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ISOLATION AND CHARACTERIZATION OF BIOMOLECULES OF *Monodon baculovirus* ISOLATES

Kamiso H.N.*, Triyanto* and Murwantoko*

ABSTRACT

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Monodon baculovirus (MBV) is one of the most important viral disease in shrimp culture because it caused high mortality rate of all shrimp life stages in wide culture regions.

The objectives of this study were to purify and characterize biomolecules of OB and MBV virion taken from shrimp hepatopancreas of Tiger shrimp (*Penaeus monodon*). Shrimp samples were taken from brackish water ponds in West Java, Central Java, and East Java provinces. Number of molecules and molecular weight of viral protein and OB, DNA from and the shape of MBV virion were determined using purified OB and extracted DNA from hepatopancreas of the shrimp.

The result indicated that shrimp samples from various sample areas was only infected by one type of virus, that is *Monodon baculovirus*. The MBV genom is DNA with size more than 10 kbp, the shape of MBV virion is rod and composed by at least nine dominant protein units.

*Key words : biomolecules, **Monodon baculovirus, Penaeus monodon***

INTISARI

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Monodon baculovirus (MBV) merupakan salah satu virus terpenting dalam budidaya udang, karena menyerang semua fase kehidupan udang, dan menyebabkan kematian yang tinggi di daerah yang sangat luas. Penelitian ini ditujukan untuk purifikasi dan karakterisasi biomolekul oklusion bodi (OB) dan virion dari hepatopankreas udang windu (*Penaeus monodon*).

Sampel udang diambil dari tambak udang di Jawa Barat, Jawa Tengah dan Jawa Timur. Jumlah molekul dan berat molekul protein virus dan OB serta bentuk virion

* Fish Diseases Laboratory, Depart. of Fisheries, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia.

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diamati menggunakan OB yang telah dimurnikan dan ektrak DNA dari hepatopankreas udang, sedangkan virion diamati menggunakan mikroskop elektron.

Hasil penelitian menunjukkan bahwa sampel udang dari semua daerah penelitian hanya terinfeksi oleh satu jenis virus yaitu MBV. *Monodon baculovirus* yang diisolasi mem-punyai genom DNA dengan ukuran lebih dari 10 kbp, sedangkan bentuk virion adalah batang yang tersusun paling tidak oleh sembilan unit protein dominan. Dalam keadaan mendesak karakterisasi OB tanpa mikroskop elektron dapat digunakan untuk diagnosis MBV secara presumtif.

*Kata kunci : biomolekul, **Monodon baculovirus, Penaeus monodon.***

INTRODUCTION

In Indonesia, intensive shrimp culture has been practiced since 1980. From 1983 to 1987 the production of culture shrimp increased approximately 67% and became one of the dominant commodities of fisheries export. However, since 1989 the production decreased due to attacks of various diseases and deterioration of evironmental conditions. Since 1989 to 1992 the lost caused by deseases was 248 millions US dollars. In 1992 alone the mortality was 41.447 ton (Anonym, 1994).

Viral disease was considered to be the main cause of high mortality in shrimp culture (Sindermann, 1990). One of the most important virus disease was *Monodon baculovirus* (MBV). The virus infected all life stages of shrimp in various shrimp culture areas and caused high mortality rates. In various countries in Asia, Africa, Europe, and America MBV infected most of *Penaeus* spp and the prevalency of infection could reach 73% to 100% (Lightner, 1996). In Indonesia the incidence of MBV infection on *Penaeus monodon* and *Penaeus merguiensis* in brood stock was 20%, in larvae 59-92%, and in rearing ponds was 29-84% (Lightner *et al.*, 1992; Larkins, 1993).

