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Intestinal Bacterial Flora of Macrobrachium rosenbergii (De Man, 1879) from Freshwater Farms in Kerala, India

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The bacterial flora in the intestine of farmed Macrobrachium rosenbergii (De Man, 1879) were examined qualitatively and quantitatively. Prawn intestine samples were analysed for total plate count, H_2S producing bacteria, faecal Streptococci, total coliforms, faecal coliforms, Escherichia coli and Staphylococcus aureus. The counts of the intestinal microflora of prawn were in the range of 10^7 to 10^8 cfu g^{-1}. Total coliform, faecal coliform and Escherichia coli levels were ° 3.0 $\log_{10}$ MPN g^{-1}. The number of H_2S producers ranged from 10^5 to 10^7 cfu g^{-1}. Faecal Streptococci had a range of 10^3 to 10^6 cfu g^{-1}. The counts of H_2S producers, faecal coliforms, E.coli and faecal Streptococci varied widely between the farms. The dominant intestinal microflora belonged to genera Aeromonas, Citrobacter, Enterobacter, Serratia, Klebsiella, Pseudomonas and Bacillus. While Acinetobacter, Shewanella, Streptococcus, Micrococcus, Staphy- lococcus and Lactobacillus were found in lower frequencies. High counts of faecal coliform bacteria and Streptococci in the intestine of farmed freshwater prawn indicates lack of good farm management practices in the fresh water farms studied.

Keywords : Macrobrachium rosenbergii, intestinal flora, freshwater farm, indicator bacteria, Enterobacteriaceae, Aeromonadaceae

Aquatic organisms often harbour a great number of bacteria in their intestinal tract, gills and body surface, which they acquire from water, sediment and/or food. The influence of the gut flora on the host is clearly of great interest in aquaculture, particularly where poor productivity and/or stock losses are widespread (Sharmila et al., 1996; Moriarty, 1997; Lavens & Sorgeloos, 2000). The maintenance of the microbiological quality of water systems used for farming is critical as faecal contamination of these systems can pose risks to human health and also results in economic losses (Sinton et al., 1998; Scott et al., 2002). Investigations on the bacterial flora in the gastrointestinal tracts of a variety of crustaceans (Yasuda & Kitao, 1980; Cahill, 1990; Sugita et al., 1996; Pinn et al., 1999; Lau et al., 2002; Shakila et al., 2006) demonstrated qualitative and quantitative variations in the intestinal microflora of prawns depending on the aqueous environment and food.

In culture system, the parameters such as water source and quality, diet, stocking density and habitat structure are different from the natural environment and it may lead to the establishment of a different gut microflora (Prieur et al., 1990; Strom & Olafsen, 1990). While studying the effect of feeding Lactobacillus-based probiotics on the gut microflora and growth and survival of postlarvae of Macrobrachium rosenbergii (de Man), Venkat et al. (2004) reported that the probiotic strains were found to have inhibitory effects against the gram-negative bacterial flora present in the gut and growth of the probiotic fed groups was significantly higher than the control group.

Gut microflora play an important role in the digestive process, growth and disease susceptibility of detritus feeders (Fenchel & Kofoes, 1976; Yingst, 1976). M. rosenbergii is commercially important as a culture species in India. Hence, the present investigation was

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carried out to determine the taxonomic composition and the numerical abundance of bacteria in the gastrointestinal tract of fresh M. rosenbergii from aquaculture farms and changes in the bacterial flora during iced storage.

Materials and Methods

M. rosenbergii was procured from four farms located in Kottayam (farms 1,2,3) and Alleppey (farm 4) districts of Kerala, India. All the ponds were fertilized at irregular intervals with organic manure (cow dung). Prawns were fed on animal protein sources like clams and livestock processing waste. M. rosenbergii samples were collected from each farm in sterile polyethene bags and transported to the laboratory in ice aseptically. Bacteriological analysis was carried out with fresh prawns within 2 to 4 h of collection. Ten representative prawns weighing between 30 and 50g from each farm were used for the analysis.

