect tissues. Early in infection BVs are produced, which are not occluded and which efficiently spread the infection from cell to cell within insects.

Humans have been aware of diseased caused by baculoviruses for over 2000 years, from the description of silk worm "*jaundice*", a disease of *Bombyx mori* that we now know is caused by a nucleopolyhedrovirus. An interesting aspect of NPV tissue tropism is that, with the important exception of the virus attacking lepidopterous insect and a single disease of cranefly, virus replication in this entire arthropod group is restricted to the midgut epithelium. Thus in mosquito, sawfly, and caddisfly larvae, their NPVs invade and produce occlusion bodies only in midgut epithelial nuclei. In the lepidopterans, the virus established a transient infection in the midgut, without producing occlusion bodies, and then invades the other tissues (see Table 1).

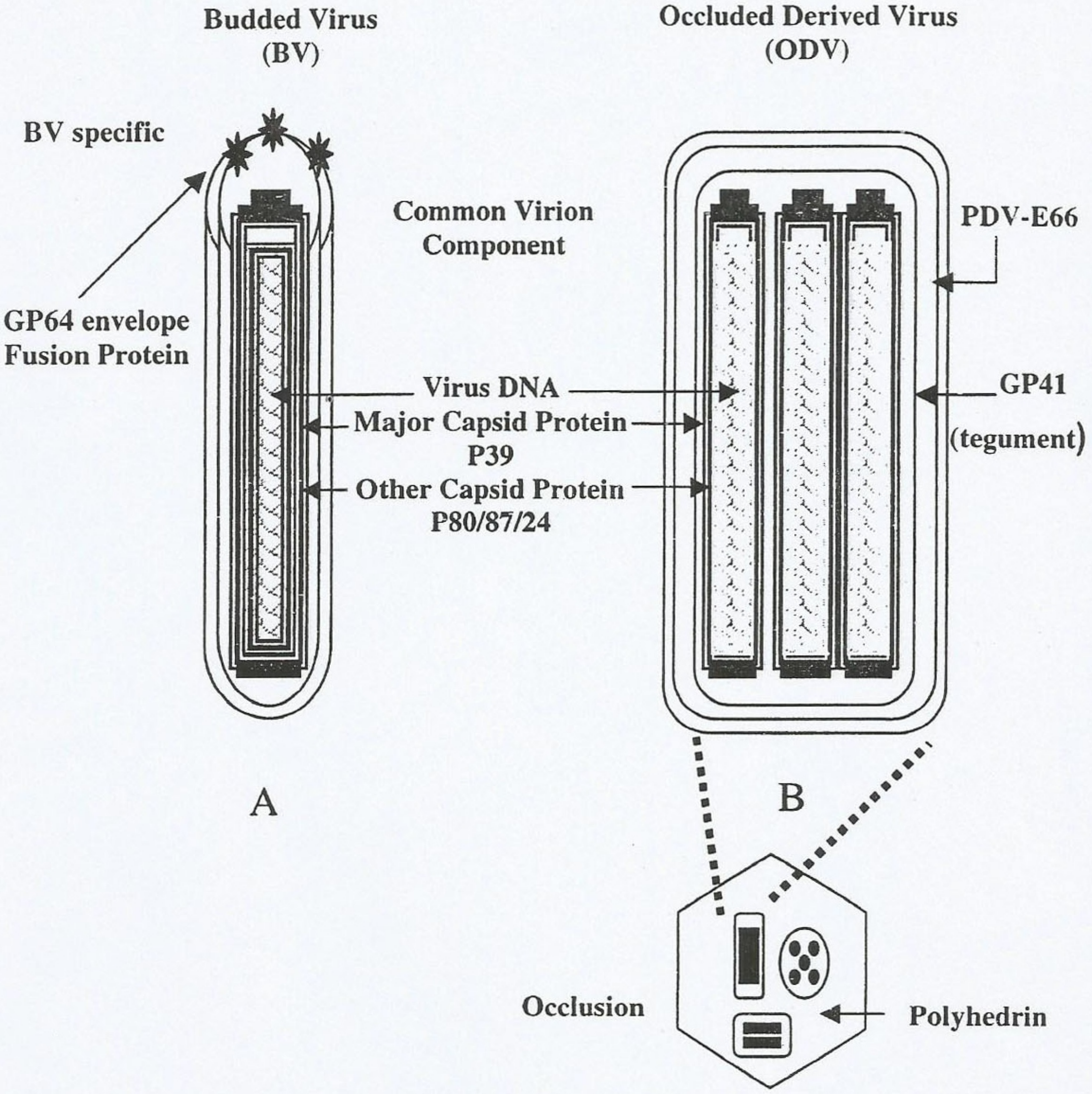

Figure 1. The location of virally encoded proteins in: (A) Budded virus and (B) Occlusion-derived virus (Redrawn from "The Baculovirus" Plenum 1997, 13 p).

Table 1. Tissue tropisms of baculoviruses

	Major tissues				
	Midgut epithelium	Hemocytes	Fat body	Epidermis	Tracheal matrix
Nucleopolyhedroviruses					
Lepidoptera (larval)	*	+	+	+	+
Hymenoptera (sawflies)	+	-	-	-	-
Diptera	+	+	-	-	-
Thysanura (silverfish)	+	-	-	-	-
Trichoptera (caddisflies)	+	-	-	-	-
Crustaceae (shrimp)	+	-	-	-	-
Granuloviruses					
Type 1 (*T. ni* GV)	*	-	+	-	-
Type 2 (*C. pomonela*)	*	*	+	+	+

Note : * = Replication of occlusion bodies may take place
+ = Replication occurs
- = No replication of occlusion bodies

The predominant route of NPV or GV infection is by ingestion of polyhedra and entry of the virus through the midgut epithelium. NPV can also be transmitted via the egg (Tanada & Kaya, 1993) or can initiate infection through the spiracles (Kirkpatrick *et al*., 1994). After ingestion, the occlusion bodies dissolve quickly, typically within seconds to at most a few minutes, in the alkaline juices at the anterior end of the midgut. After crossing the peritrophic membrane, the virion comes in contact with the microvillar membrane of columnar midgut epithelial cells by fusion (Granados & Lawler, 1981). Within 8 hr, the nucleus hypertrophies, the nucleoli enlarge, move