Various efforts had been made to control the diseases, particularly dealing with water quality management and medication using various drugs or antibiotics. However, the results were still unsatisfied. Most farmers can not detect the incidence of the disease until it has already been in advanced stage because there is no early diagnostic method that can be used. Research on MBV in Indonesia is still very limited, and isolation of MBV virion from *P. monodon* or *P merguiensis* has not been done. Diagnosis had only been conducted by observation of the pathological sign or occlusion body of wet mount from shrimp hepatopancreas and histopathology (Nash *et al.*,1988; Larkins, 1993). This method could not detect the disease in early stages before the occlusion bodies and latent stage developed (Vickers *et al.*, 1992).

The study was conducted to collect MBV isolates from varoius brackish water pond areas, purify the occlusion body and virion, characterize MBV biomolecules, and to know the shape of MBV virion by transmission electron microscope.

MATERIALS AND METHODS

Shrimp samples were collected from West Java (Karawang), Central Java (Brebes and Jepara), and East Java (Situbondo), then preserved by crushed ice and transported to Fish Diseases Laboratory at the Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Yogyakarta .

Extraction of DNA virus was conducted by using modified Pole method (1995). Destruction of membrane and protein was done by SDS 10% which was incubated in waterbath at 60°C for 10 minutes followed by proteinase K at 50°C for 15 minutes. Purification of DNA was done by combination of phenol, chloroform and isoamil alcohol. Observation of DNA molecular weight was conducted by electrophoresis with 1% agarose gell. The DNA molecular weight marker VII from Boehringer Mannheim was used as a marker. Staining was conducted with ethidium bromide 0,5 mg/ml. Observation of virions and occlusion bodies polypeptides were conducted by SDS- PAGE with 10% consentration (w : v). The markers were myosin (200 kDa), ß-galaktosidase (116,2 kDa), phosphorilase b (97.4 kDa), BSA (66,2 kDa) and ovalbumin (45 kDa). The result of electrophotesis was stained with coomasine blue.

Absorbancy was run using Beckman DU-65 spectrophotometer at 230- 300 nm. Samples were virion suspension preparation with 10 times dilution in TE buffer, and the control was TE buffer. The result was absorbancy type graph to indicate the patern of virion absorbancy to UV light (Basu & Giri, 1993).

The shape and size of virion particle was observed by transmission electron microscopy (TEM) at 80 kV with collodium as grid coating. Specimen was prepared by dripping suspension on parafilm. The use of grid which had been layered by collodium for 10 minutes, then stained with PTA 2%.

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RESULTS AND DISCUSSION

Shrimp samples taken from brackiswater pond units showed that they were infected by MBV. The pathological signs were lethar- gic, empty stomach or intestine, abnormal body color, and thin, and usually swam to the bank or the water surface.

Hepatopancreas from diseased shrimp was homogenized under steril condition. But result the showed that there are some bacteria species in the homogenized samples. In general the bacteria was Gram negative, rod shape and the colonies was round, convex and yellowish in color. There are several posibilities in regard of the occurance of the bacteria. Firstly, the bacteria was only contaminant organism in the samples. Secondly, the shrimp samples were infected by bacteria. Thirdly, the shrimp in the area was infected by bacteria and virus. It is also showed that MBV virion could not be detected in the diseased shrimps although they indicated some pathological signs. Few researchers found that bacterial infection or contaminant were often found in the hepatopancreas, most of which were *Vibrio* spp (Larkins, 1993; Chanratchakool *at al.*, 1994; Lightner, 1996). These patogenic *Vibrio* spp was sistemic in the hemolymph and the shrimp may or may not indicated pathological signs depending on the physical and physiological conditions.

The samples of hepatopancreas were easily rotten due to bacterial contamination. The success of virion purification dropped to about 50 and 25% after one and two weeks in the freezer, meanwhile for fresh samples was 75%. The purification was failed after three weeks. This finding was different from Chang *et al.* (1992) which still could find and purify the virion from hepato- pancreas after being preserved for approximately two months.

Clarification with 2200 g or 3000 rpm for 20 minutes using Beckman J-6B centrifuge was effective enough to separate the virion from com- ponents includes bacteria. Even there was bacteria in the pellet, but the supernatant was clean. This experiment could isolate occlusion body (OB) only from approximately 50 percent of shrimp samples. It was similar to Mari *et al.* (1993) which failed to found OB, but could purify MBV virion from samples. Obser- vation of OB under binocular mi- croscope at 400 times magnification could detect the OB clearly (Figure 1). The shape of OB was round similar to that of the finding of Chang *et al.* (1992).

