Bacterial and Viral Diseases of Kuruma Shrimp (Penaeus japonicus) in Japan

Yukinori Takahashi, Toshiaki Itami, Minoru Maeda
and Masakazu Kondo

Department of Applied Aquabiology, National Fisheries University,
Shimonoseki, Yamaguchi 759-6595, Japan

(Received January 30, 1998)

Three major disease problems that have been reported in kuruma shrimp (Penaeus japonicus) culture in Japan are vibriosis, penaeid acute viremia (PAV) and baculoviral mid-gut gland necrosis (BMN).

Vibriosis in kuruma shrimp was first reported in 1985, and since then it has seriously damaged the culture of this species in Japan. The characteristic signs of this disease are brown spots in the lymphoid organs and in the gills, and cloudiness of muscle in the sixth abdominal segment. This bacterium was identified as a new species in the genus Vibrio and named Vibrio penaeicida.

Outbreaks of PAV, causing serious mortality of kuruma shrimp and being closely related to white spot baculovirus infection of penaeid shrimp, have been reported in Japan since 1993. Diseased shrimp show white spots in the carapace and reddish discoloration of the body. A non-occluded bacilliform virus, penaeid rod-shaped DNA virus (PRDV), was observed under the transmission electron microscope. In order to detect the virus, primers for the polymerase chain reaction (PCR) were developed. PCR has been successfully used for diagnosis and detection of carrier state crustaceans.

BMN is the epizootic of kuruma shrimp larvae in Japan. The typical sign of this disease is a white-turbid mid-gut gland that shows remarkable cellular necrosis and no inclusion body in the section. Rod-shaped particles, resembling baculovirus, are found in the affected nuclei under the electron microscope.

Attempts were made to evaluate the efficacy of immunostimulants to kuruma shrimp as potential agents for the prophylaxis of vibriosis and PAV. The oral administration of a peptidoglycan derived from Bifidobacterium thermophilum is effective in preventing these diseases in kuruma shrimp.

Key words: PRDV, PAV, WSSV, Vibrio penaeicida, vibriosis, BMNV

Introduction

The shrimp seed production technique and the shrimp culture system of kuruma shrimp were developed by M. Fudinaga and his colleagues in the early 1960s in Japan after many ground-breaking studies over the preceding 30 years. Their techniques have been spreading throughout the world and have helped the world shrimp culture industry grow enormously.

The shrimp industry in Japan had progressed without major disease problems until the early 1980s. In 1988, the annual production of kuruma shrimp in Japan was 3,020 metric tons, which was a peak, and then decreased to 1,519 tons in 1994, which is 50.3% of the peak production. The decrease in shrimp production until 1992 was mainly due to Vibrio penaeicida infection, which caused more than 60% of the total losses. In 1993, there was a drastic decrease in shrimp production due to introduction of shrimp seeds infected with penaeid rod-shaped DNA virus (PRDV) from China in that year. According to the results of our own survey, after this year, 60–80% of the total losses was caused by PRDV.

The shrimp seed production industry had suffered from considerable losses due to baculoviral mid-gut gland necrosis (BMN) (Arimoto et al., 1995). BMN virus (BMNV) has been extensively investigated by K. Momoyama and T. Sano, so reviewing their works would point out important areas for future research.
Vibriosis

Vibriosis in kuruma shrimp was first reported by Takahashi et al. (1985a) and its causative agent was characterized by de la Peña et al. (1993) and Ishimaru et al. (1995). The typical symptoms of this disease are brown spots in the lymphoid organs (Fig. 1) and in the gills, and cloudiness of the 6th abdominal segment. The most characteristic histopathological changes were extensive necrosis caused by severe bacterial invasion and multiple formation of melanized nodules in the lymphoid organs (Egusa et al., 1988).

