Effect of Various Treatments on White Spot Syndrome Virus (WSSV) from *Penaeus japonicus* (Japan) and *P. monodon* (Thailand)

Minoru Maeda^{*1}, Jiraporn Kasornchandra^{*2}, Toshiaki Itami^{*1}, Nobutaka Suzuki^{*3},
Oscar Hennig^{*4}, Masakazu Kondo^{*1}, Juan D. Albaladejo^{*5}
and Yukinori Takahashi^{*1}

^{*1}Department of Applied Aquabiology, National Fisheries University,
Shimonoseki, Yamaguchi 759-6595, Japan

^{*2}National Institute of Coastal Aquaculture, Songkla 90000, Thailand

^{*3}Department of Food Science and Technology, National Fisheries University,
Shimonoseki, Yamaguchi 759-6595, Japan

^{*4}Faculty of Fisheries, Nagasaki University, Nagasaki 852-8131, Japan

^{*5}Bureau of Fisheries and Aquatic Resources, Department of Agriculture,
Quezon, Metro Manila 3008, Philippines

(Received February 27, 1998)

Two causative agents of white spot syndrome (WSS), penaeid rod-shaped DNA virus (PRDV) from infected kuruma shrimp (*Penaeus japonicus*) in Japan and systemic ectodermal and mesodermal baculovirus (SEMBV) from black tiger shrimp (*P. monodon*) in Thailand, were tested for their sensitivities to chemicals, temperature, drying and singlet oxygen (¹O₂). The infectivity of the treated PRDV and SEMBV was determined by challenge tests in kuruma shrimp and black tiger shrimp, respectively. Sodium hypochlorite inactivated PRDV at 1 ppm for 30 min and at 5 ppm for 10 min. SEMBV was inactivated by sodium hypochlorite at 10 ppm for 30 min. Povidone-iodine inactivated these viruses at a concentration of 10 ppm for 30 min. A high concentration of NaCl (12.5%) inactivated PRDV in 24 h at 25°C, and 15% NaCl inactivated SEMBV in 24 h at 28°C. PRDV was inactivated by heating at 50°C for 20 min, by drying at 30°C, and by using ethyl ether. PRDV in the sea water maintained its infectivity for at least 120 days at 4°C, and for more than 60 days but less than 120 days when kept at 25°C. However, PRDV, when suspended in sea water at a lower concentration, maintained infectivity for 10 days at 4°C and for 7 days at 25°C. SEMBV maintained infectivity in sea water for 5 days at 28°C. PRDV was exposed to ¹O₂, which was generated by an insoluble dye (rose bengal) and visible light. Shrimp that received ¹O₂-exposed virus showed lower mortality than control shrimp, indicating that ¹O₂ inactivated PRDV. This ¹O₂ generating system is a simple, safe and effective technique that can be applied to aquaculture.

Key words: PRDV, SEMBV, WSSV, disinfectant, singlet oxygen, *Penaeus japonicus*, *Penaeus monodon*

Penaeid acute viremia (PAV) caused by penaeid rod-shaped DNA virus (PRDV) was first observed in the early spring of 1993 in cultured kuruma shrimp *Penaeus japonicus* in Japan (Inouye *et al.*, 1994, 1996; Takahashi *et al.*, 1994). This infection causes serious mortality accompanied by symptoms of white spots on the inside of the carapace, and reddish discoloration of the body. Similar virus infections were found in black tiger shrimp *P. monodon* in Thailand, where the

causative agent of this infection was named systemic ectodermal and mesodermal baculovirus (SEMBV) (Wongteerasupaya *et al.*, 1995). PRDV (formerly named RV-PJ) was demonstrated to be closely related to SEMBV because both viruses could be detected by PCR using the DNA primers developed from PRDV (Takahashi *et al.*, 1996) and the sequences of the PCR products were identical for both viruses (unpublished data). So far, closely related viruses have been described in the literature under various names including hypodermal and hematopoietic necrosis virus (Huang *et al.*, 1995) and white spot baculovirus (Chou *et al.*, 1995; Wang *et al.*, 1995). These are now classified as