Since the number of OB was limited, purification of occluded virion from OB could not be per- formed. Therefore, purification was only made for virion outside OB (non occluded virion).

Purified OB was used to deter- mine the molecular weight of protein sub unit in the OB using electrophoresis method (Figure 2). Linear regression between distance of migration of marker band (x) and logarithmic of molecular weight (Y) of Karawang (Kr) isolate was Y = 2.4501 - 0.0133X, where r = -0.9757. The regresion equation of mixed

Figure 1. Purified occlusion body (400 x).

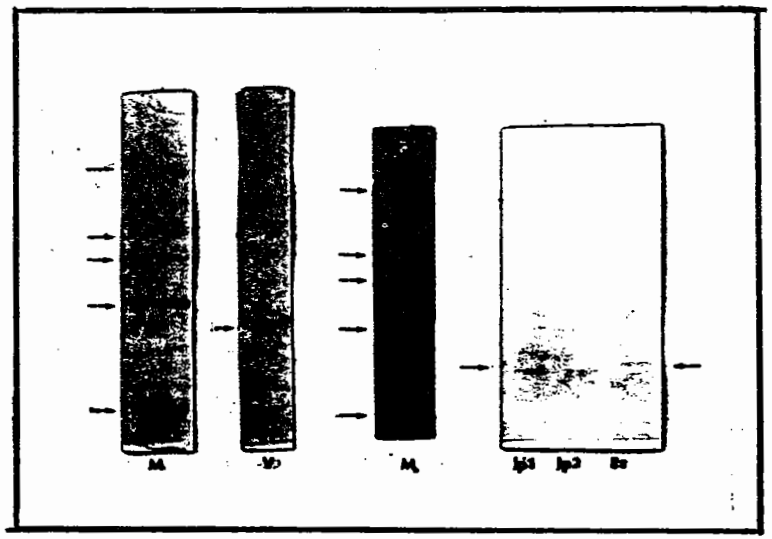

Figure 2. **Electrophoresis of occlusion body. Marker (M), molecular weight from top to bottom: 200.0, 116.25, 97.4, 66.2 and 45.0 kDa.**

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olate collected from Jepara (Jp1 id Jp2) and Brebes (Br) was Y = 1815 - 1.0144, where r = -0.9885.

Based on the equation the olecular weight of protein sub unit f OB was 62 kDa for Kr isolate and 6 kDa for Jp1, Jp2 and Br. These are pproximately the same as the result f the study conducted by Mari *et al.* 1993). Mari *et al.* (1993) using imilar method found that the nolecular weight of protein sub unit vas 58 kDa.

Centrifugation of virion suspension in sucrose gradient with SW 28 rotor found one band virion at approximately 30% of sucrose concentration (Figure 3). This result indicated that the shrimp was infected only by one type of virus. Virion band was taken by the me of Pasteur pipet and diluted in TN buffer. The suspension was harvested by centrifugasion with Ti 70 rotor and checked the molecular weight of protein by SDS polyacrylamide electrophoresis (Figure 4). The figure showed that the intensity of every band in the same well were different from one and other. It can be concluded that the MBV virus found in the samples consisted of various number of different proteins having similar pattern. It indicated that all of the isolates were of the same virus. Similar result was found in *Baculovirus oryctes*, from its viral proteins only nine bands were dominant and the most dominant protein band was the protein with molecular weight 17 kDa (Sumardiyono *et al.*, 1995). The number of protein bands were different among isolates. Brebes (Br) and Jepara (Jp2) isolates had nine dominant proteins bands. Jepara (Jp1) had eight bands and Karawang (Kr) had five bands. This difference due to the different amount of loaded protein on electrophoresis. The sample number of loaded protein caused few thin bands invisible. The corelation between rf band marker (x) and logarithmic of molecular weight (Y) of isolates Br was Y = 2,4501 - 0,0133, where r = -0,9759. The equation of Jp1, Jp2 and Kr isolates was Y = 2,4768 - 0,0162 with r = -0,9892. Based on the linear regression equation the calculated molecular weight of protein were 90.5, 86.1, 78.4, 69.9, 67.8, 56.8, 46.8, and 44.7, kDa. The missing protein band belonged to Jp1 isolated was 56.8 kDa and from Kr isolate were 83.8, 69.9, 56.8 and 44.7 kDa respectively.

