Shrimp disease management using bioactive marine secondary metabolites: an eco-friendly approach

J. Selvin and A.P. Lipton

Abstract

Vibriosis caused by opportunistic and secondary bacterial pathogens is still a serious disease problem in aquaculture of the black tiger shrimp *Penaeus monodon*. Attempts were made for controlling shrimp bacterial diseases using Marine Secondary Metabolites (MSMs). Findings indicated that the MSMs of seaweed *Ulva fasciata* and sponge *Dendrilla nigra* are effective for controlling shrimp bacterial pathogens.

Introduction

One of the most commonly cultured penaeid shrimps is *Penaeus monodon*. It is estimated that more than 50 per cent of the world production of penaeid shrimp is contributed by *P. monodon* (Anon. 1994; Roseberry 1995). In India, intensification of shrimp culture methods and improper management strategies caused the shrimps to face stress, injury and consequent diseases, which devastated the farming activities. Infectious diseases constitute the main barrier to the development and continuation of shrimp aquaculture in terms of quality, quantity, regularity and continuity (Meyer 1991; Brock 1992; Rodriguez et al. 1995).

Vibriosis has been implicated as a major mortality factor in juvenile penaeid shrimps (Lightner and Lewis 1975). Vibriosis caused by *Vibrio alginolyticus* and *V. harveyi* is still a serious disease problem in *P. monodon* culture in India even though penaeid acute viremia (white spot syndrome) prevails in Asian countries. *Vibrio* species are considered as members of the normal bacterial flora of shrimp and the culture environment (Otta et al. 1999). Often acting as an opportunistic pathogen or secondary invaders, they induce mortality ranging from slight to 100 per cent in affected population under stress (Lightner 1988).

The total sterility of rearing water during the culture period, by pretreatment, is practically impossible (Boyd 1996; Alabi et al. 1997). Thus, the opportunistic pathogenic bacteria inadvertently enter into the culture water and may cause diseases outbreaks. Initial management of such outbreaks by reducing the stocking density or carrying capacities is considered as uneconomical due to increased production costs. Pre-infection therapy such as vaccination is still under investigation for field use. Although post-infection therapy using medicated feeds with antibiotics incorporated are possible to a certain extent (de la Pena et al. 1992), the impact of antibiotics on the environment and consumer health is highly risky. Therefore, cost effective alternate treatment technologies are urgently required for controlling/ preventing such outbreaks especially due to opportunistic and secondary pathogenic invaders. Thus, this paper addresses the possibility of developing cost-effective therapeutic formulations from marine secondary metabolites (MSMs).

Materials and Methods

1. Oral intubation

Methanolic extracts containing marine secondary metabolites (MSMs) of green alga (*Ulva fasciata*) and sponge (*Dendrilla nigra*) were tested for their efficacy in controlling bacterial diseases of shrimp. Both MSMs showed potent activity in the *in vitro* antibacterial study (Selvin 2002). Therefore, they were taken up for 'in captivity' control of shrimp bacterial diseases. The commercial pelleted shrimp grower feed No. 1 (C.P. Feeds, Cochin) was used for the preparation of top-coated medicated diets. The median lethal dose of *U. fasciata* was 1 120 mg/kg of shrimp whereas *D. nigra* was 420 mg/kg of shrimp (Selvin 2002). Based on the preliminary experiments, the dose selected for *U. fasciata* was 500, 1 000 and 1 500 mg/kg of shrimp whereas for *D. nigra*, it was 250 and 500 mg/kg of shrimp. The doses were incorporated in the feed by spraying appropriate MSM on the surface of the feed at a rate of 3.2 per cent of the shrimp body weight daily using 4 per cent gelatin as binder.

P. monodon with body length of 6 - 8 cm range reared in 1 000 L fiber reinforced plastic (FRP) tanks were fed with appropriate medicated feed in three equal installments at a rate of 3.2 per cent of their body weight for a period of 15 days. On the 16th day, three groups each of healthy pre-treated shrimps (20 shrimps/ group) were challenged with median lethal dose (MLD) of bacterial pathogens and transferred to 100 L glass aquaria. For the challenge experiment, the bacterial pathogens such as two shrimp isolates (*V. harveyi* and *V. alginolyticus*), one type culture (*V. fischeri*) and one fish isolate (*Aeromonas* sp.) were used. The challenged shrimps were observed for a period of 15 days for mortality and infections.

2. Immersion route

The shrimps which showed severe infection and were at a terminal stage were used for immersion treatment. In the preliminary experiments, immersion treatment with 500 mg/l of *Ulva fasciata* or 250 mg/l of *D. nigra* was found to be ineffective. Therefore, 1 000 mg/l of *U. fasciata* and 500 mg/l of *D. nigra* were chosen for the immersion treatment.

Table 1. Effect of MSMs resistance to bacterial pathogens among P. monodon.