toward the nuclear, and then decrease in size as virogenic stroma forms within the center of the nucleus. They leave the stroma and pass through the nuclear membrane and became the budded virus (BV) by emerging from the cell. In this process, the nucleocapsid acquires a new envelope, such as the gp64, that essential for the infection of other tissues.

BACULOVIRUS ECOLOGY

The relationship between a baculovirus and its host at the individual and population level are governed by many factors. Environmental factors such as temperature affect the response of the insect to infection. Host demography distribution and the concentration of virus inoculum are also affect the virus pathogenicity and time it takes for hosts to die.

Interesting point from baculoviruses is that, there were regional differences. Comparison of the baculoviruses from the same species in different location has shown considerable variation in genetic structure (Shapiro *et al*., 1991). The presence of this variation raises some questions, particularly with regard to the maintenance of baculoviruses diversity and its affect on baculovirus-host interactions. Development of resistance to pathogens is key issues in the long-term use of baculoviruses for pest control. Population of the same species collected from different region often differs in their susceptibility. One explanation for this variation is that previous exposure to virus in different localities has selected for resistant population (Fuxa, 1993).

In order to appreciate fully the association between baculovirus and their hosts and to exploit them effectively in biological control programs, it is important to understand their interaction not only in the individual level, but also in population level. Much of the theoretical work on the temporal patterns of insect pathogen has developed from the basic model by Anderson and May (1981). The model has three dynamic variables: the density of susceptible (S) and infected host (I), and the density of infectious particles in the environment (W). This model also embodies the four basic processes of any insect host – baculovirus interaction at the population level, namely transmission (ν), virulence (or speed of kill, α), yield (λ), and persistence of the infectious particles in the environment (μ) (See Figure 2).

