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PROPERTIES OF *ORYCTES* BACULOVIRUS ISOLATED IN INDONESIA

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ABSTRACT

An Indonesian isolate of *Oryctes* baculovirus was purified from infected midguts of the rhinoceros beetle (*Oryctes rhinoceros*) by centrifugation on a 10-40% (w/v) sucrose gradient. Morphological features of nucleocapsid including a tail-like projection were very same as those previously reported. Both protein components of purified particles and restriction fragment electrophoresis profiles of viral DNA were similar to those of other isolates of *Oryctes* baculovirus, although there were some differences.

Key words : *Baculovirus oryctes*, electrophoresis

INTRODUCTION

Oryctes virus was discovered from the coconut palm rhinoceros beetle *Oryctes rhinoceros* (Huger, 1966) and is an unassigned invertebrate virus in the recent virus taxonomy (Murphy, *et al.*, 1995), although it was previously classified in non-occluded baculovirus group of the family Baculoviridae (Matthews, 1982). The virus has been successfully used as the control of rhinoceros bettle (Marschall, 1970; Zelazny, 1976; Bedford, 1980; Purrini, 1989). Virions of the *Oryctes* baculovirus are rod-shaped, single enveloped nucleocapsid (Payne, 1974). A most typical structure is the tail-like projection at one end of the nucleocapsid. The virus structural proteins were identified by SDS-polyacrylamide gel electrophoresis (Payne *et al.*, 1977; Crawford and Sheehan, 1985). A physical map of viral genomic DNA was made for the strain PV505, a cloned Philippine isolate of the *Oryctes* baculovirus (Crawford *et al.*, 1985). The genotypic variation among 12 geographical virus isolates was analyzed by endonuclease digestion and slight differences in the electrophoretic fragment profiles were found (Crawford *et al.*, 1986).

In this study, we have purified an Indonesian isolate of the *Oryctes* baculovirus and analyzed several properties.

MATERIALS AND METHODS

Purification of Oryctes virus particles

For virus purification, midguts of 11 female adults of *Oryctes rhinoceros* infected with an Indonesian isolate of *Oryctes* baculovirus were collected. The midguts were homogenized well in an equal volume of phosphate-buffered saline (PBS: 1 mM Na_2HPO_4; 10.5 mM KH_2PO_4; 140 mM NaCl; 40 mM KCl, pH 6.2) and the homogenate was centrifuged at 1,800 x g for 30 minutes at 4°C. The supernatant was than centrifuged at 85,000 x g for 1 hour at 15°C. The resulting pellet was suspended in small volume of PBS and carefully loaded on linear 10% to 40% sucrose gradient. After centrifugation at 30,000 x g for 30 minutes at 15°C, a thick, white band containing virus particles was collected and diluted with TE buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0). The virus pellet obtained by centrifugation at 85,000 x g for 1 hour at 15°C was resuspended in 1 ml of TE buffer and stored at -20°C.

Electron microscopy

Purified virus particles were negatively stained with 2% uranyl acetate and observed using JEOL JEM 100CX electron microscope.

SDS-PAGE

The proteins of virus particles were analyzed by SDS-PAGE (12%) under reducing conditions (Laemmli, 1970).

Purification of viral DNA

The virus particles were disrupted by incubation for 2 hours in a solution containing SDS (0.1 %) and proteinase K (1 mg/ml). The digested virus was then extracted twice with phenol, once with phenol:chloroform: isoamyl-alcohol (25:24:1) and once with chloroform: isoamylalcohol (24:1). The extracted viral DNA was analyzed overnight at 4°C against 1 l of TE buffer with two changes. The DNA concentration was quantified by measuring the OD_{260} and OD_{280} of the dialyzed solution and then stored at 4°C.

Restriction of viral DNA

The purified viral DNA was digested with either *Eco*RI or *Hind*III restriction endonucleases (Takara). The restriction fragments were resolved on 0.8% agarose gels, stained with ethidium bromide, and examined with a u.v. transilluminator.

DISCUSSION

Morphology of Virus Particles

Purified preparation of the Indonesian isolate of *Oryctes* baculovirus contained empty nucleocapsids, measuring 210 x 80 nm shaped as cylinders with rounded ends, and disrupted viral envelopes (Fig. 1). The tail-like projections, measuring 310 x 15 nm, were associated at one end of the nucleocapsids. A fibrous network probably composed of released viral DNA was also observed.

Structural Protein of Virus Particles

A total of 19 virus structural proteins were detected in a Coomassie brilliant blue-stained polyacrylamide gel (Fig.2). Among this proteins, 10 proteins (69, 59, 47, 45, 33, 31, 28, 26, 17 and 16 kDa) were very abundant. There was no similarity in SDS-PAGE profiles of virus proteins between *Oryctes* baculovirus and *Bombyx mori* nuclear polyhedrosis virus, an occluded baculovirus.