Extract concentration (mg/kg)	% Infection/ mortality within 15 days							
	Vibrio fischeri		*V. alginolyticus*		*V. harveyi*		*Aeromonas* sp.	
	Infection*	Mortality	Infection*	Mortality	Infection*	Mortality	Infection*	Mortality
Ulva fasciata								
500	60	0	80	0	80	0	40	0
1 000	20	0	20	0	0	0	0	0
1 500	10	0	20	0	10	0	0	0
Dendrilla nigra								
250	80	0	90	0	80	20	60	0
500	0	0	20	0	10	0	20	0
Control	0	100	0	100	0	100	0	100

*Shell disease but not culminated in mortality within 15 days of post-challenge

The treatment was continued for a period of seven days or until the shrimps recovered from infection.

Results

1. Oral intubation

The results of lower, median and higher dose of *Ulva* and lower and higher dose of *Dendrilla* are presented in Table1. *P. monodon* treated with a lower dose of MSMs (500 mg of *U. fasciata* or 250 mg of *D. nigra* per kg of *P. monodon*) did not provide protection against bacterial infection. At this dose, the shrimp inoculated with MLD of *V. fischeri* was infected to the extent of 60 per cent in the *U. fasciata* treated group and 80 per cent in the *D. nigra* treated group. The shrimp isolates such as *V. harveyi* and *V. alginolyticus* caused infection to the extent of 80 per cent in the *U. fasciata* treated shrimps. In the case of *D. nigra* treated shrimps, *V. harveyi* caused 80 per cent infection and *V. alginolyticus* caused 90 per cent infection. The fish isolate *Aeromonas* sp. infected 40 per cent and 60 per cent of the shrimps treated with *U. fasciata* and *D. nigra* respectively.

The experimental group, inoculated with median (1 000 mg/kg shrimp) and higher (1 500 mg/kg shrimp) doses of *Ulva fasciata* diet exhibited more or less similar protection. The shrimps fed with a median dose of *U. fasciata* diet, followed by pathogen inoculation (MLD) of *V. harveyi* and *Aeromonas* sp. showed no infection. *P. monodon* that was inoculated with *V. fischeri* and *V. alginolyticus* caused 20 per cent infection in the treated groups. The shrimps which were treated with a higher dose of *U. fasciata* diet and subsequently challenged with *V. harveyi* and *V. fischeri* exhibited mild infection (10 per cent) whereas *V. alginolyticus* caused 20 per cent infection. There was no infection observed in the treated *P. monodon* inoculated with fish pathogen. Based on these findings, it could be inferred that treatment with 1 000 mg/kg of *U. fasciata* diet will provide higher protection compared to other doses. In the case of *D. nigra* treated shrimps, the propagation of *V. fischeri* was completely prevented in the shrimps treated with a higher dose of medicated feed. However, pathogens such as *V. alginolyticus*, *V. harveyi* and *Aeromonas* sp. were virulent enough to cause mild infections to the extent of 10 - 20 per cent even in the higher dose group.

2. Immersion route

With the dip treatment, the 'shell disease' was cured only after moulting (Plates 1 and 2). The dip treatment was thus found to be an effective method of post infection therapy. Either *U. fasciata* and/or *D. nigra* showed more or less the same level of efficacy, while the complete recovery was quick in combined medication (Table 2). The days of dip treatment given for shrimp varied even on an individual basis and most of them recovered within 10 - 15 days of dip treatment.

Discussion

The study revealed that low doses of *U. fasciata* and *D. nigra* might not have the sufficient active principles to elicit complete protection against bacterial infection. However, they elicited a low level of protection when compared to the control group. This implicated the possible prevention of bacterial propagation to attain infectivity dose. The higher doses of *U. fasciata* (1 000 and 1 500 mg/kg shrimp) elicited more or less similar protection. Therefore the median dose (1 000 mg/kg) was considered as a recommended

Plate 1. Infected shrimp which was given immersion treatment.

Plate 2. Shrimp which recovered from the symptoms after the immersion treatment.

Table 2. Efficacy of immersion treatment to the infected P. monodon

Group	Extract Dose (mg/l)	Period of dip (min)	% recovery
Infected by *V. fischeri*	*U. fasciata* 1 000	30	100
Infected by *V. harveyi*	*D. nigra* 500	30	100
Infected by *V. alginolyticus*	*U. fasciata* 1 000 and *D. nigra* 500	15	100

treatment level for field use. However, *D. nigra* was less effective compared to *U. fasciata*. Invariably the *P. monodon* treated with both microorganisms and challenged subsequently with MLD of appropriate bacterium resulted in higher survival than the control group.