The causative agent is a gram-negative and short-rod bacterium (Fig. 2), being classified as a member of Vibrio (Takahashi et al., 1985a), and was tentatively designated Vibrio sp. PJ by de la Peña et al. (1993). Ishimaru et al. (1995) determined that this bacterium is a new member of Vibrio and proposed the name Vibrio penaeicida. The LD50 value of the isolates was less than $1.35 \times 10^3 \text{--} 1.6 \times 10^4$ cells/g of body weight by injection (Takahashi et al., 1985a; Itami et al., 1989) or $1 \times 10^2 \text{--} 10^3 \text{ cfu/shrimp (13-22 g)}$ (de la Peña et al., 1993). This indicates an extremely high pathogenicity of this pathogen, compared with other vibrio species reported in penaeid shrimp (Jaravanichpaisal et al., 1994). With respect to the mode of oral infection, de la Peña et al. (1995) reported that the shrimp started dying from 48 h- and 72 h-post inoculation of pathogen which was administered orally to the shrimp at a concentration of $10^4 \text{ cfu/shrimp}$. The orally administered pathogen multiplied in the stomach in the early infection stage, and then grew in the hepatopancreas and lymphoid organs in the latter phase of infection resulting in systemic infection. These results suggest that the shrimp dying from vibriosis are a source of infection, since shrimp have a strong propensity for cannibalism.

A field survey of this bacterium was conducted by de la Peña et al. (1992) who detected the bacterium in the rearing water of the shrimp ponds and in apparently healthy shrimp by a conventional culture method. Recently, the technique of 16S rRNA-targeted reverse transcription polymerase chain reaction (RT-PCR) was developed to detect this bacterium with high sensitivity (a 10-fg detection limit) and species specificity (Genmoto et al., 1996), being verified to detect latent V. penaeicida in asymptomatic carrier kuruma shrimp (Nakai et al., 1997).

With respect to chemotherapy, we demonstrated the efficacy of oxytetracycline (OTC) tablets and showed that administration of 50-100 mg OTC/kg body weight/day for 4–6 days had excellent therapeutic effects (Takahashi et al., 1985b). In order to prevent the shrimp from getting the disease, attempts were made to develop vaccines (Itami et al., 1989, 1992a, b) and immunostimulants (Itami et al., 1994; Itami et al., 1998). A vaccine was prepared from formalin-killed cells of V. penaeicida and application of the vaccine by injection, immersion and spray techniques all reduced mortality of the shrimp challenged by V. penaeicida 30 days later. Hemocytes in contact with blood homogenate from immunized shrimp produced chemokinetic factor(s) that induced other hemocytes to migrate through a membrane in a Boyden chamber. Two types of immunostimulants, one was β-1,3-glucan prepared from Schizophillum commune (Itami et al., 1994) and the other was peptidoglycan derived from Bifidobacterium thermophilum (Itami et al., 1998), had a prophylactic efficacy against V. penaeicida infection after oral ad-
ministration for at least one week. The mode of action of immunostimulants is to activate the phagocytic activity of hemocytes, and activated hemocytes ingest and kill the invading microorganisms (Takahashi et al., 1995; Itami et al., 1994; Itami et al., 1998).

**Penaeid Acute Viremia**

Penaeid acute viremia (PAV) causes serious problems for kuruma shrimp farmers (Inouye et al., 1994, 1996; Takahashi et al., 1994, 1996) and was also reported in the seedling production of greasyback shrimp (Metapenaeus ensis) in Japan (Momoyama et al., 1997). The causative agent of PAV is the penaeid rod-shaped DNA virus (PRDV, formerly named RV-PJ). The infected shrimp show white spots in the carapace and reddish discoloration on the body. PRDV was first introduced from China when kuruma shrimp juveniles were imported into Japan and spread to cultured kuruma shrimp held in nearby grow-out ponds (Nakano et al., 1994). The major histopathological changes of PAV are nuclear hypertrophy in the infected cells in the tissues of mesodermal and ectodermal origin (Momoyama et al., 1994). Electronmicroscopy revealed long ovoid-shaped virions with a loosely surrounding envelope (Takahashi et al., 1994; Inouye et al., 1994). PRDV was proven to possess non-segmented, double-stranded DNA molecules of approximately 163 kbp, indicating that it is a member of the unassigned rod-shaped ds-DNA viruses which were previously classified in the Nudibaculovirinae, a subfamily of the Baculoviridae (Inouye et al., 1996).