Address for Correspondence: Dr. Toshiaki Itami, 2-7-1, Nagata-Honmachi, Shimonoseki, Yamaguchi 759-6595, Japan
Fax :+81-832-86-7435
E-mail: itamit@fish-u.ac.jp

unassigned viruses, but were previously classified in the Nudibaculovirinae, a subfamily of the Baculoviridae (Murphy *et al.*, 1995). It has been proposed that this group of closely related viruses be called the white spot syndrome virus (WSSV) group (Nunan and Lightner, 1997). WSSV has been reported to infect many penaeid species and other arthropods (Lo *et al.*, 1996; Maeda *et al.*, 1997; Momoyama *et al.*, 1997; Wang *et al.*, 1997; Kasornchandra *et al.*, 1998). In this article, we will refer to the WSSV group viruses in Japan and Thailand as PRDV and SEMBV, respectively. Information on the biological characteristics of the WSSV group is important for prevention and treatment. Accordingly, the present study was undertaken to develop effective countermeasures for controlling PRDV and SEMBV in penaeid shrimp culture.

Materials and Methods

Viral preparation

PRDV-infected shrimp, weighing an average of 12 g, were obtained from a farm located in Yamaguchi Prefecture, Japan, and SEMBV-infected shrimp, weighing an average of 26 g, were collected from a farm located in Songkla, Thailand. Lymphoid organs of 3 infected shrimp were collected, homogenized in 3 ml sterilized sea water and centrifuged at $10,000 \times g$ for 10 min at 4°C. The supernatant was filtered through a 0.45 µm membrane filter and the filtrate was used as the viral preparation (VP) stock for the experiments. VP stock was prepared individually for each experiment.

Virucidal effect of sodium hypochlorite and povidone-iodine

VP stock (0.1 ml) was added to 50 ml sterilized sea water followed by addition of sodium hypochlorite and povidone-iodine to achieve final concentrations of active ingredients at 0, 1, 5, 10, 25 and 50 ppm for sodium hypochlorite, and at 0, 1, 5, 10, 20, 50 and 100 ppm for povidone-iodine. The preparations were incubated at 25°C for 10 min or 30 min for PRDV, and at 28°C for 30 min for SEMBV. The preparations were then neutralized with N/100 or N/1000 sodium thiosulfate and injected into shrimp (*P. japonicus* for PRDV and *P. monodon* for SEMBV) for challenge trials.

Inactivation by heat treatment

VP stock (0.1 ml) was added to 10 ml sterile sea water which was previously filtered through a 0.20 µm membrane and the preparations were heated at 25, 40, 50, 60,

70 or 80°C for 20 min, and then the cooled samples were used for the challenge trials. Only PRDV was used in this experiment.

Inactivation by drying

VP stock (0.1 ml) was absorbed to sterile filter paper (Toyo No. 2, Toyo Roshi Kaisha, Ltd, Japan). The filter paper was then incubated at 30°C for 0, 0.5, 1.0, 1.5 and 2.0 h, before being cut into small pieces and immersed in 50 ml sterile sea water with stirring for 10 min. The paper suspensions were subsequently centrifuged and the supernatant was used for the challenge tests. As a control, VP stock (1 ml) in a stoppered test tube was incubated at 30°C for h. Immediately after 0.1 ml of this solution was absorbed to sterile filter paper, the paper was cut into small pieces and the supernatant for challenge tests was prepared in the same procedure mentioned above. Only PRDV was used in this experiment.

Tolerance to ethyl ether

VP stock (4.0 ml) was added to 1.0 ml ethyl ether and incubated at 4°C for 18 h. Sterile sea water was used to replace ethyl ether for the control. After incubation, reaction mixtures were stirred sufficiently to remove ethyl ether and were used for the challenge tests. Only PRDV was used in this experiment.

Tolerance to NaCl

VP stock (0.1 ml) was added to 50 ml of distilled water containing NaCl at concentrations ranging from 0.03 to 25.0%. The mixtures were incubated at 25°C for 24 h. Each solution was subsequently diluted 1:10 with sterile sea water before being used for the challenge tests.