Restriction Endonuclease Digestion of Viral DNA

The viral DNA was cleaved with *Eco*RI and *Hind*III into 39 *Eco*RI fragments and 22 *Hind*III fragments (Fig. 3). All fragments were observed clearly, although 7.6 kb *Hind*III fragment was less stained. The sizes of the fragments are shown in Table 1. The calculated genom size of the virus was approximately 130 kb.

Table 1. Restriction endonuclease fragments of *Oryctes* baculovirus (Indonesia isolate)

Fragment	*Eco*RI	*Hind*III
A	19,0	21,0
B	11,0	12,5
C	6,0	12,0
D	6,0	10,5
E	6,0	10,0
F	5,2	9,2
G	5,2	7,6
H	5,0	7,0
I	4,6	5,8
J	4,6	5,4
K	4,3	5,0
L	3,7	4,8
M	3,3	4,3
N	3,3	4,1
O	3,3	3,9
P	3,0	3,5
Q	3,0	0,68
R	3,0	0,58
S	2,5	0,58
T	2,3	0,50
U	2,3	0,36
V	2,2	0,31
W	2,2	
X	2,0	
Y	2,0	
Z	1,8	
a	1,8	
b	1,4	
c	1,4	
d	1,3	
e	1,2	
f	1,2	
g	1,0	
h	1,0	
i	0,51	
j	0,48	
k	0,46	
l	0,41	
m	0,34	
Total	129,30	129,61

Electron microscopic observation revealed that the preparation of Indonesian isolate of *Oryctes* baculovirus purified from infected midguts was composed of empty nucleocapsids and virus envelopes (Fig.1). Payne *et al.* (1977) obtained the similar preparation of nucleocapsids by treating enveloped viral particles with the detergent (NP 40). Although we did not use any kind of detergents during the virus purification, the purified virus particles must be damaged by several repetition of freezing and thawing before electron microscopic observation.

The nucleocapsids of Indonesian isolate showed the same morphological features including the long tail-like projection at one end of the nucleocapsid as those previously reported (Payne *et al.*, 1977). The sizes of the nucleocapsid (210 x 80 nm) and the tail-like projection (310 x 15 nm) determined for our preparation, however, were slightly larger than those (160 x 50 nm and 270 x 10 nm respectively) of Payne *et al.* (1977). In other preparations, the nucleocapsids averaged 180 x 65 nm in size (Huger and Krieg, 1991). The small variation in size among preparations may not reflect virus strains used but conditions of preparation.

Among 19 structural proteins identified by SDS-PAGE, molecular weights of most proteins were consistent well with previously reported for strain PV505 (Crawford and Sheehan, 1985). One of the exceptions was the most abundant protein, whose molecular weight was 17 kDa in the Indonesian isolate but 13 kDa in strain PV505.

Although the Indonesian isolate was not cloned, all restriction fragments resulted from digestion of viral DNA with both *Eco*RI and *Hind*III were observed clearly and except 7.6 kb *Hind* III fragment there were no obvious submolar fragments (Fig. 3). In addition, the calculated genom size of approximately 130 kb was consistent with that for the cloned strain PV505 (Crawford *et al.*, 1985). Therefore the Indonesian isolate is expected to be highly homogenous for its genotype.

Crawford *et al.* (1986) examined genotypic variation among 12 isolates of *Oryctes* baculovirus and found slight differences in their restriction fragment electrophoresis profiles. Most of common fragments among these isolates were also found in the Indonesian isolate. However, parts of fragment profiles such as presence of 19 kb *Eco*RI fragment and absence of 3.3 kb *Hind* III fragment were specific to the Indonesian isolate.

Our results indicate that the Indonesian isolate of *Oryctes* baculovirus shares most properties commonly identified in other geographical isolates from Philippines, Tanzania, Seychelles, Malaysia and India. Detection of several properties specific to the Indonesian isolate, however, means it is a geographical variation of *Oryctes* baculovirus. Further comparative studies will reveal the relationship between the Indonesian isolate and other geographical isolates of *Oryctes* baculovirus.