Similar virus diseases, closely related to PAV, have been reported from Asian countries and the USA in different names (Chou et al., 1995; Huang et al., 1995; Wang et al., 1995; Wongteerasupaya et al., 1995; Lightner, 1996; Kasornchandra et al., 1998). These diseases are characterized by the presence of white spots in the carapace, hypertrophied nuclei of infected cells, and the rod-shaped morphology of the enveloped virion. The syndrome is generally known as white spot syndrome (WSS) and its agent(s) as white spot syndrome baculovirus (WSSV) (Durand et al., 1997).

The name WSSV is widely used in the world, while PRDV and PAV are the standardized names of fish diseases in Japan (Inui, 1996). Table 1 compares selected characteristics of the WSSVs. The sizes of the virions found in ultra-thin sections were different among the

<table>
<thead>
<tr>
<th>Viruses</th>
<th>Histological changes</th>
<th>Size of the virions (nm)</th>
<th>Tail-like structure</th>
<th>Restriction enzyme pattern</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cross section material</td>
<td>negatively stained material</td>
<td>EcoR I</td>
<td>BamH I</td>
</tr>
<tr>
<td>PRDV (RV-PJ)</td>
<td>hypertrophied nuclei</td>
<td>83 × 275</td>
<td>111 ± 10.7 × 374.9 ± 8.1</td>
<td>Yes</td>
<td>○</td>
</tr>
<tr>
<td>PRDV (RV-PJ)</td>
<td>hypertrophied nuclei</td>
<td>130 × –</td>
<td>152 ± 16.8 × 404 ± 25.6</td>
<td>No</td>
<td>○</td>
</tr>
<tr>
<td>WSSV or WSBV*3</td>
<td>hypertrophied nuclei, inclusion body</td>
<td>100 × 250</td>
<td>300–420 × 110–140</td>
<td>Yes</td>
<td>○</td>
</tr>
<tr>
<td>SEMBV</td>
<td>hypertrophied nuclei, inclusion body</td>
<td>111 ± 8 × 29 ± 29</td>
<td>121 ± 9 × 276 ± 26</td>
<td>Yes</td>
<td>○</td>
</tr>
<tr>
<td>HHNBV</td>
<td>hypertrophied nuclei</td>
<td>120 × 360</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

*1: Similar profile was found among the same symbols.
NT: Not tested.
–: Unable to measure.
viruses and those that had a negative stain were different among them. These differences might be attributed to different methods of preparing the samples and to the different measurement techniques being used (Durand et al., 1996). A tail-like structure was observed in all the virus strains except PRDV that was reported by Kimura et al. (1995) and Inouye et al. (1996). However, according to our observation of negatively stained PRDV virions, tail-like structures were observed in the purified viruses by sucrose continuous density gradient (Fig. 3). Although there were some confusions in the comparison of restriction enzyme analyses (Inouye et al., 1996; Durand et al., 1996; Lo et al., 1996), the patterns of EcoRI-digested DNAs of PRDV and WSBV are at least similar to SEMBV (K. Inouye and D. V. Lightner, personal communications). The DNA sequences of PCR products amplified from PRDV-infected shrimp DNA were identical to those amplified from SEMBV-infected shrimp DNA when the primers, which were developed from PRDV, were applied to both diseased shrimp (Maeda et al., unpublished data). The histopathological changes of infected organs listed in this table are similar among viruses of different names. These results indicate that these viruses are at least closely related if not identical.

For diagnosis of PAV, Momoyama et al. (1995) developed dark-field microscopic techniques for detecting the virus particles in the hemolymph with Brownian movement and the hypertrophied nuclei in stomach cuticular epidermis. These techniques are practical for the diagnosis for heavily infected shrimp when mass mortality occurred.

In order to detect the virus in the early infection stage, polymerase chain reaction (PCR) amplification was successfully applied (Kimura et al., 1996; Takahashi et al., 1996). Two-step PCR detected PRDV with high sensitivity (10 fg of genomic DNA) and high specificity in asymptomatic kuruma shrimp (T. Kimura, personal communication), in excretions from infected shrimp, and in other crustaceans in an infected shrimp culture pond (Kimura et al., 1996). The primer sets that we developed did not cross react with normal shrimp tissues and the major shrimp disease viruses, (e.g. BMNV, yellow-head disease virus, Baculovirus penaei), in Asia and Japan, and detected PRDV in kuruma shrimp and SEMBV in black tiger shrimp (Takahashi et al., 1996).