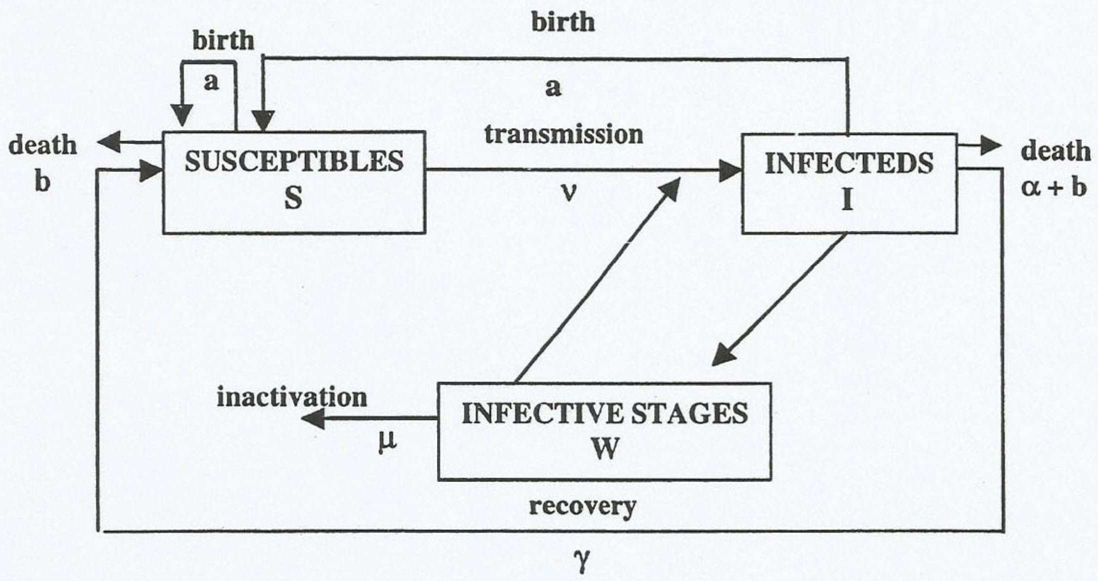

Figure 2. Three dynamic variables: the density of susceptible hosts (S), infected hosts (I), and the density of infectious particles in the environment (W). Other parameters are transmission coefficient (or rate at which infective stages successfully infect host) (ν), virulence (or disease induced mortality rate)(α), rate of production of infective stages per host (λ), and mortality rate of the infectious particles in the environment (μ), host birth rate (a), natural mortality of host (b), and rate of host recovery from infection (γ) (From Anderson & May, 1981).

DEVELOPMENT OF IMPROVED VIRUSES

The ability of foliar-applied baculoviruses to protect crops from insect pest damage is dependent on two fundamental issues: (1) effective dose acquisition and (2) the speed of action of the acquired dose. For chemical insecticides that are contact poisons, it can be achieved when a target insect simply walks across a treated leaf surface. For a baculovirus, however, effective dose acquisition is achieved when the target insect ingests a dose of virus that is sufficient to initiate a productive systemic infection. These becoming a relatively complex process that is affected by the rate of baculovirus application, the stability (residual activity) of the applied dose on the treated leaf surface over time, the feeding behavior of the pest (Black *et al.*, 1997; Kunimi *et al.*, 1997)

The other parameter is the time required for the virus to curb the feeding activity of the infected pest insect. Speed of action has been one of the most visible weaknesses of baculovirus based insecticides. Naturally occurring baculoviruses take from 4 to 7 days to kill their host, and some viruses can take up to 10 to 20 days. During this time the infected insects continue to feed and may cause substantial crop damage (Black *et al.*, 1997).

A critical factor for the performance of any baculovirus insecticide is how well the host range of the baculovirus matches the spectrum of pest insects that it must be able to control in any given crop treatment. Armyworms are frequently found in association with other lepidopteran pest, such as budworm or ballworm that lie outside the host range of the armyworm NPV. In this case, a narrow-range baculovirus insecticide is of little use. The simplest way to modify product host range is to find naturally occurring baculovirus whose economic host range includes the key members of the pest complex one need to control. This is possible because of the extraordinary host range diversity that exists among baculoviruses.