Duration of infectivity in sea water

VP stock (1.0 ml) of PRDV was placed in sterile test tubes and maintained at 4°C or 25°C. The tube contents were sampled periodically for use in challenge tests. Diluted VP stock (10^{-7}) was treated in the same way and also sampled periodically for use in challenge tests.

Inactivation by singlet oxygen (1O_2)

VP stock (1.0 ml) of PRDV was mixed with 4 mg of rose bengal (an insoluble, immobilized dye) and aerated for 10 min as a pretreatment. The mixture was then exposed to visible light for 20 min with aeration. By this irradiation, 1O_2 , a molecular species of active oxy-

gen with killing activity against microorganisms, was generated from the dye. As a control to examine the toxicity of $^1\text{O}_2$ and trace dye dissolved in sea water, a suspension of the dye without the virus was exposed to light. These samples were then used for challenge tests and the results were analyzed by χ^2 tests. Only PRDV was used in this experiment.

Challenge tests with treated virus solutions

The shrimp used in this study were obtained from a shrimp culture farms where PAV or SEMBV was not endemic. The average body weights of kuruma shrimp and black tiger shrimp were 9 g and 30 g, respectively. Treated viral preparations and control solutions were injected into the muscle of the third abdominal segment at a dose of 1% (v/w) of shrimp body weight. The injected kuruma shrimp were subsequently kept in aquaria at 21–23°C and black tiger shrimp were kept at 28–30°C. Mortality was monitored every day for 10 days after injection. Both species were fed commercial diets. The experiments on kuruma shrimp and black tiger shrimp were carried out in Japan and Thailand, respectively.

Results and Discussion

PAV is the second virus infection to cause serious economic loss in Japan. The first virus infection was baculoviral mid-gut gland necrosis (BMNV) (Sano *et al.*, 1981), which affected mysis and post-larvae of kuruma shrimp (Momoyama *et al.*, 1988). On the other hand, there are many viruses that cause economic losses in Thailand, such as hepatopancreatic parvo-like virus (HPV), monodon baculovirus (MBV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and WSSV (Flegel, 1997). However, only limited information is available on the biological characteristics of these viruses.

The effects of various disinfectants and treatments on BMNV were investigated in detail by Momoyama (1989a, b, c, d). He determined the effects of these disinfectants by observation of the typical disease sign developed in kuruma shrimp challenged with the treated virus. In the present study, the effects were examined based on the difference in mortality rates of shrimp between treated-virus-injected group and control. Generally, injection of the PRDV or SEMBV can result in nearly 100% mortality within 4–6 days because of their high pathogenicity. We, therefore, used survival rate of challenged shrimp to evaluate the efficacy of the

treatments.

Due to the lack of the cell lines to grow PRDV and SEMBV, it is not possible to measure viral concentrations and to standardize the initial preparations of VP stock. Hence, a comparison between these viruses in sensitivity to various treatments is not appropriate in this experiment.

Virucidal effects of sodium hypochlorite and povidone-iodine

PRDV was inactivated by a 10-min exposure to 5 ppm sodium hypochlorite, and by a 30-min exposure to 1 ppm sodium hypochlorite. SEMBV was inactivated by a 30-min exposure to 10 ppm sodium hypochlorite (Table 1). A 30-min exposure to 10 ppm povidone-iodine inactivated PRDV and SEMBV (Table 2).

Inactivation by heat treatment

PRDV lost its infectivity by being heated at 50°C or higher for 20 min in sterile sea water (Table 3).

Inactivation by drying

The water content of the filter paper that had absorbed the virus suspension decreased from 86.2% to 3.4% within 30 min during drying treatment at 30°C (Table 4). The virus absorbed in the filter paper was inactivated within 1 h at 30°C but not in 30 min, although the water content in both filter papers was the same. The control, in which the virus was kept in sterile sea water at 30°C for 1 h, showed high pathogenicity.

Table 1. Virucidal effects of sodium hypochlorite on PRDV and SEMBV

Concentration (ppm)	Number of shrimp dead		
	PRDV*		SEMBV**
	10 min	30 min	30 min
50	0	0	0
25	0	NT	0
10	0	0	0
5	0	0	1
1	NT	0	4
0	8	8	10

* The temperature during the treatment was 25°C. PRDV was injected into 10 kuruma shrimp in each test.

