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Bacterial Flora Associated with the Larval Rearing Environment and Larvae of Giant Freshwater Prawn *Macrobrachium Rosenbergii*

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Water samples from seed transportation bag, larval rearing tank and *Artemia* collected from rearing operation of *Macrobrachium rosenbergii* were analysed to characterize the microflora associated with these samples. The larvae were also analysed to see the microflora. It was found that *Vibrio* was the dominant genera in all the samples. Since the mortality was high in the hatchery operation, the water samples from the rearing tank were analysed for total heterotrophic bacterial (THB) load during five cycles. THB ranged between 8 x 10^3 and 3.2 x 10^5 cfu per ml. Microbial diversity was high in the water samples from larval rearing tank, which included various genera such as *Alcaligenes, Vibrio, Bacillus, Pseudomonas, Moraxella* and *Micrococcus* apart from members of the family Enterobacteriaceae.

Key words : *Macrobrachium rosenbergii*, rearing water microflora, larval mortality.

Aquaculture has been expanding rapidly, in an attempt to increase production. Many different species are now being cultured. Prawns are the most important by virtue of their high value and persistent demand. With the development of an export market and considerable price rise, the value of Giant fresh water prawn, *Macrobrachium rosenbergii* (De Man 1859) is now being realised and there is a growing interest in the establishment of hatcheries and systematic culture for increased production both for internal consumption and export market. Although global farmed freshwater prawn production has gone up expanded by a factor of 35% in the 7 years from 1984 to 1991, it has declined significantly or stagnated since 1992. This was caused mainly due to disease outbreaks resulting from environmental degradation as well as inbreeding problems.

Disease is an important aspect in the culture of any organism. It becomes particularly prevalent when animals are reared intensively in hatchery situations. Juveniles and adult rearing phase experience less disease problem than hatchery phase (Soundarapandian & Kannupandi, 1998). Though the reasons for the reduced larval survival are attributed to various physical, chemical and biological parameters of the rearing environment, the most important one is the microbial infections of the larvae (Baticados *et al.*, 1991; Lightner, 1988). Though the newly hatched larval gut is sterile, it soon absorbs microorganisms from the environment by way of drinking and feeding from surrounding environment (Hansen & Olafsen, 1989; Olafsen, 1984). It was also reported that the intestinal microflora of fish reflects the bacterial content of ingested food and of the water (Seki, 1969;

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Horsley, 1977; Tanasomwang & Muroga, 1988).

In the present investigation an attempt has been made to characterise the microflora associated with the larval rearing environment and the larvae of *M. rosenbergii* as the mortality was very high during hatchery operations.

Materials and Methods

Samples were collected from state owned hatchery, Veliyankode during the period September, 1999 to November 1999. The samples included water from the seed transporting bag and the larval rearing tank, brine shrimp (*Artemia salina*) and larval samples of *M. rosenbergii*. The water samples from the seed transporting bag were from the private hatchery which used to supply the seeds. These water samples were collected just before releasing the seeds into the tanks maintained at hatchery set up. The larval rearing tank water samples were collected from the rearing tanks where the larvae were grown to further size.

Water samples from the larval rearing tanks and the seed transporting bag were collected in sterilised conical flask. Water samples were collected about a feet below the surface from the larval rearing tank. The samples were brought to the laboratory in an ice box and analysed within 2 h of collection (Anon, 1978). Several samples of 100 – 200 brine shrimps used to feed larvae and 150 – 200 larvae from different larval rearing tanks were also collected in sterile polyethylene bags and transported to the laboratory in live condition.

The water samples from the rearing tank were analysed for heterotrophic bacteria, both qualitatively and quantitatively, for 5 cycles. The water samples collected were serially diluted as required using sterile 9ml saline (0.85% NaCl) dilution blank. Various dilutions were then plated aseptically in nutrient agar by pour plate method and incubated at room temperature (28 + 2°C) for 48 hours. Isolation and characterisation of bacteria from *Artemia* and larval samples were also obtained by the same method, except that the samples were surface washed in a sterile 0.1% Benzalkonium chloride saline solution (BZC) for 1 min, then thoroughly rinsed in sterile saline (0.85% NaCl) water (Muroga *et al.*, 1987; Blanch *et al.*, 1997) and were homogenized in a glass homogeniser before serial dilution.

For quantitative estimation, plates with 30 – 300 colonies were used for counting. The plates were examined after incubation and morphologically different colonies from various samples were isolated, purified and maintained on nutrient agar vials. Thirty six isolates were selected at random from each sample for further characterisation studies. The bacterial isolates were then characterized up to generic level following various tests such as Gram staining, spore staining, motility, Kovac's oxidase, catalase and oxidation fermentation tests (Buchanan & Gibbons, 1974). Members of the family Enterobacteriaceae were not characterized further because of the time limitation. The ability of the isolates to elaborate hydrolytic enzymes such as amylase, protease and lipase were also analyzed by plate assay (Harrigan & McCance, 1976) using starch, gelatin and tween 80 as respective substrates.

Results and Discussion

In the present investigation bacterial flora associated with the hatchery environment and larvae has been analysed for its characterisation, morphological and physiological grouping. During hatchery operations temperature, pH and salinity of larval rearing water varied between 28 – 32°C, 7 – 8 and 8 - 12 respectively.

Mean and range of the total heterotrophic bacteria (THB) of the rearing water collected from various tanks during 5 batches of operation is presented in Table 1. Bacterial load was higher in water samples of 1st and 2nd batches with a mean population

Table 1. Mean and range of population of Total Heterotrophic Bacteria (THB) associated with the larval rearing water samples.

