CHAPTER - 5

CONCLUSION

*Macrobrachium rosenbergii* (de Man) is native to Thailand and other Southeast Asian countries including India and has been a prominent fishery in these regions. However, as a result of over fishing and destruction of habitat and spawning grounds, the landings have been dwindling rapidly over the years and the species is now a luxury food item; production no longer meets consumer demand. Meanwhile, as a matter of fact, hatchery production of postlarvae of *M. rosenbergii* could be made a reality and it initiated augmented efforts for producing cultured prawn. However, inspite of best of the efforts made, consistent production of sufficient quantity of healthy prawn seed at the right time as per the requirements of country's grow out systems could not be achieved with sustainability. This is still a concern of aquaculturists not only of India but the world over. This instability in the production process is mainly due to diseases and the consequent mass mortality of larvae at different stages; among which vibriosis is the most prominent bacterial disease. It is disappointing to note that despite two decades of research, a viable solution to the global problem has not yet been evolved and the situation continues to be serious demanding much more concerted efforts as quickly as possible. It has been pointed out that lack of adequate information in this systemic infection caused by several species of *Vibrio* is one of the main reasons for the above situation. Nevertheless, vibriosis remains still the major disease in hatcheries, and prompt, specific and rapid detection and identification of the pathogens becomes an absolute essentiality. It has to be borne in mind that conventional microbiological and biochemical analyses need three working days and the information obtained after such a prolonged period is of no use to protect the stock. This invariably makes the rapid detection methods an important requirement. At disease management level, to control vibrios in hatcheries, so far prophylactic and therapeutic use of antibiotics has been the choice especially in commercial establishments. Unequivocally, this practice led to the development of resistance in the pathogens with a possible spread in the environment.
This situation demands an alternate management measure, which includes the introduction of selected bacterial cultures/products as probiotics with antagonistic properties. The present work was undertaken with the realizations that to stabilize the production process of commercial hatcheries an appropriate, comprehensive and foolproof technology is required primarily for the rapid detection of *Vibrio* and subsequently for its management. Results obtained and conclusions made out of this endeavour are summarized as follows:

- Nine species of *Vibrio* such as *V. cholerae*, *V. nereis*, *V. vulnificus*, *V. alginolyticus*, *V. mediterranei*, *V. parahaemolyticus*, *V. splendidus* II, *V. proteolyticus*, *V. fluvialis* and *Aeromonas* sp. have been found to be associated with larvae of *M. rosenbergii* in hatchery.

- Haemolytic assay of the *Vibrio* and *Aeromonas* on prawn blood agar showed that all isolates of *V. alginolyticus* and *Aeromonas* sp., from moribund, necrotized larvae were haemolytic and the isolates of *V. cholerae*, *V. splendidus* II, *V. proteolyticus* and *V. fluvialis* from the larvae obtained from apparently healthy larval rearing systems were non-haemolytic.

- Hydrolytic enzymes such as lipase, chitinase and gelatinase were widespread amongst the *Vibrio* and *Aeromonas* isolates.

- Dominance of *V. alginolyticus* among the isolates from necrotic larvae and the failure in isolating them from rearing water strongly suggest that they infect larvae and multiply in the larval body and cause mortality in the hatchery. This was also supported by a pathogenicity test where *M. rosenbergii* larvae when challenged with a representative isolate of *V. alginolyticus* at $10^6$, $10^7$ and $10^8$ cfu/ml, mortalities of 80, 87 and 100 per cent respectively were observed within 96 h. This observation suggested that the isolate *V. alginolyticus* was a pathogen to the larvae of *M. rosenbergii*. 

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Although variations in the antibiotic resistance patterns could be observed among the bacterial isolates, practically all of them proved to be resistant to erythromycin, the antibiotic frequently employed in hatcheries as a prophylactic agent. The incidence of bacterial resistance to oxytetracycline was comparatively higher (40%) followed by ampicillin (24%) and streptomycin (22%). It has to be pointed out that oxytetracycline is the next widely used antibiotic in hatchery.

In order to meet the requirements of rapid detection of *V. alginolyticus* in the larval rearing systems of *M. rosenbergii*, an indirect fluorescent antibody technique (IFAT) based on polyclonal antibodies was developed. Selection of the isolate of *V. alginolyticus* (MRNL 3) was based on the fact that the above isolate had caused severe larval mortality in the pathogenicity test.

The homologous agglutination titre of the antiserum raised against *V. alginolyticus* in rabbits was 4096, determined by the slide agglutination method, where as the titre of affinity- purified antiserum was only 32.

The undiluted antiserum reacted with all isolates of *V. alginolyticus* and *V. parahaemolyticus* whereas the diluted antiserum (1:100) did not react with *V. parahaemolyticus* and also did not cross-react with *Aeromonas* sp, *Pseudomonas* sp, *E. coli*, *Micrococcus* sp, *Bacillus* sp and *Salmonella typhi*.

Un-purified and purified antiserum, diluted to 1:128 and 1:32 respectively with PBS was found to give good fluorescence in IFAT when compared to other dilutions. The IFAT developed was found to be very selective in detecting *V. alginolyticus* alone in a batch of vibrios tested.

For developing antagonistic probiotic bacterial preparation a large collection of heterotrophic bacteria from different environments was screened against *Vibrio* spp. and *Aeromonas* sp. isolated from prawn larval rearing systems. As a result five isolates of antagonistic bacteria, which could inhibit the target pathogens, were segregated and four of them were identified as *Micrococcus* sp and one a
Pseudomonas sp. However, an appropriate antagonistic bacterium could not be isolated for two isolates of V. splendidus II.

All the isolates of Micrococcus exhibited gelatinase, amylase and cellulase activity but no lipase and chitinase production. The antagonistic Pseudomonas was lipase, gelatinase and cellulase producer having no potential for amylase and chitinase activity.

Co-culture experiments showed that the growth of V. alginolyticus was inhibited by Pseudomonas MCCB103 and Micrococcus MCCB104 inoculated at an initial count of $10^5$ to $10^7$ cfu/ml.

The preliminary characterization of inhibitory substance after treatments with α-chymotrypsin, trypsin, proteinase K, pronase E, lysozyme, lipase, catalase and α-amylase suggested that it was not a protein, lipid or carbohydrate, and did not contain hydrogen peroxide or any compound of alkaline or acidic in nature. Thermostability tests indicated that they were stable at 100°C.

In vivo effect of Pseudomonas MCCB103 and Micrococcus MCCB104 on vibrios was tested by adding the culture to prawn larval rearing water. The total Vibrio count determined subsequent to the addition of inoculum was significantly lesser ($p < 0.05$) than that of the control and the treatment resulted in increased per cent conversion of larvae to post-larvae, with slightly higher mean weight and mean length.

To sum up, through this work, nine species of Vibrio and genus Aeromonas associated with M. rosenbergii larval rearing systems could be isolated and segregated based on the haemolytic activity and the antibiotic sensitivity profile was determined. Highly specific and stable polyclonal antibodies (PAbs) for use in diagnosis or epidemiological studies could be produced, based on a virulent culture of V. alginolyticus. This could possibly replace the conventional biochemical tests for identification. As prophylaxis to vibriosis, four isolates of Micrococcus spp. and an isolate of Pseudomonas sp. could be obtained which could possibly be used as antagonistic probiotics in the larval rearing system of M. rosenbergii.