Once the parameter affected the baculovirus performance can be optimized, then the baculoviruses-based insecticide will become a significant and effective tool for insect pest management.

Gene deletion and insertion. Identify and remove from the viral genome any nonessential genes that might act to prolong the life of the host is one of the strategy to improve the speed of action of baculoviruses. Many viruses contain genes that alter the host organism's physiology for the purpose of maximizing the yield of transmission of viral progeny at the end of the infection cycle. One of the genes is the viral ecdysteroid glucosyltransferase gene (*egt*), which was discovered in *Autographa californica* NPV (AcMNPV) (O'Reilly & Miller, 1990). Larvae infected with the *egt*$^{-}$ mutants of AcMNPV die faster, up to 30% faster, than those infected with the wild type-virus and the significant reduction of food consumed (O'Reilly & Miller, 1991).

The insertion of insect-specific toxin holds promise for delivering a commercially viable baculovirus insecticide. The first test of this strategy involved the use of an insect-specific toxin from the scorpion *Androctonus australis* (Darbon *et al.*, 1982). It should be pointed out, however, that while insect-selective neurotoxin can dramatically enhance the speed of action of AcMNPV and related baculoviruses, they do not alter the intrinsic infectivity of the virus on permissive and semi-permissive host species or alter its natural host range.

Viral enhancing factor. Tanada (1959) first reported viral gene products, which enhance baculovirus infection of insect larvae. Later, he also reported a synergistic factor that present in the capsules of the Hawaiian strain of a granulovirus, which has the ability to enhance the *per os* infection of the NPV in the armyworm *Pseudaletia unipuncta* (Tanada & Hukuhara, 1971). Spheroids of an entomopoxvirus (EPV) have also been reported to enhance the infection of armyworm NPV. Armyworm larvae that have been administered with the mixture of NPV and EPV spheroid showed that its ID_{50} was reduced about 7000–9000 times (Xu & Hukuhara, 1992).

Wijonarko *et al.* (2000) which successfully purified this enhancing factor by means of immuno affinity chromatography, showed that the enhancing factor (EF) expand the host range of the AcMNPV, the most widely known among the NPVs. This finding will open new window to develop more effective and efficient way to use baculovirus as bioinsecticide.

FUTURE PROSPECT IN INDONESIA

Indonesia was listed as the second country after Brazil, to have the highest level of biodiversity. Therefore there are still so many probabilities and chance to explore its nature and finding the most promising microorganism, including insect viruses to be developed as bioinsecticide candidate. Without winter breaks that makes its possible for plant to be seed almost at any time, make the room for finding a new strain or even a new species of baculovirus is widely open in Indonesia.

There was report from several developing countries that use the NPV to control insect pest. *Spodoptera litura* NPV has been used to control *S. litura* and *S. littoralis* in India, Africa, and Southeast Asian countries. Also the use of Pieris GV to control cabbage worms in China. This example can be used to conclude that baculovirus can be used successfully in developed countries, because economic conditions are so different. The other factor is that, what makes the baculovirus production as bioinsecticide does not work in developed countries is the high cost of production.

In case for Indonesia in regard with the recent economic condition, where chemical pesticides price become enormously high, it will be promising to plan and assess carefully, whether baculovirus based insecticide may somehow economically be able to be produced. Commune and farms could share the cost for production, while research institution provides assistance. For consumer, this will be one leap ahead, and one more choice to have more "green-product".

REFERENCES

Anderson, R.M. & R.M. May. 1981. The Population Dynamic of Microparasites and Their Invertebrata Hosts. *Philos. Trans. R. Soc. Lond. Biol. Scie*. 291: 451–452.

Black, B.C., A. Brennan, P.M. Dierks, & I.E. Gard. 1997. In Miller, L.K (ed.), *The baculoviruses*, p. 341–387. Plenum, New York.

