Promising strategies against WSSV for

kuruma shrimp in Japan

Kuruma shrimp, also called kuruma prawn or kurumaebi (*Marsupenaeus japonicus*) is one of Japan's most favored shrimps.

A native to the Indian Ocean and southwestern Pacific Ocean from Japan to Australia, these tasty creatures are chiefly consumed by the Japanese who utilize them in, among other things, a dish called dancing shrimp because the shrimps are served live. In Japan, live kuruma shrimp can fetch as much as \$100 per kilogram.

A hardy adaptable variety of shrimp, kuruma shrimp were among the first shrimp species to be cultured. However, outbreaks of the white spot syndrome (WSS) have been causing serious mortality to kuruma shrimp culture in Japan since 1993. Production declined from 3020 metric tons in 1988 to 1500 metric tons in 1994.

In 1993, about 80 % of kuruma shrimp production loss in Japan was due to white spot syndrome virus (WSSV) infection. The

loss was estimated to be US\$ 20 million. Other losses recorded were due to *Vibrio*, *Fusarium*, and other unidentified infections.

WSSV can be easily detected by routine examination using 2-step PCR even in its early state of infection, but, it is highly pathogenic and can be easily transmitted either through vertical (spawners to eggs) or horizontal (from other crustaceans and shrimp to shrimp) mode of transmission.

On the contrary, the use of prophylaxis was recently proven to be effective against WSSV infection. Dr. Toshiaki Itami of the Department of Aquabiology, National Fisheries University, Japan recently developed a new prophylaxis strategy for WSSV: oral administration of peptidoglycan and fucoidan in shrimp diets.

Use of peptidoglycan (PG)

Peptidoglycan (PG) is an immunostimulant derived from *Bifidobacterium thermophilum*. Oral administration of PG was found to enhance the defense activity of kuruma shrimp against WSSV thus making it more resistant to infections.

In order to confirm the prophylactic efficacy of peptidoglycan to kuruma shrimp, Dr. Itami fed juvenile shrimp with PG at a concentration of 0.2mg/kg body weight/day with a 7-day intermittent schedule in which PG was fed for 4 days followed by 3 days of control diet (diet with no PG).

This was administered for 30 days. Shrimp were challenged with WSSV in this period by being exposed to a WSSV-infected effluent seawater.

Survival rate of PG-fed shrimp was 97.6%, whereas survival for the control diet was only 19%. The latent virus was monitored using 2-step PCR. Examinations showed that the shrimps were still virus-positive but WSS did not become infectious.

Culture was continued after 1 month but the PG diet was replaced with the control diet. The shrimps started dying within 20 days after the replacement of feed. Final survival rate was 7.2% after 33 days of termination of PG feeding.

These results showed that PG enhanced the resistance of shrimp

against WSSV because shrimp fed with PG exhibited a higher survival rate than did the control in the challenge trial, and because the shrimp started dying once the feeding of PG was stopped during the challenge.

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In another experimental set-up, the quantity of WSSV in shrimp that survived infection after feeding with PG in the convalescent phase was studied using 2-step PCR in order to identify the exact period to clear the virus from shrimp.

Results showed that the virus was still detectable by PCR within 60 to 95 days of feeding with PG. On the other hand, no virus was detected after 95 to 125 days of feeding with PG. It was concluded that it would take time to clear the virus under the detection limit of 2-step PCR.

Shrimp fed with PG and survived after 30 days still carry a small number of virus parti-

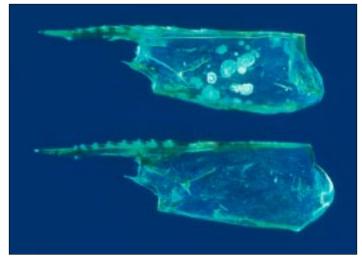
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Dr. Toshiaki Itami presenting his paper at the National Shrimp Congress held at Bacolod City, Philippines from July 1 to 5, 2002

A brown algae is the source of fucoidan developed as prophylaxis for WSSV [cf. Ohno M, Critchley AT. 1993. Seaweed cultivation and marine ranching (1st ed). Japan International Cooperation Agency. p 51]

White spots on the carapace of kuruma shrimp PHOTO COURTESY OF DR. ITAMI



cles and could be a source of infection. Hence, shrimp that survived WSSV infection by PG-administration should continue to receive PG for at least 2-3 months. This was to clear the virus by shrimp defense factors that have been strengthened by PG.

Use of Fucoidan

Sulfated polysaccharides are previously shown to inhibit virus adsorption or attachment to the surface of animal cells. The sulfates in fucoidan structure were shown to play an important role in the binding of WSSV. It also controls infection by inhibiting replication of enveloped viruses. Thus, it is a potent prophylactic agent against virus infections, WSSV included.

Fucoidan is a kind of sulfated polysaccharide obtained from Okinawa-mozuko, *Cladosiphon okamuranus* a brown sea alga.

To test the efficacy of fucoidan in inhibiting WSSV infection in kuruma shrimp, Dr. Itami prepared shrimp diets with different levels of fucoidan and administered it to kuruma shrimp.

Fucoidan were fed at concentrations of 10, 20, 60 and 100 mg/kg bodyweight/day for 15 days. The control group was fed with 0 mg fucoidan. Four days after the first feeding, the shrimps were immersed in WSSV suspension. Mortality was monitored.

Results showed that survival rates of 100 mg fucoidan-fed group was 82.3%, 60 mg group 78.9%, 20 mg group 46.1%, 10 mg group 38.4% and control group 12.4%. A dose of fucoidan at 60 mg/kg bodyweight/day or higher is effective in preventing WSS in kuruma shrimp.

In another experiment, shrimps that were fed intermittently with fucoidan showed a lower survival rate. Shrimps that were fed fucoidan everyday exhibited the highest survival rate. It was concluded that fucoidan should be fed everyday to prevent infection.

Conclusion

Peptidoglycan, an immunostimulant and fucoidan, a viral inhibitor are both effective in prophylaxis against WSS as shown in experiments conducted by Dr. Itami. Significant effects on survival had been recorded when they were both incorporated in the diet of WSSV-infected shrimps.

Dr. Itami stressed the importance of being vigilant in translocating shrimp or crustaceans from other regions or countries into ponds to avoid entry of non- indigenous diseases. He said that some Japanese shrimp farmers committed this mistake by importing shrimp fry, which they think looked healthy and cheap. However, it was later found out that these shrimp fry were disease-carriers. Only a few farmers did this but it led to the collapse of the entire shrimp industry in Japan. This is the reason why Japanese farmers are now experiencing economic crisis in the shrimp farming business. "Learn from our failure!" Dr. Itami emphasized.

On its practical use, immunostimulants and viral inhibitors in shrimp farming is not a cure-all. Good health management, excellent pond management and stocking of genetically disease-resistant shrimp fry are still required to complement and increase the effectiveness of these prophylaxis strategies.

For details on use of PG and Fucoidan as prophylaxis against WSS contact:

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Mr. Kramer thanked the support of his colleagues, Negros Prawn Producers Marketing Cooperative, Inc. (NPPMCI), and SEAFDEC/AQD. "Special thanks to Southeast Asian Fisheries Development Center for having to certify the use of MMI and their efforts to help resuscitate the much beleaguered shrimp farming," Mr. Kramer said.

The use of probiotics in aquaculture is hoped to boost shrimp production for sustainable aquaculture, not only in the Philippines, but also in the region. *-CBL*