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Characterization of the White Spot Syndrome Baculovirus (WSBV) Infection in Fresh Shrimp, *Penaeus chinensis*, Cultured in Korea

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The virions of causative virus for white spot syndrome in cultured Fresh shrimp, *Penaeus chinensis* were rod-shaped, double envelope. An average size of the virion was 70 nm in diameter and 250~300 nm in length. Histopathological test of affected stomach, heart, and lymphoid organ revealed nuclear hypertrophy. Infectivity trials carried out by injection and feeding with purified virus revealed high cumulative mortality to healthy shrimp. The twenty one different protein species were detected in the analysis of virion. The length of total DNA from the purified virus particles were detected as a single band, double-stranded DNA molecule of approximately 114 kb.

Key words – fresh shrimp, *penaeus chinensis*, white spot syndrome.

The species of shrimp living in Korea generally called shrimp is largely divides into Pennaeidae and Caridae. Penaeidae genus in Penaeidae family species includes *Penaeus japonicus* from Japan and *Penaeus chinensis* from China. In Korea, Penaeidae farming was begun for the first time on the western coast in 1960 but the lack of technology and experience caused a lot of difficulties, in the 1980s the artificial hatching production technology and the increase of demand in the domestic market developed the cultured shrimp industry, and thus in the 1990s the number of shrimp farms in the western and southern coasts comes to around 500. However even in Korea like neighboring shrimp culturing nations, in incurable epidemic has broken out every year since 1993 and is dealing a big blow to shrimp farming on the Western Coast. The virus species causing contagious disease among shrimp number almost 15 and these viruses are different in their occurrence rates and areas depending on environment, water temperature, region, and host specificity. Therefore, Since 1993, mass mortalities among cultured fresh shrimp, *Penaeus chinensis*, have been observed in Korea[3]. The cephalothrax and body surface was showed to white spots by infected shrimp and changed to reddish in the color. So, the name of the disease is generally called white spot syndrome diseases (WSSD), and the serious mortality rate of shrimp due to this kind of symptom is occuring all over Asia[2,13]. Most species of economically important shrimps such as redtail prawn, *P. penicillatus*, giant tiger prawn, *P. monodon*, kuruma shrimp, *P. japonicus*, and fresh shrimp, *P. chinensis*, are affected by the virus. The causative viral agent, named white spot baculovirus (WSBV) as baculovirus associated with white spot syndrome disease (WSSD).

The present study reports on histopathological changes in the tissues of infected shrimp, morphological characters of the white spot syndrome virus in the diseased shrimp as revealed by electron microscopy, protein, and nucleic acid characters of causative virus. This study was also aimed to provide the fundamental information for characterization of the White spot syndrome Baculovirus (WSBV) infection in the fresh shrimp, *Penaeus chinensis*.

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Materials and Methods.

Source of specimens

Shrimp infected from WSBV were collected from farm in the nearest yellow and southern sea in Korea from July 2001 to July 2002. Healthy fresh shrimp for use in virus infection were obtained from a farm at Taean South Chungcheong Province, and acclimated in the laboratory for three month. During this experimentally infected period, the virus-infected shrimps were reared in tanks at 23℃ for three week. Hemolymph and stomach tissues were harvested from those of artificially infected shrimps by WSBV and used for virus purification, electron microscopic observation and histopathological changes in the tissues.

Observation by electron microscopy and histopathology

The stomach epithelium and hemolymph of shrimp in-

fected from WSBV were used for the ultrathin section. The tissues were fixed by 2.5% glutaraldehyde in cacodylate buffer, post fixed in 1% osmium tetroxide in the same buffer. Treated tissues were dehydrated in ethanol and embedded in Spurr's resin[11]. Ultrathin sections were cut on a Reichert-Jung ultramicrotome at 60 nm and stained with uranyl acetate and lead citrate according to Reynolds [9]. For histological studies, stomach epithelium, gills, heart, and lymphoid organ of shrimp infected from WSBV were preserved in Davidson's fixative solution for 48 hr and then transferred to 60% ethyl alcohol for storage. Tissues were prepared for light microscopy using routine paraffin techniques method and then were stained with hematoxylin and eosin[1].

Challenge Studies

Challenge experiment were conducted using virus-free healthy shrimp obtained from shrimp pond where no virus infection had been determined by PCR[6].

Ten shrimps were used in each experimental group and control group for ten days. Each experimental group were kept in tank with aeration, and water was maintained at a daily exchange rate nearly 8~10% and fed by commercial meat. Water temperature during experiment were maintained 23~25℃ for ten days.

Virus purification

The purification of virions of WSBV from moribund shrimp was carried out as follows. Pooled hemolymph from moribund shrimps were first rinsed with cold 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), then was centrifuged at 1,000 xg for 20 min at 4℃. The supernatant was centrifuged again at 35,000 xg for 60 min at 4℃. The pellet was resuspended in 1 ml TE buffer at 4℃. This suspension was layered onto the top of continuous sucrose gradient (20 to 50%) and ultracentrifuged at 125,000 xg for 2 hr at 4℃ using Beckman ultracentrifuge (SW L7).