Darbon, H., E. Zlotkin, C. Kopeyan, J. Van Rietschoten, & H. Rochat. 1982. Covalent Structure of the Insect Toxin of the North African Scorpion *Androctonus australis* Hector. *Int. J. Peptide Protein* res. 20: 320–330.

Engelhart, E.K. & L.E. Volkman. 1995. Developmental Resistance in Fourth Instar *Trichoplusia ni* Orally Inoculated with *Autographa californica* MNPV. *Virol.* 209: 384–389.

Fuxa, J.R. 1993. Insect Resistance to Viruses, p. 197–209. *In* N.E. Beckage, S.N. Thompson, & B.A. Federici (eds.), *Parasites and Pathogens of Insects*, vol. 2. *Pathogens*. Acad. Press, San Diego.

Granados, R.R. & K.A. Lawler. 1981. In Vivo Pathway of *Autographa californica* Baculovirus Invasion and Infection. *Virology*. 108: 296–301.

Hukuhara, T., T. Hayakawa & A. Wijonarko. 1999. Increased Baculovirus Susceptibility of Armyworm Larvae Feeding on Transgenic Rice Plants Expressing an Entomopoxvirus Gene. *Nature Biotech.* 17: 1122–1124.

Kirkpatrick, B.A., J.O. Washburn, E.K. Engelhard, & L.E. Volkman. 1994. Primary Infection of Insect Tracheae by *Autographa californica* MNPV. *Virology*. 203: 184–188.

Kunimi, Y., J.R. Fuxa, J.R. & A.R. Richter. 1997. Survival Times and Lethal Doses for Wild and Recombinant *Autographa californica* Nuclear Polyhedrosis Viruses in Different Instars of *Pseudoplusia includens*. *Biol. Control.* 9: 129–135.

Miller, L.K. 1995. Genetically Engineered Insect Virus Pesticides: Present and Future. *J. Invertebr. Pathol.* 65: 211–216.

O'Reilly, D.R. & L.K. Miller. 1990. Regulation of Expression of a Baculovirus Ecdysteroid UDP-glucosyltransferase Gene. *J. Virol.* 64: 1321–1328.

O'Reilly, D.R., & Miller, L.K. 1991. Improvement of a Baculovirus Pesticide by Deletion of the *egt* gene. *Biotechnology* 9: 1086–1089.

Shapiro, M., J.R. Fuxa, H.D. Braymer, & D.P. Pashley. 1991. DNA Restriction Polymorphism in Wild Isolates of *Spodoptera frugiperda* Nuclear Polyhedrosis Virus. *J. Invertebr. Pathol.* 58: 94–98.

Tanada, Y. 1959. Synergism between Two Viruses of the Armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera, Noctuidae). *J. Insect. Pathol.* 1: 215–231.

Tanada, Y. & T. Hukuhara. 1971. Enhanced Infection of a Nuclear Polyhedrosis Virus in Larvae of the Armyworm, *Pseudaletia unipuncta*, by a Factor in the Capsule of a Granulosis Virus. *J. Invertebr. Pathol.* 17: 116–126.

Tanada, Y. & H.K. Kaya. 1993. *Insect Pathology*. Acad. Press. San Diego. 870 p.

Teakle, R.E., J.M. Jensen, J.M. & J.C. Mulder. 1985. Susceptibilty of *Heliothis armigera* (Lepidoptera:Noctuidae) on Sorghum to Nuclear Polyhedrosis Virus. *J. Econ. Entomol.* 78: 1373–1379.

Wijonarko, A., T. Hukuhara, & Y. Kunimi. 2000. Alteration of *Autographa californica* MNPV Host by Enhancing Factor (EF). In Press.

Xu, J. & T. Hukuhara. 1992. Enhanced Infection of a Nuclear Polyhedrosis Virus in Larvae of the Armyworm, *Pseudaletia unipuncta*, by a Factor in the Spheroids of an Entomopoxvirus. *J. Invertebr. Pathol.* 60: 259–264.