Sample source	Mean population (cfu/ml)	Range of population (cfu/ml)
1st Batch	1.80×10^5	$6.0 \times 10^4 - 2.9 \times 10^5$
2nd Batch	1.32×10^5	$8.0 \times 10^3 - 3.2 \times 10^5$
3rd Batch	2.48×10^4	$8.0 \times 10^3 - 3.2 \times 10^4$
4th Batch	2.10×10^4	$1.7 \times 10^4 - 2.5 \times 10^4$
5th Batch	1.30×10^4	$1.0 \times 10^4 - 1.6 \times 10^4$

value of 1.8×10^5 cfu/ml and 1.32×10^5 cfu/ml respectively. After a few days from sampling these two batches were discarded when large scale mortality was observed. The sample collected from the 5th batch showed the lowest count with a mean population of 1.3×10^4 cfu/ml. Some authors have reported that the bacterial flora of aquatic animals, especially that of fish and shellfish is reflection of their environmental microflora (Geldreich & Clarke, 1966). It is also reported that the establishment of gut microflora of the larvae is mainly influenced by the rearing water (Ringo & Vadestein, 1997). The deformities of the larvae resulting from the chemical treatment of water as well as injury during transportation due to chemical treatment and injury, helps the pathogenic bacteria of the rearing medium to invade the tissue of the host. Table 2 represents the various morphological and physiological groups of bacteria from the various samples. Gram-negative rods were dominant in all samples.

Percentage distribution of different genera associated with the larval rearing water samples, water from the seed transportation bag, *Artemia* and larvae are presented in Table 3. *Vibrio* was found to be the dominant genera in all the samples. Other predominant genera encountered were the members of the family Enterobacteriaceae in the water from larval rearing tank as well as in the larvae, *Alcaligenes* in Artemia and in the water collected from seed transportation tank.

The relationship of the microflora associated with the developing prawn was significantly influenced by environmental conditions and diet (Sugita *et al.*, 1992). *Vibrio* was first isolated from brine shrimp and from seawater and was evident in zoea to post larval stages. In our findings *Vibrio* was the dominant genera identified in brine shrimp sample. The studies conducted by Blanch *et al* (1997) concluded that live food is the main source of bacteria for colonization of the gut.

The generic composition of the larval samples revealed dominance of *Vibrio* followed by the members of the family Enterobacteriaceae. So it is hypothesized that high mortality was caused mainly due to the disease vibriosis. The role of *Vibrio* as an etiologic agent of many diseases in

Table 2. Percentage incidence of different morphological and physiological groups in various samples collected from the hatchery.

Sample	Source	Gram +ve (%)	Gram -ve (%)	Rods (%)	Cocci (%)	Amylolytic (%)	Lipolytic (%)	Gelatinolytic (%)
Water	Seed transporting bag	10	90	100	-	72	84	81
Water	Larval rearing tank	7	93	98	2	46	78	67
Artemia	Artemia rearing tank	0.0	100	100	-	65	100	100
Larvae	Larval rearing tank	0.0	100	100	-	66	92	88

Table 3. Percentage distribution of various genera collected from larval rearing tank water, Seed transporting water, *Artemia* and Larvae.

Genera	Larval rearing tank water	Seed trans-porting water	Artemia	Larvae
Vibrio	29%	70%	72%	50%
Alcligenes	9%	10%	28%	–
Enterobacteriaceae [a]	16%	–	–	33%
Bacillus	4%	10%	–	–
Pseudomonas	11%	–	–	–
Moraxella	6%	–	–	–
Micrococcus	2%	–	–	–
Unidentified	22%	10%	–	17%

[a]Members of the family Enterobacteriaceae

hatchery operations of fish and shellfish is well documented (Masumura *et al.*, 1989; Sedano *et al.*, 1996). The investigation supports the view that the indigenous microflora contribute to protect the fish from invasion of pathogenic microorganisms (Onarheim & Raa, 1990; Olsson *et al.*, 1992). It has been reported that *Sparus aurata* larvae infected with *Vibrio* strains were suffering from high mortality and that they did not possess an indigenous microbiota associated with their intestinal mucosa and therefore more susceptible to adhesion by pathogenic vibrios (Sedano *et al.*, 1996). They had also observed rapid mortality in the larvae experimentally infected with *Vibrio* strains. In the present study high mortality was observed among the zoeal stages of prawn larvae. It is concluded that one of the transmission route of disease may be through feeding.

Under intensive rearing conditions, survival ratio of the larval forms are highly variable and unpredictable due to the fact that larvae are hatched into, and participate as a part of the same ecosystem as potential bacterial pathogens and there are evidence that high larval mortality is associated with the presence of bacterial pathogens (Iwata *et al.*, 1977; Kusuda *et al.*, 1986; Masumura *et al.*, 1989; Tamsomvang & Muroga, 1989).

The ability of isolates to elaborate various hydrolytic enzymes revealed that proteolytic forms were high in all samples. This was followed by lipolytic and amylolytic forms. These enzymes favour the microbial invasiveness of the host tissues. The presence or these activities in the strains degrade the tissue, since several activities such as amylase, lipase and gelatinise are very important factors in the virulence of some microorganisms.

This investigation was carried out to elucidate the relation of larval microflora with respect to its live feed and hatchery environment. From these studies it seems reasonable to conclude that live food is the main source of bacteria for the colonization of larval microflora. Similar observation has been reported in turbot (*Scophthalmus maximus*) larvae (Blanch *et al.*, 1997). The presence of *Vibrio* sp associated with brine shrimp reinforces the need to monitor the quality of the batches of brine shrimps and the control of the bacterial population in larval rearing to increase the survival rate of larvae.

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