The survival rate of *P. monodon* treated with *U. fasciata* was 100 per cent against the infection caused by MLD of shrimp isolates, type cultures and fish isolates. The *U. fasciata* medication elicited effective protection against the bacterial propagation and consequent infection in the host. The mechanism of antibiosis alone might not be adequate to elicit this complete protection against infection in the treated group. The possible explanation for this reason is in the *in vitro* antibacterial studies, the crude extract of *U. fasciata* was not found to be a potent bacteriostatic agent against *Aeromonas* sp. (CF2) and shrimp isolates. However, in contrast to these *in vitro* findings, the same extract produced significant control against the same bacteria in the *in vivo* system. Therefore, in the light of the *in vivo* results, it could be assumed that the extract could have influenced the host defense mechanisms.

Although *D. nigra* gave 100 per cent survival against *Vibrio* sp., it was reduced to 60 per cent survival against *Aeromonas* sp. This could be due to the narrow spectrum vibriostatic activity of *D. nigra*. Therefore, the protection elicited by *D. nigra* could be due to its antibiotic effect rather than its influence on the host defense system.

Among the shrimp isolates, *V. harveyi* was a virulent opportunistic pathogen and *V. alginolyticus* was a secondary pathogen of the White Spot Syndrome Virus (WSSV) infection (Selvin 2002; Selvin and Lipton 2002). Invariably, both of the species caused 'shell disease' in the infected shrimps (Selvin 2002). In the present study, both pathogens were effectively controlled with MSMs incorporated in medicated diets. Therefore, the shrimp disease management using MSMs could form a package of practice for sustainable shrimp farming.

Acknowledgements

Authors are thankful to Dr. Mohan Joseph Modayil, Director and Dr. R. Paul Raj, Head, PNP Division, Central Marine Fisheries Research Institute, Kochi, India for the facilities and encouragement. This paper is a part of the Ph.D thesis work of J. Selvin.

References

Alabi, A.O., Yudiati, E., Jones, D.A. and Latchford, J.W. 1997. Bacterial levels in Penaeid larval culture, p. 381-388. ***In*** Diseases in Asian Aquaculture III. Flegel, T.W. and MacRae, I.H. (Eds). Fish Health Section. Asian Fisheries Society. Manila.

Anon. 1994. Aquaculture production. 1985-1992. FAO fisheries circular No. 815 Rev. 6. Rome, Italy. 213 p.

Boyd, C.E. 1996. Chlorination and water quality in aquaculture ponds. World Aquacult. 20(3): 41-45.

Brock, C.A. 1992. Current diagnostic methods for agents and diseases of farmed marine shrimp, p. 209-233 ***In*** Diseases in cultured Penaeid shrimps in Asia and the United States. Fulko, W. and Main, K.L. (eds). Proceedings of a workshop in Honolulu, Hawaii, 27-30 April 1992. The Oceanic Institute, Honolulu, Hawaii.

de la Pena, L.D., Momoyama, K., Nakai, T. and Muruga, K. 1992. Detection of the causative bacterium of vibriosis in kuruma prawn, *Penaeus japonicus*. Fish Pathol. 27: 223-228.

Lightner, D.V. 1988. Diseases of cultured penaeid shrimp and prawns, p. 8-127 ***In*** Disease diagnosis and control in North American marine aquaculture. Second edition. C. J Sinderman and D. V. Lightner (eds.) Elsevier, Amsterdam.

Lightner, D.V. and Lewis, D.H. 1975. A septicaemic bacterial disease syndrome in penaeid shrimp. Mar. Fish. Rev. 37: 25-28.

Meyer, F. 1991. Aquaculture disease and health management. J. Anim. Sci. 69: 4201-4208.

Otta, S.K., Karunasagar, I. and Karunasagar, I. 1999. Bacterial flora associated with shrimp culture ponds growing *Penaeus monodon* in India. J. Aqua. Trop. 14(4): 309-318.

Rodriguez, J., Boulo, V., Mialhe, E. and Bachere, E. 1995. Characterization of shrimp haemocytes and plasma components by monoclonal antibodies. J. Cell. Sci. 108: 1043-1050.

Roseberry, B. 1995. World shrimp farming 1995. Shrimp News Int., San Diego, USA. 68 p.

Selvin, J. 2002. Shrimp disease management using bioactive secondary metabolites isolated from marine organisms. Ph.D thesis submitted to M.S. University, Tirunelveli, India. 204 p.

Selvin, J. and Lipton, A.P. 2002. Pathogenicity, antibiogram and biochemical characteristics of luminescent *Vibrio harveyi*, associated with 'black shell disease' of *Penaeus monodon*. Aquaculture (Submitted)

J. Selvin is from the Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakavilai – 629165, Kanyakumari District, India (email: selvinj@rediffmail.com) and **A.P. Lipton** is from the Marine Biotechnology Laboratory, Central Marine Fisheries Research Institute, Vizhinjam – 695 521, Thiruvananthapuram, India.