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Investigation on bacterial flora of farmed freshwater prawn (*Macrobrachium rosenbergii* de Man) in Bangladesh

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Abstract

Total bacterial load in the haemolymph of freshwater prawn *Macrobrachium rosenbergii* varied from $6.2x10^4$ to $1.9x10^7$ CFU/ml whereas in the hepatopancreas, bacterial load varied from $1.9x10^3$ to $2.9x10^5$ CFU/g. The total bacterial load in the pond water varied from $2.6x10^2$ to $4.1x10^5$ CFU/ml. The isolated bacterial genera in the haemolymph and the hepatopancreas of prawn were *Streptococcus, Acinetobacter, Micrococcus, Aeromonas, Vibrio, Flavobacterium, Staphylococcus* and *Pseudomonas,* whereas the detected bacterial genera in pond water were *Micrococcus, Streptococcus, Vibrio, Flavobacterium, Staphylococcus, Pseudomonas* and *Aeromonas*. Among the detected genera, *Vibrio* and *Staphylococcus* were found to be dominant genera in the haemolymph of the sampled prawn throughout the study period whereas *Staphylococcus* and *Pseudomonas* were dominant in pond water.

Key words : *M. rosenbergii*, Haemolymph, Hepatopancreas, Bacterial flora

Introduction

Now-a-days freshwater prawn farming has turned to be a promising sector in Bangladesh since return from export earning of this commodity is considerable high. With the rapid development in *Macrobrachium* hatchery production and number of prawn grow-out farms, good husbandry and environmental problems developed as prawn were stressed and weakened under adverse conditions. Moreover, shrimp is a highly putrescent fishery commodity as microbial activity is the most effective in producing undesirable odours and appearance in shrimp. Considering the importance of prawn fishery and the recent outbreak of shrimp disease in the country, a preliminary investigation was designed to investigate the bacteriological status in farmed prawn, *Macrobrachium rosenbergii* to know the monthly distribution pattern of bacteria in different organs of prawn.

Materials and methods

Water and prawn samples

Grow-out ponds under Jhalak Fish Farm- a private entreprenuer at Gouripur in Mymensingh were selected for sampling of freshwater prawn, *Macrobrachium rosenbergii*. Prawn samples were collected twice in a month and carried to the Fish Disease Laboratory of BAU in a plastic container containing the same pond water of the respective prawn samples.

Prawn organs for bacterial isolation

The prawn were stopped their movement by a simple hurt to the head. A disposable syringe was inserted into the cephalo-thorax region under the carapace to collect haemolymph from the heart of the prawn. An amount of 0.3 ml haemolymph was mixed with 2.7 ml of physiological saline solution (0.85% NaCl) to make a stock suspension. Samples of hepatopancreas were collected with a sterile loop in a pre-weighted test tube. Suspension of hepatopancreas containing bacteria was made in physiological saline solution with the help of a vortex mixer. Necessary dilution were made by ten-fold dilution method. Inoculum (0.1 ml) was spread on TSA plates, incubated at 25^0C and colonies were counted after two days of incubation.

Determination of bacterial isolates

Total number of bacteria were determined by counting the developed colonies on the triplicate plates of the same dilution after incubation. For determination of percentage composition, twenty colonies were seperated unbiasly from the total colonies grown in each of the plate having the number within 30 to 300 to obtain the pure culture and to characterize the isolates. From these plates of pure culture the isolates were preserved on TSA agar slant at a temperature of 4^0C and successive studies were performed for characterization of the isolates to identify them upto genus/group level according to the methods described by Cowan and Steel (1975) and Thoesen (1994) with slight modification. After the identification of bacterial genera/groups the percentage composition were determined on the basis of twenty isolates from pond water, haemolymph and hepatopancreas of the sampled prawn.

Results and discussion

The total bacterial load during the sampling period as varied from $6.2x10^4$ to $1.9x10^7$ CFU/ml of haemolymph, $1.9x10^3$ to $2.9x10^5$ CFU/g of hepatopancreas and $2.6x10^2$ to $4.1x10^5$ CFU/ml of pond water (Table 1). Total number of bacteria in the haemolymph of prawn varied with the months. The bacterial load of pond water was found to vary with months but they did not differ distinctly among the ponds studied. In pond water highest number of bacterial population was found in the month of July and the lowest in the month of November.

Table 1. Monthly variation of the total bacterial load in the haemolymph, hepatopancreas of *M. rosenbergii* and pond water

Months	Total load of the bacterial genera					
	Pond-1			Pond-2		
	Haemolymph (CFU/ml)	Hepatopancreas (CFU/g)	Water (CFU/ml)	Haemolymph (CFU/ml)	Hepatopancreas (CFU/g)	Water (CFU/ml)
July	$1.7x10^5$	$4.9x10^4$	$2.5x10^4$	$1.9x10^7$	$2.9x10^5$	$4.1x10^5$
August	$7.0x10^4$	$3.0x10^3$	$1.6x10^3$	$2.6x10^6$	$3.7x10^4$	$1.9x10^4$
September	$1.3x10^5$	$4.6x10^3$	$2.2x10^3$	$1.3x10^6$	$3.6x10^4$	$8.5x10^3$
October	$2.3x10^6$	$4.6x10^3$	$1.7x10^3$	$9.6x10^4$	$4.2x10^4$	$2.4x10^3$
November	$6.2x10^4$	$5.0x10^3$	$8.6x10^2$	$4.6x10^5$	$8.4x10^3$	$2.6x10^2$
December	$3.9x10^6$	$1.9x10^3$	$2.3x10^3$	$2.7x10^4$	$3.3x10^4$	$1.6x10^3$