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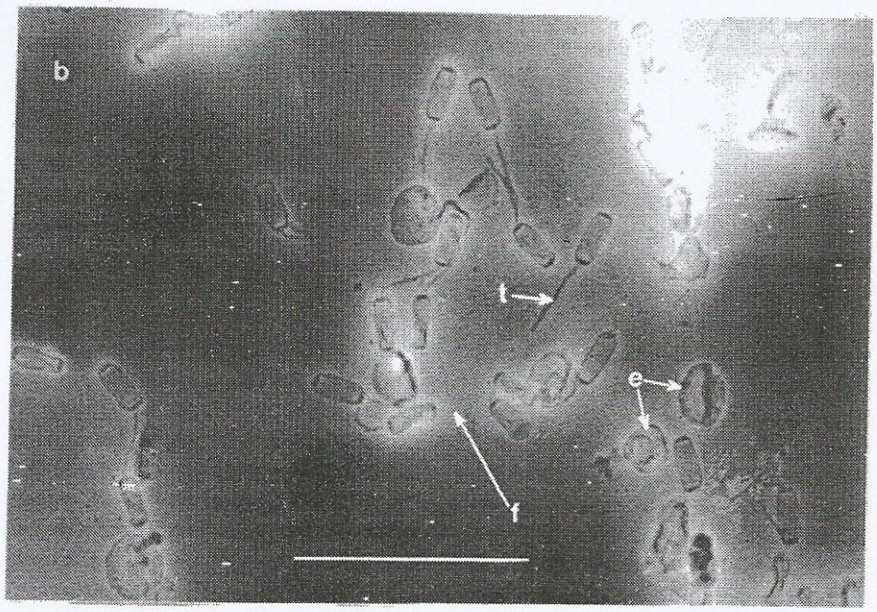

Figure 1. Electron micrograph of *Oryctes* baculovirus (Indonesian isolate). Disrupted viral envelop (e), the tail-like projection (t) at one end of cylindrical nucleocapsid and the fibrous network (f) were indicated with arrows. Bar = 1 μm.

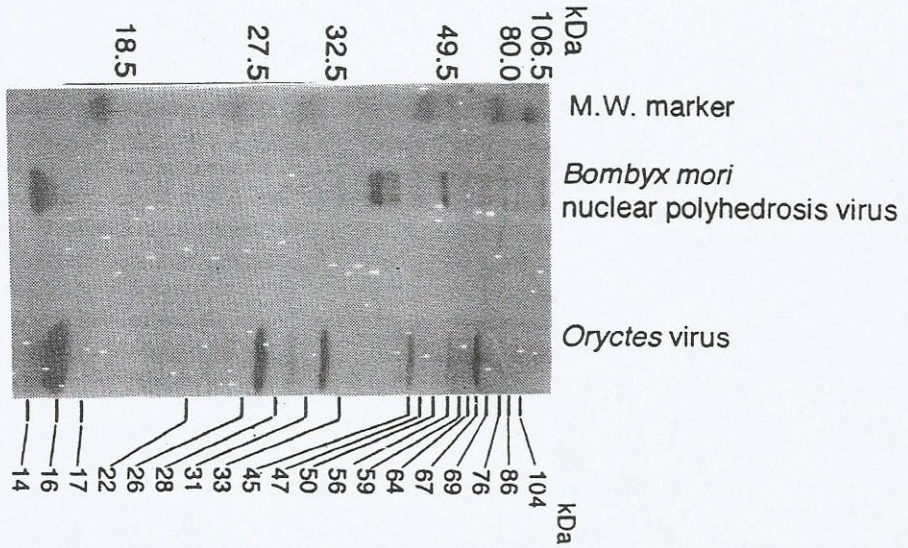

Figure 2. Structural proteins of *Oryctes* baculovirus (Indonesian isolate). Purified virus particles were electrophoresed on 12 % SDS-polyacrylamide gel. For comparison, purified occluded virions of *Bombyx mori* nuclear polyhedrosis virus were also lectrophoresed. Numbers were molecular weights (kDa).

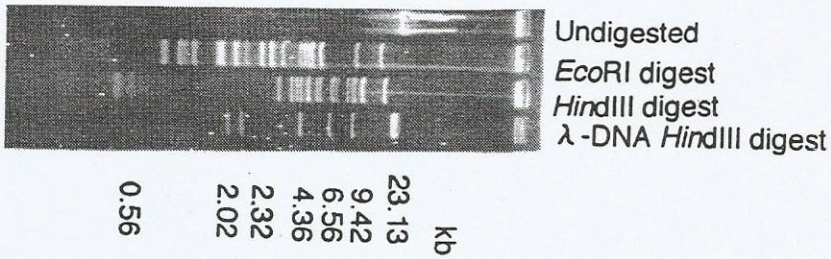

Figure 3. Restriction endonuclease fragment profiles of DNA from *Oryctes* baculovirus (Indonesian isolate). Both *Eco*RI fragments and *Hin*dIII fragments were electrophoresed on 0.8% agarose. Numbers were sizes of DNA fragments (kb).