Protein analysis

Electrophoresis to analyze the virion protein infection of shrimp was conducted according to the method of Laemmli[7]. Specifically, the concentrated and purified shrimp-infecting virus was mixed with the same volume of SDS-sample buffer (2.3% SDS, 0.05 mM Tris, pH 6.8, 10% glycerol (w/v), 5% 2-mercaptoethanol), then it was boiled water for 2 min. After centrifuge at 10,000 xg for 10 sec, supernatant was used for electrophoresis in 10% polyacrylamide gel. It was separated to electrophoresis at 200 volt for 3 hr. The gel was strained with coomassie blue R-250, and its color was destained with discoloring solution. The molecular weight of the virus was decided to compared with the moving distance of standard protein.

Nucleic acid analysis

The purified virions from moribund shrimp were treated at 65℃ for 2 hr in the presence of proteinase K and SDS at the final concentration of 1 mg/ml and 1%, respectively, and then extracted with phenol twice, phenol/chloroform three times and chloroform once. The viral nucleic acids were precipitated with ethanol and dissolved in a TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.6). The purified viral nucleic acids and their restriction endonuclease digests were electrophoresed on a 0.8% agarose gel using a Tris-acetate buffer (0.04 M Tris-acetate, 0.001 M EDTA, pH 7.6)[10].

Results and Discussion

Virus morphology by electron microscopy

Many virus particles within the lymphoid organ, stomach and its cuticle epithelial cell of virus-infected shrimp and were observed through an electron mircroscope (Fig. 1). Virion by electron microscope is a rod-shaped form, 250~300×50~70 nm, and is comprised of a nucleocapsid and an envelope. The fine structure of the virion in the present study is similar to that of the rod-shaped baculovirus found in Kuruma shrimp, *P. japonicus*, and chinese shrimp, *P. orientalis*[4,12,15]. All these viruses posses a rod-shaped morphology, an double envelope, and do not contain occlu-

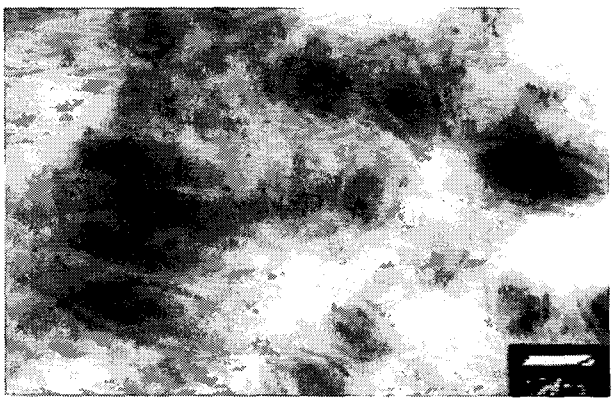

Fig. 1. Electron microscopy of the lymphoid organ of naturally infected fresh shrimp (*Penaeus chinensis*). Scale bar is 100 nm.

sion bodies. The virion of Kuruma shrimp is 275 nm in length of the and 83 nm in diameter[12]. The virion of Chinese shrimp is 365 nm in length and 107 nm in diameter [15]. The sizes of the virion in Fresh shrimp are similar to Kuruma shrimp and are smaller than those in Chinese shrimp.

Therefore, these Kuruma shrimp and Fresh shrimp causative agent infected by WSBV probably are same species, are possibly involved in infection in same host.

Histopathological observation

Stomach epithelium, gills, heart, and lymphoid organ of naturally infected shrimp exhibited similar histopathological change. Large numbers of hypertrophic nuclei were observed stomach epithelium and lymphoid organ (Fig. 2). The characteristic of histopathological changes was the conspicuous hypertrophy of the infected nucleus.

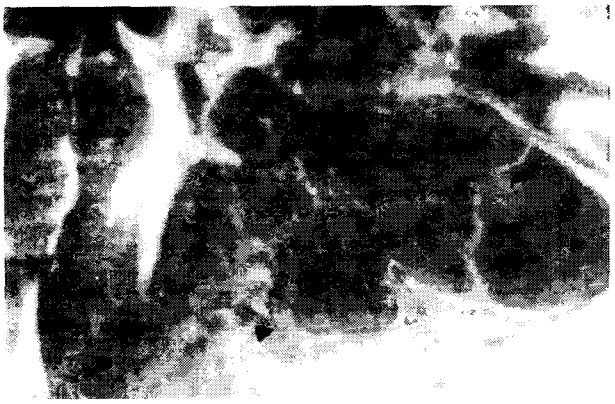

Fig. 2. Histological section of the lymphoid organ of an infected *Penaeus chinensis*, showing that hypertrophic nuclei (arrow head). H-E stain. (×400).

Momoyama et al.[8] and Zheng et al.[15] have described similar histopathological changes in cultured Kuruma shrimp (*P. japonicus*) and Chinese shrimp (*P. orientalis*) infected by rod-shaped virions, and it was similar to the present study on the histopathological changes of the Fresh shrimp (*P. chinensis*) that the notable hypertrophy of the infected nucleus was seriously damaged.