The bacterial genera/groups detected in the haemolymph and hepatopancreas of the prawn were tentatively *Streptococcus*, *Acinetobacter*, *Micrococcus*, *Aeromonas*, *Vibrio*, *Flavobacterium*, *Staphylococcus* and *Pseudomonas* (Table 2), whereas the detected bacterial genera in pond water were *Micrococcus*, *Streptococcus*, *Vibrio*, *Flavobacterium*, *Staphylococcus*, *Pseudomonas* and *Aeromonas* (Table 3). The identified bacterial genera recovered from the haemolymph were found throughout the study period except *Acinetobacter*, and *Vibrio* was found to be dominant genera in the month of August. *Flavobacterium* and *Staphylococcus* were found to be dominant in the hepatopancreas of prawn.

Table 2. Monthly distribution of bacterial genera in the haemolymph and hepatopancreas of cultured *M. rosenbergii*

Months	Prevalence of the bacterial genera															
	Haemolymph								Hepatopancreas							
	Str.	Aci	Mi.	Ae.	Vi	Fl.	Sta	Ps.	Str	Aci	Mi.	Ae	Vi	Fl	Sta	Ps.
July.	11	Nd	14	14	20	19	08	12	15	03	13	12	03	24	26	08
Aug.	12	Nd	13	14	37	03	20	05	17	05	13	13	07	13	23	13
Sep.	09	12	11	10	19	18	20	07	09	15	19	13	Nd	14	21	10
Oct.	11	24	03	11	10	Nd	20	24	09	13	19	17	15	14	22	11
Nov.	08	12	13	17	14	07	10	20	18	09	13	12	07	13	24	13
Dec.	10	03	12	20	23	05	20	09	15	03	11	13	03	23	24	11

Str : *Streptococcu* Aci : *Acinetobacter* Mi : *Micrococcus*
Ae : *Aeromonas* Vi : *Vibrio* Fl : *Flavobacterium*
Sta : *Staphylococcus* Ps : *Pseudomonas* Nd : Not detected

Table 3. Monthly distribution pattern of bacterial genera in pond water

Months	Prevalence of the bacterial genera											
	Pond-1						Pond-2					
	Mi.	Str.	Vi.	Fl.	Sta.	Ps.	Mi.	Ae.	Vi.	Fl.	Sta.	Ps.
July	07	06	20	07	30	30	22	12	Nd	28	38	Nd
August	35	10	12	13	15	15	12	08	10	30	40	Nd
September	20	08	12	15	22	23	13	15	08	27	32	05
October	13	22	08	12	23	22	15	23	Nd	27	27	08
November	15	30	10	15	15	15	03	22	05	20	25	25
December	10	12	18	20	20	20	10	27	Nd	22	18	23

Mi : *Micrococcus* Str : *Streptococcus* Vi : *Vibrio*
Fl : *Flavobacterium* Sta : *Staphylococcus* Ps : *Pseudomonas*
Ae : *Aeromonas* Nd : Not detected

Qualitative and quantitative composition of bacteria in different organs of the prawn investigated were found to vary with months, organs and pond water also. In the study period, the bacterial load in the haemolymph of prawn were higher than those in hepatopancreas or pond water. Environmental factors such as water temperature, pH, dissolved oxygen, nutrients, organic substances etc. might be the causal factors of these variations. Besides these supplementary feed to prawn were the sources of organic substances. Bacterial growth in water was influenced by availability of carbon and energy source (Marshal 1985). Udea *et al.* (1992) mentioned that the microflora associated with the growing prawn significantly influenced by the environmental conditions. Total load of bacteria in the haemolymph of prawn was higher in summer than in winter due to the differences in the ambient temperature (Zuberi *et al.* 1992, Fonseka and Ranjini 1995).

Poor water quality and high organic loading are associated with shell lesion including bacterial growth in grow-out prawn. A variety of bacteria, producing extracellular lipases or protease such as *Aeromonas, Pseudomonas, Vibrio, Benekea* spp. have been implicated in shell disease (Cook and Lofton 1973). Organic matter supplied as feed to pond and manure increased the bacterial load in pond water (Moriarty 1986). In most of the months, *Vibrio* and *Pseudomonas* were high in the haemolymph of prawn investigated which is similar with the findings of Lombardi and Labao (1991a and 1991b), mentioned *Pseudomonas, Vibrio* etc. as the possible causative agents of prawn diseases. Although pathogenicity and antibiotic test were not performed in the present study, the isolated bacteria *Pseudomonas, Vibrio* and *Aeromonas* could be pathogens to the cultured prawns which needs further study.

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