** The temperature during the treatment was 28°C. SEMBV was injected into 10 black tiger shrimp in each test.

NT: not tested.

Table 2. Virucidal effects of povidone-iodine on PRDV and SEMBV

Concentration of povidone-iodine* (ppm)	Number of shrimp dead	
	PRDV **	SEMBV ***
100	0	0
50	0	0
10	0	0
5	7	6
1	8	8
0	10	10

* Exposure time was 30 min.

** Water temperature during the treatment was 25°C. PRDV was injected into 10 kuruma shrimp in each test.

*** Water temperature during the treatment was 28°C. SEMBV was injected into 10 black tiger shrimp in each test.

Table 3. Effect of heat treatment on the infectivity of PRDV to kuruma shrimp

Temperature (°C)	Number of shrimp dead
	PRDV*
25	10
40	10
50	0
60	0
70	0
80	0

* PRDV was exposed to the temperature for 20 min. PRDV was injected into 10 kuruma shrimp in each test.

Table 4. Effect of drying treatment on the infectivity of PRDV to kuruma shrimp

Drying time* (hour)	Water content in filter paper (%)	Number of shrimp dead	
		Dry-treatment	Control
0	86.2	10	NT
0.5	3.4	2	NT
1.0	3.4	0	10
1.5	3.4	0	NT
2.0	3.4	0	NT

* The samples were placed in an oven at 30°C, and injected into 10 kuruma shrimp in each test.
NT: not tested*Tolerance to ethyl ether*

Ethyl ether-treated PRDV lost its infectivity (Table 5). Generally, a virus having an envelope loses its pathogenicity when treated with ethyl ether because of the fat-soluble envelope, and this agrees with the existence of an envelope in PRDV (Takahashi *et al.*, 1994). BMNV was also sensitive to ethyl ether (Momoyama, 1989c).

Tolerance to NaCl

NaCl concentrations of 12.5% or higher inactivated PRDV and NaCl concentrations of 15.0% or higher inactivated SEMBV in 24 h at 25°C (Table 6). It is clear that both viral types are sensitive to high concentrations of NaCl.

Table 5. Effect of diethyl ether on the infectivity of PRDV to kuruma shrimp

Treatment*	Number of shrimp dead
Yes	0
No	10

* Virus was treated with 20% diethyl ether in sterile sea water for 18 at 4°C, and injected into 10 kuruma shrimp in each test.

Table 6. Effect of NaCl concentration on the infectivity of PRDV and SEMBV to kuruma shrimp and black tiger shrimp, respectively

NaCl concentration* (%)	Number of shrimp dead	
	PRDV**	SEMBV***
0.03	7	9
0.3	7	NT
0.5	NT	10
1.0	NT	10
3.0	10	10
6.0	6	7
12.5	0	NT
15.0	NT	0
25.0	0	0

* The viruses were exposed to different concentrations of NaCl for 24 h.

** The temperature during the treatment was 25°C. PRDV was injected into 10 kuruma shrimp in each test.

*** The temperature during the treatment was 28°C. SEMBV was injected into 10 black tiger shrimp in each test.

NT: not tested.

Duration of infectivity in sea water

VP stock of PRDV maintained its infectivity in the sea water for at least 120 days at 4°C (Table 7). When the same preparations of virus was kept at 25°C, the infectivity lasted more than 60 days but was lost by 120 days (Table 7). By contrast, a 10^{-7} dilution of the VP stock of PRDV maintained its pathogenicity for only 10 days at 4°C and for 7 days at 25°C. A 10^{-7} dilution of the VP stock of SEMBV maintained its pathogenicity for 5 days at 28°C (Table 8). This result suggests that these viruses in sea water can not keep their infectivities longer than 5–7 days, and that they are easily inactivated in sea water when their concentration is low. However, at high concentration, PRDV can maintain its

infectivity against shrimp for at least 60 days at 25°C or for 120 days at 4°C.