Challenge studies

The two challenge group experiments by injection with purified virus obtained from spontaneously diseased shrimp were performed by intramuscular injection and feeding (Table 1 and 2). The first challenge experiment group by intramuscular injections was started to be killed 5 days after inoculation and cumulative mortality reached 100% within 12 days. In the second challenge test, injected shrimp started to be killed 6 days after inoculation and cumulative mortality reached 60% with 15 days. The mortality of two control groups was 0%. After infection, the symptoms were similar to those shown by naturally infected shrimp. Cumulative mortality in two group reached over 95% and 60%, respectively.

Protein and Nucleic acid analysis

The analysis of virion protein consisting of 21 proteins were confirmed in the range of 14 to 190 kDa in molecular weight and this is very similar to virion protein of HHNBV (hypodermal and hematopoietic necrosis baculovirus)[2]

Table 1. Mortality of *Penaeus chinensis* by intramuscular injection with purified virus obtained from spontaneously diseased shrimp.

Experimental group	Shrimp used (N)	Dead shrimp (N) 3* 4 5 6 7 8 9 10 11 12 13 14 15	Total Mortality (%)
Injection A	10	2 2 1 1 2 1 1	100
Control A	10		0
Injection B	10	1 2 3 2 1 1	100
Control B	10		0

* Days after inoculation

Table 2. Mortality of *Penaeus chinensis* by feeding with purified virus obtained from spontaneously diseased shrimp.

Experimental group	Shrimp used (N)	Dead shrimp (N) 3* 4 5 6 7 8 9 10 11 12 13 14 15	Total Mortality (%)
Feeding A	10	2 1 1 1 1	100
Control A	10		0
Feeding B	10	1 1 1 2 1	100
Control B	10		0

* Days after inoculation

(Fig. 3). From results of *Bam*HI digestion of viral nucleic acid chromosome of shrimp virus was approximately 114 kb in length (Fig. 4). According to the data reported so far in Japan the nucleic acid of PRDV (penaeid rod-shaped DNA virus), cause virus of penaeid acute vireamia (PAV), which bring about a serious mortality of prawn, is 163 kb in length[5], in the Southeast Asian region the nucleic acid of SEMBV (systemic ectodermal and mesodermal baculovirus), which causes a serious mortality of *Penaeus monodon*, is 168 kb in length[14]. When compared to these two kinds of virus, the nucleic acid of the virus used in this experiment is a bit smaller in size. However since generally the nucleic acid of baculovirus is 90~230 kb in size, it is thought to be a kind of baculovirus. Therefore, according to the experiment result mentioned earlier the virus isolated in this study is WSBV and is thought to be very similar to PRDV which is the causal virus of shrimp PAV (penaeid acute viremia) isolated from prawn farms in Japan.

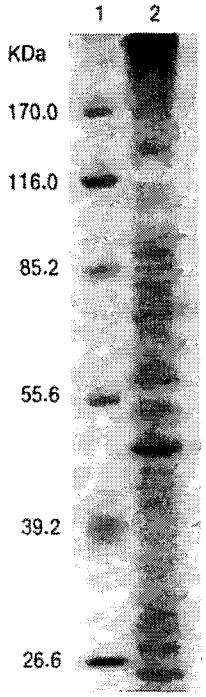

Fig. 3. SDS-polyacrylamide gel electrophoresis of viral proteins. Lane 1: Standard molecular weight marker. Land 2: Viral protein.

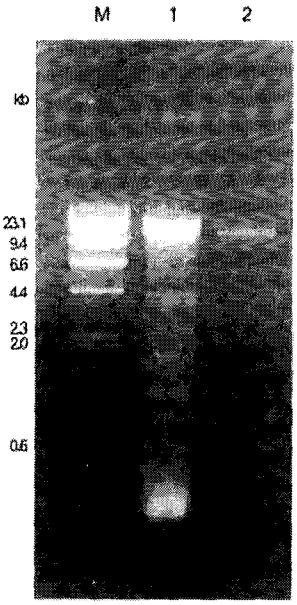

Fig. 4. Agarose gel electrophoresis of viral nucleic acids. Lane M: λ-Hind III marker. Lane 1: WSBV nucleic acids digested with Bam HI. Lane 2: undigested WSBV nucleic acids.

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초록 : 한국의 양식대하에서의 흰반점증상 바이러스감염의 특징

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대하 새우양식장의 대하에 흰반점 증상을 나타내는 원인바이러스는 막대형의 이중막을 가지고 있었으며, 전자 현미경 관찰 결과 평균 크기가 250~300×70 nm였고, 조직학적 병변은 위상피 등에서 핵이 비대해지는 것이 관찰되었다. 공격실험에서는 건강체의 새우에 많은 누적 폐사율을 보였다. 원인 바이러스 단백질은 21개의 밴드를 보였으며, 핵산 분석 결과 total 분자량은 114 kb로 나타났다.