DNA could be extracted from four isolates and all of them showed one band at the same position. It indicated that all isolates were of the same type of virus (Figure 5). Linear regression equation between rf DNA marker (x) and the logarithmic of

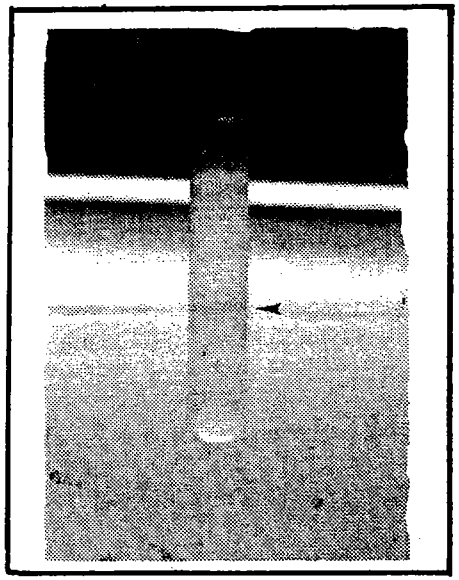

Figure 3. Virion band in sucrose gradient by ultracentrifugation.

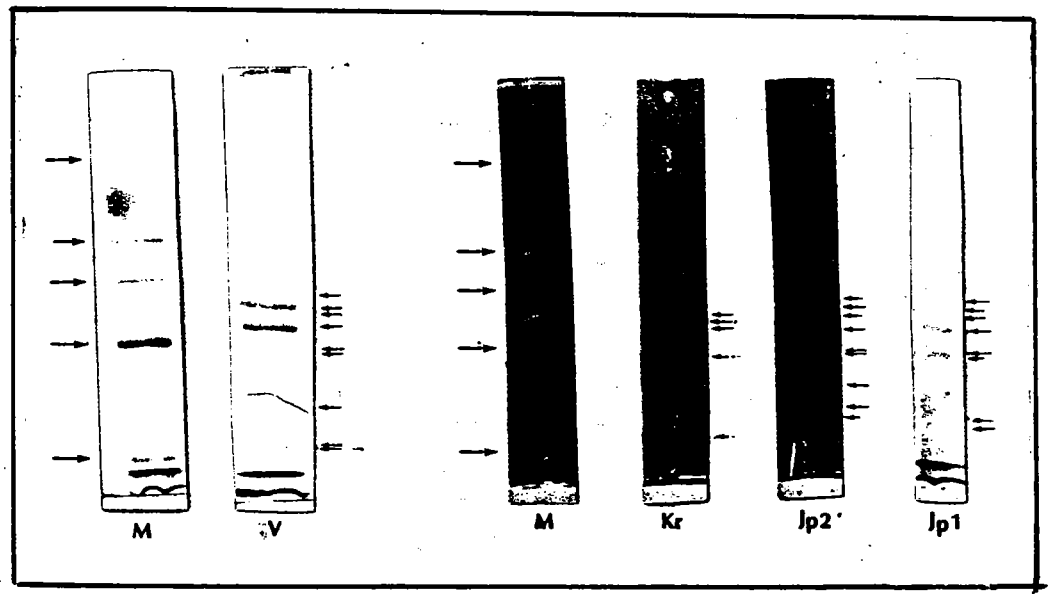

Figure 4. SDS-PAGE of MBV virion. Marker (M), molecular weight from top to bottom: 200.0, 116.25, 97.4, 66.2 and 45.0 kDa.