Inactivation by single oxygen (1O_2)

In an attempt to develop a new technique to inactivate PRDV, we exposed it to singlet oxygen (1O_2) that was generated from rose bengal upon irradiation with visible light (Gollnick, 1968; Suzuki *et al.*, 1990). The mortality rate of the shrimp that received the 1O_2 -treated virus was significantly lower than that of the control shrimp ($P < 0.001$) (Table 9), indicating that 1O_2 inactivated PRDV. Recently, application of UV light irradiation and ozonation have been developed for disinfecting sea water for aquaculture (Brown and Russo, 1979; Kobayashi *et al.*, 1993). These techniques have been demonstrated to be effective in inactivation of fish pathogens (Sugita *et al.*, 1992). However, the equipment for both methods is expensive in initial investment. Moreover, ozonation produces residual oxidants coupled with Br and Cl that are toxic to animals and have a long half-life. Hence, these toxic residues in sea water must be removed by activated charcoal before the sea water is transferred to aquaria (Sugita *et al.*, 1992). On the other hand, the 1O_2 used in this study was proven to be an effective disinfectant, having a short half-life (Saito and Matsugo, 1988), and the process is simple and inexpensive. Therefore, we believe that this 1O_2 generating system has a high potential for future application at least to shrimp and fish hatcheries where they use smaller volume of water. The proportion of dye to water volume used in this experiment was rather large and the direct application of this proportion would be difficult for grow-out ponds. If we could find more efficient dye that can produce more 1O_2 with smaller

Table 7. Duration of PRDV infectivity in sea water against kuruma shrimp

Periods* (days)	Number of shrimp dead	
	4°C	25°C
0	10	10
5	10	10
10	10	10
15	10	10
30	10	9
60	10	6
120	10	0

* PRDV sea water suspensions prepared from homogenates of infected lymphoid organs were kept at 4°C or 25°C, and injected into 10 shrimp after indicated periods.

Table 8. Duration of PRDV and SEMBV infectivity when suspended at lower concentrations in sea water

Period* (days)	Number of shrimp dead		
	PRDV**		SEMBV***
	4°C	25°C	28°C
0	10	10	10
1	6	6	10
3	10	7	8
5	8	6	2
7	10	4	0
10	6	0	0
15	0	0	0

* The virus concentration used in this experiment was a 10^{-7} dilution of the original solution used in the previous experiment (Table 7).

** PRDV was injected into 10 kuruma shrimp.

*** SEMBV was injected into 10 black tiger shrimp.

Table 9. Effect of singlet oxygen (1O_2) on the infectivity of PRDV against kuruma shrimp

Treatment*	1O_2 generation**	Number of shrimp (No. dead/No. tested)
PRDV + rose bengal + visible light	Yes	4/21 ^a
PRDV + visible light	No	22/22 ^b
PRDV + rose bengal	No	22/22 ^b
rose bengal + visible light	Yes	4/21 ^a

* Treatment was done at room temperature.

** 1O_2 was generated from rose bengal by irradiation of visible light.

a, b: Significant differences between different letters ($P < 0.001$).

Table 10. Comparison of inactivation conditions between WSSV (PRDV, SEMBV) and BMNV

Treatments	Reaction conditions	PRDV*	SEMBV*	BMNV**
Sodium hypochlorite	10 min	5 ppm	NT	5 ppm
	30 min	1 ppm	10 ppm	NT
Povidone-iodine	10 min	NT	NT	25 ppm
	30 min	10 ppm	10 ppm	NT
Heat	50°C	20 min	NT	30 min
Drying	30°C	1.0 h	NT	1.5 h
NaCl concentrations	24 h	12.5%	15.0%	12.5%
Duration	28°C	NT	7 days	NT
	25°C	10 days	NT	7 days
	4°C	15 days	NT	NT

NT: not tested

* Present study

** Momoyama (1989a, b, c, d)

amounts of dye, this method would become practical for aquaculture industries. We need to collect more information on the quality of the dye, the intensity of the light and the $^1\text{O}_2$ itself in shrimp culture ponds with heterotrophic populations of microorganisms and high concentrations of organic materials.

