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PROPAGATION AND PURIFICATION OF *BACULOVIRUS ORYCTES* HUGER

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ABSTRACT

An isolate of *Baculovirus oryctes*, a possible biological control agent for coconut beetle (*Oryctes rhinoceros* Huger) from East Java was propagated and purified. The virus could be transmitted by feeding the imago with 10% sucrose containing virus from homogenate of infected beetles. Effectivity of virus transmission by food contact was 53 %, while one virus-infected male beetle was able to transmit the virus to 9 healthy females by sexual copulation. Virus could be successfully purified by a method of Payne.

Key words: *Baculovirus oryctes*, transmission, purification

INTRODUCTION

A large number of viruses have been reported to occur as insect pathogens in nature. Such viruses, together with other natural enemies, such as parasites,parasitoids, predators, and other insect pests, play an important role as pest suppressant in natural ecosystem. Some viral diseases identified as insect pathogen in Indonesia, such as *Baculovirus* of Oryctes has been proved as valuable agent in controlling coconut rhinoceros beetle (*Oryctes rhinoceros* (L)) (Huger, 1966; Matthews, 1982; Huger and Krieg, 1991). Among the features that make the *Baculovirus* attractive as one of the biological control agent are their efficacy and specificity. Baculovirus induces lethal epizootics which can reduce host insect population and infect only a limited member of coleoptera and no effects on other species or invertebrates have been demonstrated. The virus is not known to infect mammals, other vertebrates, or any plant species, and is environmentally benign. Thus *Baculovirus oryctes* is attractive as an alternative biocide to the conventional chemical pesticides to develop integrated pest management (IPM) in coconut plantation.

While the need for implementation of *Baculovirus oryctes* in IPM in Indonesia increases, field application of the virus in the coconut plantation has been faced problem with the lack of accurate methods for virus detection. Thus, it is very difficult to monitor the effectiveness of any viral related programs.Recent advantages in biotechnology, particularly the development of techniques for the preparation of monoclonal antibodies (Kohler and Milstein, 1975), could increased the possibility of using better serological detection. Monoclonal antibodies have already proved useful in virus detection of animal and plant viruses. We describe here a method for antigen preparation which finally will be used to produce monoclonal antibody-based virus detection.

MATERIALS AND METHODS

Virus isolate.-- The virus isolate was kindly provided by Field Laboratory Biological Control, Estate Crop Service, Jombang, East Java. The virus has already been used to control the beetle in field. Virus propagation was done at Field Laboratory of Biological Control, Wonocatur, Yogyakarta.

Preparation of beetle.-- Four thousands of third instar larvae were collected from the field in Yogyakarta and were selected visually to obtain the diseases-free larvae. The healthy larvae were then reared individually in the bottle containing coconut sawdust. The adult beetles were reared using a pice of sugarcane.

Preparation of inoculum.-- Freshly dissected beetle midguts showing advanced symptoms of infection of East Java strain of *Baculovirus oryctes* were homogenized in phosphate buffer saline (PBS). The homogenate

was then passed through a 0,45 µm milipore to remove contaminating bacteria and fungi. The filtrate was than added with sucrose to the concentration of 10%, and was stored at -20 C.

Method of inoculation to beetle.-- Adult beetles (imago) reared in the laboratory were infected by feeding them a few drop (about 25 ml/beetle) 10% sucrose containing virus from homogenized beetle guts (Bausat, 1992). The beetles were subjected to a 24 h fast before and after feeding with the virus. The inoculated beetle were ready to be used in the experiment for transmission by sex and food contacts.

Virus purification.-- Virus was purified basically using a method reported by Payne (1974). Almost all steps of the purification procedure were done at the temperature of 4-6 C. Midgut from infected beetles were homogenized in phosphate buffer saline (PBS). After squeezing through two layers of gauze, the homogenate was centrifuged at 2,000 g for 10 min. in Sigma 3K30 angle 12156 rotor. The supernatant was then centrifuged at 50,000 g for 60 min. The pellet was resuspended in 1% Natrium thioglycolat and centrifuge 2,000 g for 10 min. The supernatant was subjected to zonal centrifugation at 45,000 rpm for in Hitachi RP-65 T rotor tubes containing 20-50% sucrose. The virus-containing zones were collected and diluted in PBS. Purified virus was stored at - 20 C before be used as immunogen or antigen in assays.

RESULTS AND DISCUSSION

Natural infection of virus.-- When the field collected larvae were observed, it was found that more than 75% of the larvae showed symptom of infection by virus or fungi (data not shown). The same experience was also reported by Crawford (1985) who observed high virus infection of rhinoceros beetle in Sulawesi. It is interesting to note that the high infection of *Baculovirus* was in some time associated with serious outbreak of the beetles. This finding indicated that *Baculovirus* found in this region has become less pathogenic or originally derived from weak strains.

Transmission through food contact.-- To know the possibility of transmission of the virus through food contact, one artificially infected male beetle was reared together with healthy male beetles for 20 days in the same container. The results are given in Table 1. It was found that the virus could be transmitted through food contact at the rate of 53 %.

Table 1. Inoculation of *Baculovirus oryctes* to beetles by food contact[1)]

Experiment	Result of inoculation[2)]	Percentage of infected beetle
1	1/3	33
2	2/3	66
3	2/3	66
4	1/3	33
5	2/3	66
Average		53

Notes: 1)As the control, 15 healthy beetles were reared without contact with infected beetles.
2)Numerator = Number of infected beetles
Denominator = Number of healthy beetles reared together with an infected beetle

Transmission of virus through sex copulation.-- Table 2 showed that one infected male beetle was able to transmit virus at least to nine female beetles. In view of the difficulty to propagate virus, the transmissibility of the virus by sex-copulation could be manipulated either in application of the virus in field or in propagation of the virus in the laboratory.

Table 2. Effectivity of male beetle to transmit *Baculovirus oryctes* to *Oryctes rhinoceros* [1)]

Experiment	Transmission infectivity (%) of the virus when one infected male beetle was reared with healthy female at a number of				
1	1	3	5	7	9
1	100	100	100	85	88
2	100	100	100	100	77
3	100	100	80	85	77
Average	100	100	93	90	85

Note :[1)] Effectivity of virus transmission was mentioned in percent of infected female to number of healthy female reared together with one infected male

Purification of the virus.– In the preliminary experiment to purify the virus, 10 infected beetles were used as the starting material. Unfortunately, after finishing sucrose gradient centrifugation virus band could not be observed. The problem was then solved by using dissected beetle midguts. After subjected to gradient centrifugation, several band could be observed on the tubes. The band between 30-40% sucrose contained *Baculovirus* as determined by spectrophotometer and electrophoresis. The characteristics of the virus were the same as those reported by Monsarrat *et al.* (1973) and Zellazny *et al.* (1985).

An experiments are now being conducted for producing polyclonal and monoclonal antibodies by using the purified virus.

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