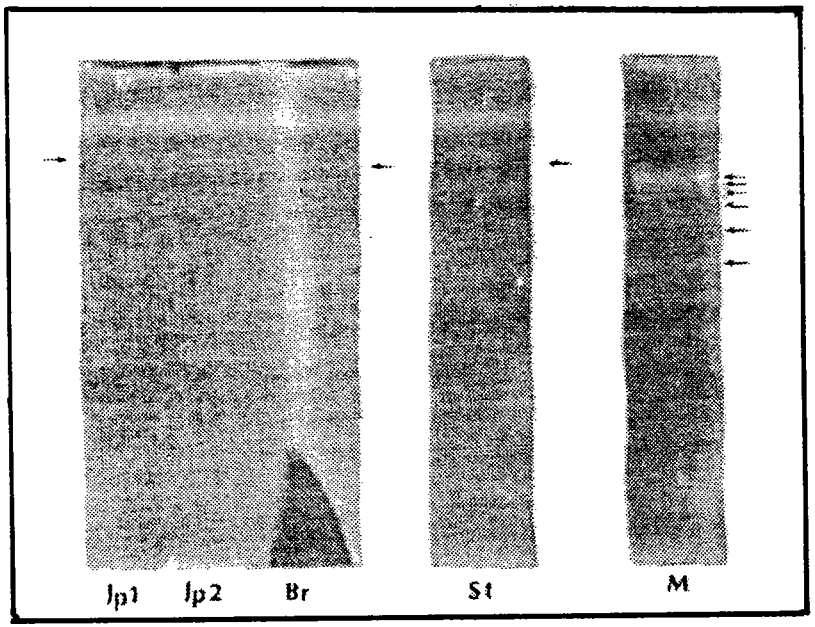

Figure 5. DNA of isolated MBV on agarose gel electrophoresis. Marker (M), molecular weight from top to bottom : 8.0, 7.1, 6.0, 4.8, 3.5 and 2.7 kbp.

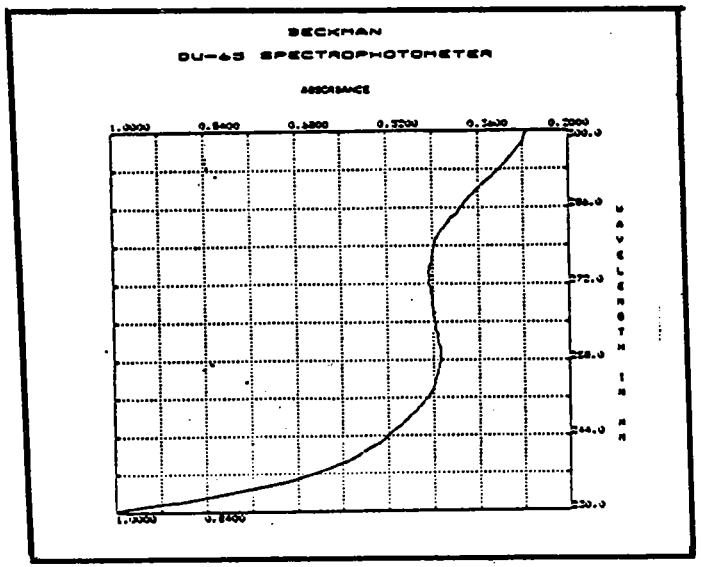

Figure 6. Type of UV light absorbancy of MBV virion.

base pair size (y) was Y = 1.4090702 - 0.037696 X, where r = - 0.98859. Based on the equation the size of DNA virion was aproximately 10 kbp. This result was different from Mari *et al.* (1993) where the size of DNA virion from isolated MBV was 43 kbp. The difference may be due to the difference in the strains, since MBV has various strains. Lightner (1996) found that the size of nucleocapsid of isolates from Indo-Pasific were 39-45 x 231-261 nm, but isolates from Australia were 45-52 x 260 - 200 nm.