Attempts were made to detect the latent PRDV in shrimp and other crustaceans by 2-step PCR by Maeda et al. (1998a) to clarify whether the virus is vertically or horizontally transmitted. We examined wild-caught spawners collected from 4 ports in Kyushu island and found the percentage of PRDV-positive shrimp ranging from 13.3% to 55.0%, with an average of 25.3% (in total, 51 out of 202 shrimp tested positive). Forty-five of 272 immature shrimp (16.5%) were PRDV-positive by 2-step PCR. The percentage of males and females that were PRDV-positive were about the same (15.6% and 14.5%, respectively). With respect to the virus infection in crustaceans in shrimp ponds, 11 crustacean species (4 species of shrimp, 6 species of crabs and 1 species of mud shrimp, Upogebia major) out of 14 collected from kuruma shrimp ponds were found to be positive by 2-step PCR when the samples were obtained during and after an epizootic of PAV. The shore crab Helice tridens had an especially high frequency of positive reaction (66.7%, 26 out of 39 crabs). The virus detected by 2-step PCR was confirmed to be pathogenic by infection trials using injection, co-habitation and stress application. These trials showed that the latent PRDV in carrier states in shrimp and other crustaceans can be transferred both vertically and horizontally to cultured shrimp.

Information on the biological characteristics of PRDV is important for developing effective countermeasures. PRDV is inactivated by sodium hypochlorite (1 ppm for 30 min, at 10 ppm for 10 min), povidone-iodine (10 ppm for 30 min), a high concentration of NaCl (12.5%), heat treatment (at 50°C for 20 min), and desiccation (30°C for 1 h). PRDV in the seawater could maintain its pathogenicity for at least 120 days at 4°C, and the patho-

---

**Fig. 3.** Electronmicrograph of PRDV purified by sucrose continuous gradient. (Scale bar: 100 nm).
Shrimp diseases in Japan

361

genicity lasted more than 60 days but was lost by 120
days when kept at 25°C. However, PRDV suspended
at low concentration (a 10⁻⁷ dilution of the above solu-
tion) maintained its pathogenicity for only 7 days at 25°C
and for 10 days at 4°C. The singlet oxygen (¹Ο₂) which
is generated from an insoluble dye, rose bengal, by
irradiation of visible light inactivates the virus. These
results indicate that shrimp ponds, instruments and tools
can be disinfected by these treatments to prevent the
shrimp from becoming infected with PRDV. The ¹Ο₂
generating system is believed to have a high potential
for future use in the shrimp and fish culture industries
(Maeda et al., 1998b).

In an attempt to develop a prophylaxis against PAV,
the potency of an oral administration of peptidoglycan
(PG) derived from Bifidobacterium thermophilum was
examined. In that experiment, PG was administered
at a concentration of 0.2 mg/kg b.w./day in the feed for
at least 7 days and challenge tests were carried out by
the water-born method. The final survival rates of the
PG-fed groups were significantly higher than the survival
rate of the control group (p < 0.01). These results
revealed that oral administration of PG is effective in
preventing PAV in kuruma shrimp (Itami et al., 1998).

Baculoviral mid-gut gland necrosis

Several aspects of baculoviral mid-gut gland necrosis
virus (BMNV) have been described, including a DNA
analysis, transmission routes, diagnostic techniques,
countermeasures, and host ranges (Sano et al., 1981,
1985; Sano and Momoyama, 1992; Momoyama, 1983,
1988, 1989a, b, c, d; Momoyama and Sano, 1988, 1989,
1996; Arimoto et al., 1995).