The present results showed that PRDV and SEMBV are sensitive to disinfectants, drying, high temperature and high concentrations of NaCl. Table 10 summarizes the inactivation conditions for WSSV (PRDV, SEMBV) and BMNV (Momoyama, 1989a, b, c, d). As mentioned in the previous section, the comparisons among these viruses are not possible because of the inability of the quantification of these viruses. These viruses, however, were inactivated by similar conditions, even though the concentration of these viruses were not standardized. In general, shrimp ponds and instruments are tainted by organic materials originated from residual feeds, feces, and decomposed aquatic animals and plants. Therefore, the concentration of the disinfectants for practical use in shrimp farms should be higher than those mentioned in this paper because organic materials reduce the virucidal effect of these chemicals.

References

- Brown, C and D. J. Russo (1979): Ultraviolet light disinfection of shellfish hatchery sea water. I. Elimination of five pathogenic bacteria. *Aquaculture*, **17**, 17–23.
- Chou, H-Y., C-Y. Huang, C-H. Wang, H-C. Chiang and C-F. Lo (1995): Pathogenicity of a baculovirus infection causing white spot syndrome in cultured penaeid shrimp in Taiwan. *Dis. Aquat. Org.*, **23**, 165–173.
- Flegel, T. W. (1997): Major viral disease of the black tiger prawn (*Penaeus monodon*) in Thailand, In “New approaches to viral diseases of aquatic animals”, NRIA International Workshop, National Research Institute of Aquaculture, Nansei, Mie, Japan, pp. 218–228.
- Gollnick, K. (1968): Photooxygenation reactions in solution. In “Advances in photochemistry” (ed. by W. A. Noyes Jr *et al.*). John Wiley & Sons, New York, pp. 1–122.
- Huang, J., X. Song, J. Yu and C. Yang (1995): Baculoviral hypodermal and hematopoietic necrosis-study on the pathogen and pathology of the explosive epidemic disease of shrimp. *Mar. Fish. Res.*, **16**, 1–10. (In Chinese)
- Inouye, K., S. Miwa, N. Oseko, H. Nakano, T. Kimura, K. Momoyama and M. Hiraoka (1994): Mass mortality of cultured kuruma shrimp *Penaeus japonicus* in Japan in 1993: Electron microscopic evidence of the causative virus. *Fish Pathol.*, **29**, 149–158.
- Inouye, K., K. Yamano, N. Ikeda, T. Kimura, H. Nakano, K. Momoyama, J. Kobayashi and S. Miyajima (1996): The penaeid rod-shaped DNA virus (PRDV), which causes penaeid acute viremia (PAV). *Fish Pathol.*, **31**, 39–45.
- Kasornchandra, J., S. Boonyaratpalin and T. Itami (1998): Detection of white spot syndrome in cultured penaeid shrimp in Asia: Microscopic observation and polymerase chain reaction. *Aquaculture*, **164**, 243–251.
- Kobayashi, T., H. Yotsumoto, T. Ozawa and H. Kawahara (1993): Closed circulatory system for mariculture using ozone. *Ozone Sci. Eng.*, **15**, 311–330.
- Lo, C-F., C-H. Ho, S-E. Peng, C-H. Chen, H-C. Hsu, Y-L. Chiu,