Type of UV light absorbancy indicated that pure virus has maximum UV light absorbancy 275 nm and minimum 258 nm with A maximum/A minimum value 1.0087 and the ratio of optical density (OD) 280/260 was 1.01 (Figure 6).

The scanning of MBV virion by Transmission Electron Microscope (TEM) with 31.000 magnification indicated that the shape of MBV virion was rod. Unfortunately the size could not be measured due to equipment defect (Figure 7).

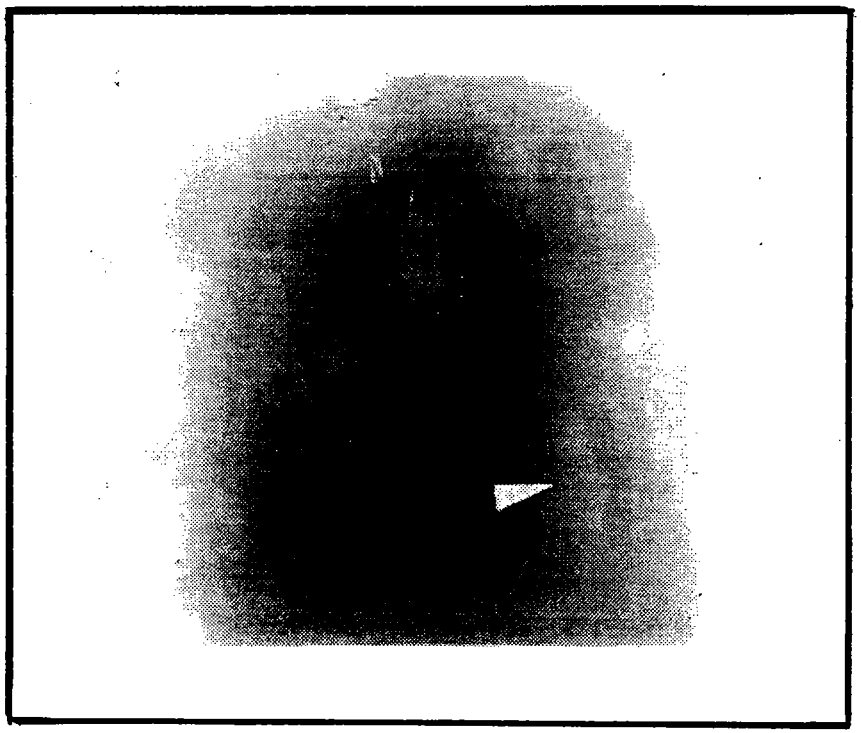

Figure 7. Transmission Electron Microscopy of MBV virion (50.000 x).

CONCLUSIONS AND SUGGESTIONS

1. All shrimp samples from Jepara, Brebes and Karawang were infected by the same virus, i.e. *Monodon baculovirus* (MBV).
2. The shape of occlusion body (OB) was round, composed by single sub unit proteins with molecular weight 56-62 kDa.
3. The position of MBV virion was at approximately 30% of sucrose concentration (w : v) and it was rod in shape.
4. The structure of MBV virion consited of various proteins with different concentrations among types of proteins. there were nine dominant protein types with size 90.5, 86.1, 83.8, 78.4, 69.9, 67.8, 56.8, 46.8 and 47.7 kDa.
5. Genetic Material of MBV Was DNA With 10 Kbp In Size.
6. Pure Virus has maximum and minimum absorbancy of UV light 275 nm and 258 nm respectifely with minimum A max/a minimum value was 1.0087 and the ratio optical density (OD) 2880/260 was 1.01.
7. The characterization of OB without Electron Microscope can be used as presumptive diagnosis of MBV.

SUGGESTIONS

1. Purification of OB and virion should be conducted as soon as possible to prevent bacterial contamination in the samples of hepatopancreas.
2. To obtain enough concentration of OB and virion it is suggested to collect enough infected shrimp samples or to propagate them either *in vivo or in vitro* using susceptible cell culture

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