For ice sorage study, 3-5 prawns (each weighing 200-350g) were packed in individual plastic bags and stored in flake ice in the ratio of 1:1(w/w) in insulated thermocole boxes and kept in a cold room maintained at 2-4^{o}C for 26 days. Ice was topped up every day after draining the melt water. Samples were withdrawn after every 1 or 2 days of storage for the first week and thereafter every 3 or 4 days and analysed for bacterial parameters.

Intestine of prawns were excised aseptically, weighed and placed in sterile bags containing enough normal saline (NaCl, 0.85% w/v) to make 1:10 dilutions and homogenized in a stomacher (Lab blender 400, Seward Medical, London.) for 60 sec at room temperature. Decimal dilutions in normal saline were prepared and plated on agar or poured in to tubes for MPN method.

Prawn intestinal homogenates, serially diluted were plated on Tryptone Soya Agar (TSA, Oxoid, U.K.) for total aerobic plate counts (TPC) (Austin & Al–Zahrani, 1988) and incubated at 37^{o}C and 30^{o}C (2-3 d) and 20^{o}C (3-5 d). Psychrotrophic counts were determined on TSA incubated at 7^{o}C for 10 days. Hydrogen sulphide (H_2S) producing bacteria were enumerated as per the method of Gram et al. (1987), faecal Streptococci and Staphylococcus aureus counts by the plating method (FDA, 1998) and total coliforms, faecal coliforms and Escherichia coli by the three tube MPN method (APHA, 1998).

All colonies from a sector or whole plates of TSA and Iron Agar (IA) (20^{o}C) were isolated, purified and stored on TSA slants. A total of 86 factorial cultures were isolated. The strains were tested for gram reaction, catalase and oxidase reactions, motility, oxidative/fermentative metabolism and presence of spores. They were then grouped according to the taxonomic schemes of Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984; Sneath et al., 1986), further tested for the most relevant characteristics of each group and identified using the schemes proposed by Dainty et al., (1979), Valera & Esteve, (2002).

The microbial counts were expressed as $\log_{10}$ cfu g^{-1} for analysis. Analysis of Variance was performed using the statistical tool package of Microsoft Excel 97 software. 'Student's t test' analysis was used to evaluate the significance of differences between means of microbial counts at 37, 20 and 7^{o}C.

Results and Discussion

Intestinal microflora of fresh prawn

The mean counts ($\log_{10}$ cfu g^{-1}) of total aerobic bacteria (37and 30^{o}C), H_2S producing bacteria, faecal Streptococci, S. aureus, total coliforms, faecal coliforms and E. coli in the intestine of M. rosenbergii from four different farms are presented in Table 1. The total aerobic bacterial population ranged from 7.2-8.52 $\log_{10}$ cfu g^{-1}. Higher counts were recorded in prawn obtained from two farms, one located at Kottayam (farm 1) and the other at Alleppey (farm 4). Total aerobic counts at 37 and 30^{o}C did not differ significantly. However, counts at 7^{o}C (Fig. 1) were significantly lower than those at 37^{o}C ($p< 0.01$) indicating that a significant fraction of the microflora is mesophilic in nature. Lalitha and Surendran (2004) reported aerobic counts of 3.6 and 4.83 $\log_{10}$ cfu/g for farm water and freshwater prawn surface respectively. These values are much lower than that of the intestine indicating that intestine

provnIcs i fivoritlc ccologncil enchc for somc ticacrni (Goezilcz et al., 1999). Thc TPC vilucs otscrvcI ne ahc prcscea sauIy wcrc compiritlc ao ahia rcporacI for firmcI tricknshwiacr shrnmp (NiyyirihimmcI et al., 1995) ieI firmcI frcshwiacr fnsh (SurceIrie et al., 1995) ne IeIni ieI frcshwiacr fnsh from rnvcr ne Jipie (Sugnai et al., 1983). Shrnmps, tcneg toaaom Iwcllneg Icarnaus fcclcrs, oface hivc hnghcr ticacrnil populianoe ahie fnefnshcs.