- C-F. Chang, K-F. Liu, M-S. Su, C-H. Wang and G-H. Kou (1996): White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. *Dis. Aquat. Org.*, **27**, 215–225.
- Maeda, M., T. Itami, M. Kondo, O. Hennig, Y. Takahashi, I. Hirano and T. Aoki (1997): Characteristics of penaeid rod-shaped DNA virus of kuruma shrimp. In “New approaches to viral diseases of aquatic animals”, NRIA International Workshop, National Research Institute of Aquaculture, Nansei, Mie Japan, pp. 218–228.
- Momoyama, K. and N. Sano (1988): A method of experimental infection of kuruma shrimp larvae, *Penaeus japonicus* Bate, with baculoviral mid-gut gland necrosis (BMN) virus. *J. Fish. Dis.*, **11**, 105–111.
- Momoyama, K. (1989a): Virucidal effect of some disinfectants on baculoviral mid-gut gland necrosis (BMN) virus. *Fish Pathol.*, **24**, 47–49.
- Momoyama, K. (1989b): Inactivation of baculoviral mid-gut gland necrosis (BMN) virus by ultraviolet irradiation, sunlight exposure, heating and drying. *Fish Pathol.*, **24**, 115–118.
- Momoyama, K. (1989c): Tolerance of baculoviral mid-gut gland necrosis (BMN) virus to ether, NaCl concentration and pH. *Fish Pathol.*, **24**, 175–177.
- Momoyama, K. (1989d): Survival of baculoviral mid-gut gland necrosis virus (BMNV) in infected tissues and in sea water. *Fish Pathol.*, **24**, 179–181.
- Momoyama, K., M. Hiraoka, K. Inouye, T. Kimura, H. Nakano and M. Yasui (1997): Mass mortalities in the production of juvenile greasyback shrimp, *Metapenaeus ensis*, caused by penaeid acute viremia (PAV). *Fish Pathol.*, **32**, 51–58.
- Murphy, F. A., C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo and M. D. Summers (1995): Virus taxonomy, 6th report of the international committee on taxonomy of viruses. *Arch. Virol. Supplementum* **10**, 1–586.
- Nunan, L. M. and D. V. Lightner (1997): Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV). *J. Virol. Methods*, **63**, 193–201.
- Saito, I. and S. Matsugo (1988): Chemistry of active oxygen species. *Protein, Nucleic Acid and Enzyme*, **33**, 2665–2677. (In Japanese)
- Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno (1981): Baculovirus infection of cultured kuruma shrimp *Penaeus japonicus* in Japan. *Fish Pathol.*, **15**, 185–191.
- Sugita, H., T. Asai, K. Hayashi, T. Mitsuya, K. Amanuma, C. Maruyama and Y. Deguchi (1992): Application of ozone disinfection to remove *Enterococcus seriolicida*, *Pasteurella piscicida* and *Vibrio anguillarum* from seawater. *Appl. Environ. Microbiol.*, **46**, 1157–1162.
- Suzuki, N., T. Nomoto, I. Misumoto, M. Kobayashi, M. Wada, B. Wada and H. Inaba (1990): Chemiluminescence of luminol with singlet oxygen generated from a rose bengal system. *Chem. Express*, **5**, 505–508.
- Takahashi, Y., T. Itami, M. Kondo, M. Maeda, R. Fujii, S. Tomonaga, K. Supamattaya and S. Boonyaratpalin (1994): Electron microscopic evidence of bacilliform virus infection in kuruma shrimp (*Penaeus japonicus*). *Fish Pathol.*, **29**, 121–125.
- Takahashi, Y., T. Itami, M. Maeda, N. Suzuki, J. Kasornchandra, K. Supamattaya, R. Khongpradit, S. Boonyaratpalin, M. Kondo, K. Kawai, R. Kusuda, I. Hirano and T. Aoki (1996): Polymerase chain reaction (PCR) amplification of bacilliform virus (RV-PJ) DNA in *Penaeus japonicus* Bate and systemic ectodermal and mesodermal baculovirus (SEMBV) DNA in *Penaeus monodon* Fabricius. *J. Fish. Dis.*, **19**, 399–403.
- Wang, C-H., C-F. Lo, J-H. Leu, C-M. Chou, P-Y. Yeh, H-Y. Chou, M-C. Tung, C -F. Chang, M-S. Su and G-H. Kou (1995): Purification and genomic analysis of baculovirus associated with white spot syndrome (WSBV) of *Penaeus monodon*. *Dis. Aquat. Org.*, **23**, 239–242.
- Wang, C-S., Y-J. Tsai, G-H. Kou and S-N. Chen (1997): Detection of white spot disease virus infection in wild-caught greasy back shrimp, *Metapenaeus ensis* (de Haan) in Taiwan. *Fish Pathol.*, **32**, 35–41.
- Wongteerasupaya, C., J. E. Vickers, S. Sriurairatana, G. L. Nash, A. Akarajamorn, V. Boonsaeng, S. Panyim, A. Tassanakajon, B. Withyachumnarnkul and T. W. Flegel (1995a): A non-occluded, systemic baculovirus that occurs in cells of ectodermal and mesodermal origin and causes high mortality in the black tiger prawn *Penaeus monodon*. *Dis. Aquat. Org.*, **21**, 69–77.