BMN is the epizootic of kuruma shrimp larvae in
Japan during May to September (Sano et al., 1981).
The disease causes high mortality in laval and postlarval
stages. The susceptibility to infection tends to decrease
with advancing stages of development from the zoea
stage to the 10-day-old postlarval stage (Sano et al.,
1985; Momoyama and Sano, 1989). Typical gross sign
of the disease is a white-turbid mid-gut gland in an
advanced stage of infection. The pathological changes
are remarkable cellular necrosis and collapse of the mid-
gut gland, accompanying hypertrophied nuclei of the
mid-gut gland epithelium. The tissue sections contain
no inclusion bodies. Numerous rod-shaped particles,
resembling baculovirus, can be seen in the affected
nuclei under the electron microscope. The average
length and diameter of the virions are 310 nm and 72
nm, respectively, and the average length of the nucleo-
capsids is 250 nm (Sano et al., 1981). Arimoto et al.
(1995) reported that the viral genomic DNA is digested
with BamHI and Sau3AI, and that the molecular weight
is approximately 85.1 × 10⁶ Da. They proposed to
name this “PjNOB”, according to the guidelines by the
International Committee on Taxonomy of Viruses
(Murphy et al., 1995).

Rapid and simple diagnostic techniques, using fresh
squash preparation or stained squash preparation of the
mid-gut gland, were developed for presumptive diagno-
sis (Momoyama, 1983). BMNV is inactivated by
hypochlorite (5 ppm), povidone-iodine (25 ppm),
formalin (0.5%), ethanol (30%), benzalkonium
chloride (100 ppm), benzethonium chloride (100 ppm)
(Momoyama, 1989a), and ethyl ether (Momoyama,
1989c). Ultraviolet irradiation (4.1 × 10⁵ μW • sec/ cm²),
sunlight exposure (3-h exposure to summer sunlight),
heat treatment (45°C within 120 min) and desic-
cation (1.5 h) inactivate the virus (Momoyama, 1989b).
BMNV in the seawater losess its pathogenicity within 4
days at 30°C, and within 20 days at 15°C (Momoyama,
1989d). With respect to the host range of BMNV, P.
monodon larvae are demonstrated to have a high sus-
ceptibility to the virus that is nearly as high as that of P.
japonicus larvae. The larvae of P. chinensis and P.
semisulcatus have lower susceptibility, showing no
growth retardation and no significant mortality. How-
ever, Metapenaeus ensis and Portunus trituberculatus
did not show any evidence of infections (Momoyama
and Sano, 1996).

According to the epizootiological investigations, la-
tently infected spawners and cultured shrimp are the
sources of infection for the vertical and the horizontal
transmission routes (Momoyama, 1988). BMNV is
contained in the excretions of spawners and acts as an
infection source. In order to prevent the larvae from
becoming infected with BMNV, Sano and Momoyama
(1992) developed a technique for rinsing eggs and ap-
plicated this to the seed production program. This resulted
in no outbreak of BMN in the facilities where this tech-
nique was used. Therefore, this rinsing treatment is
highly effective in preventing the virus infection.

Conclusions

Shrimp culture in Japan has been seriously damaged
since 1988, when the maximum production was
achieved. Three major diseases, vibriosis, PAV and
BMN, are the primary causes of the decrease in produc-
tion, and are of a great concern to the shrimp culture industry.

To control vibriosis, oxytetracycline and oxolinic acid are available in Japan and widely used. To avoid the appearance of resistant strains to these two drugs, the proper use of these drugs should be strongly recommended.

In order to reduce the incidence of PAV and BMN, and other virus diseases, avoiding exposure to the virus is most important. Therefore, an early and accurate diagnosis is needed to detect the virus in latently infected shrimp and in the virus-carrying crustaceans before the explosive infection occurs. In this respect, PCR and 2-step PCR are the most useful methods for PRDV detection in hatcheries and grow-out farms.

Even though these effective detection methods are available, it should be emphasized that the close attention needs to be paid to the basic techniques for shrimp health management and shrimp pond management. For example, the quality of the bottom sand can remarkably affect shrimp health conditions, as well as water quality.

Establishment of disease-resistant shrimp strains will provide one possible solution to the disease problems that are now threatening the world shrimp industry. Because artificial breeding techniques of kuruma shrimp have not been sufficiently developed yet, this will be an important area for future research.

References