Table 1. Intestinal microflora of farmed Macrobrachium rosenbergii from four farms in Kerala

Microbial parameters	Microbial count (log_{10}cfu/g) Farm 1*	Farm 2	Farm 3	Farm 4
TPC 37°C	8.30	7.20	7.77	8.52
30°C	8.32	7.20	7.76	8.45
H_2S producers	6.26	5.30	6.07	7.04
Streptococcal count	4.46	3.04	5.64	6.73
Staphylococcus aureus	3.60	3.47	3.53	3.07
Total coliforms	4.14	3.65	5.14	5.14
Faecal coliforms	4.04	3.39	3.60	5.14
Escherichia coli	4.04	3.39	3.60	5.04

*Farm 1- Vallakom I, Farm 2 - Vallakom II, Farm 3 - TV Puram, Farm 4 - Vayalar

Thc H_2S proIucneg ticacrnil coueas wcrc ne ahc riegc of 10^5 ao 10^7 cfu g^{-1}. Hnghcr coueas of H_2S proIucneg ticacrni ieI ficcil Sarcpaococcn ($p>0.01$) wcrc rccorIcI ne priwe otainecI from firm 4. Thc S. aureus coueas ne priwe neacsanec wcrc ne ahc riegc of 3.0 –3.6 log_{10} cfu g^{-1}. Buris et al. (1987) hivc showe ahia ticacrnil lcvcls of poeI-rcircI fnsh miy necrcisc coesnIcritly ne cIntlc anssucs ifacr i ahrcshholI lcvcl his tcce surpisscI ne ahc cevnroemcea.

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Intestinal flora of prawn stored in ice

A 1-2 log necrcisc ne ahc icrotnc-mcso-phnlnc, psychroarophnc ieI H_2S proIucneg ticacrnil coueas wis eoanccI ne ncc saorcI simplcs tcawcce 0 ieI 5 Iiys (Fng. 1). Ia ns possntly ciuscI ty ahc loegcr coolneg anmc eccIcI ao rcIucc ahc acmpcriaurc of priwe pickcI ne plisanc tigs from 29±2°C ao 0°C. WnIc flucauianoes wcrc eoanccI ne psychroarophnc ieI H_2S proIucneg ticacrnil coueas up ao Iiy 19. Growah of ficcil Sarcpaococcn wis eoanccI from Iiy 3 oewirIs ieI coueas rcichcI 7.8 log_{10} cfu g^{-1} ia ahc ceI of saorigc (Fng. 2). A 1 log necrcisc wis eoanccI ne ficcil colnform ieI E. coli coueas ty 3 Iiys ieI ahcrcifacr rcminecI ia ahia lcvcl.

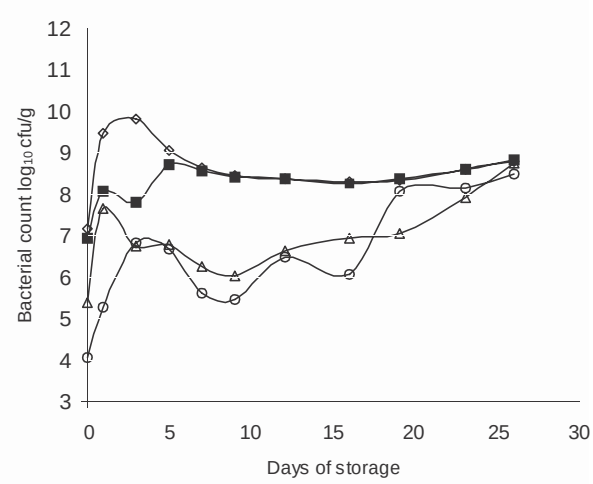

Fig. 1 Changes in the total aerobic and H_2S producing bacterial populations in the intestine of farmed Macrobrachium rosenbergii during iced storage.

--- -- TPC at 37°C — - — TPC at 20°C
— Δ— TPC at 7°C — •— H S producers

2

Taxonomic composition of the intestinal microflora

Gua mncroflori cxhntnacI i grciacr ticacrnil Invcrsnay ieI ahcnr pcrcceaigc necnIcecc ns gnvce ne Titlc 2. Bicacrni nsoliacI from ahc simplcs wcrc prcIomn- eiealy grim ecgianvc roI (66-76%) ieI ahc flori wis IomneiacI ty Eeacroticacrniccic (22-40%), followcI ty AcromoeiIiccic (17-30%). Thc pcrcceaigc necnIcecc of gcecri Bacillus and Micrococcus/Staphylococcus wis 8-19% ieI 6-17% rcspccanvcly. Oahcr gcecri vnz. Pseudomonas/Xanthomonas/Shewanella/

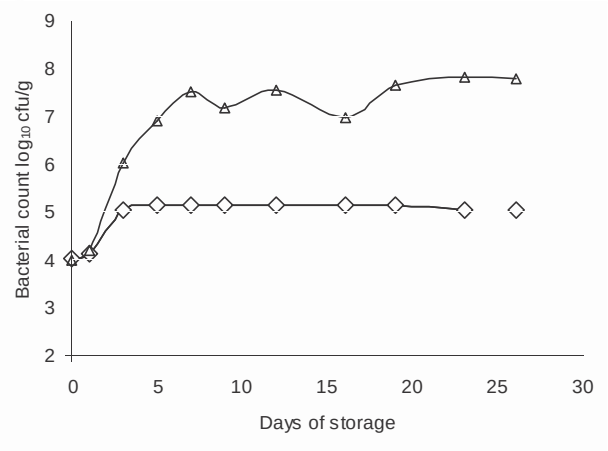

Fig. 2. Changes in the Indicator bacterial populations in the intestine of farmed Macrobrachium rosenbergii during iced storage.

--- --- Faecal coliforms — .— E. coli —A— Faecal Streptococci

Cytophaga/Acinetobacter/Moraxella/Arthrobacter and Streptococcus were also isolated in small numters. The dominant gram-negative species found in the gut microflora of prawn telong to Aeromonas hydrophila, A.veronii tivar sobria, A. schubertii, Enterobacter cloacae and Citrobacter freundii. Among enterococci, Streptococcus faecalis and S. faecium were isolated from all the farms. The microflora of ice stored prawn comprised mainly of A.

Table 2. Intestinal microflora of farmed Macrobrachium rosenbergii from farms located in Kerala, India.

Genera	Microflora (%)			
	Farm 1	Farm 2	Farm 3	Farm 4
Enterobacteriaceae	40	22	31	30
Aeromonadaceae	20	17	19	30
Pseudomonas/ Xanthomonas/ Shewanella	10	-	6	8
Flavobacterium	0	11	13	-
Acinetobacter/Moraxella	3	11	-	8
Cytophaga	0	6	-	-
Bacillus	10	11	19	8
Streptococcus	7	5	6	-
Micrococcus/ Staphylococcus	7	17	6	8
Arthrobacter	0	-	-	8
Corynebacterium	3	-	-	-

hydrophila, A. veronii tiovar sobria A. veronii tiovar veronii and A. jandaei, Enterococcus spp., Pseudomonas and Shewanella. The presence of Aeromonas spp. in the gut microflora of M. rosenbergi indicates a potential risk.

Bacteria isolated from prawn intestine were metatolically active exhititing lipase, amylase and gelatinase activity (Tatle 3). Aeromonas spp. recovered from prawn intestine were proteolytic, amylolytic and haemolytic indicating that they are metatolically active and potentially pathogenic as reported earlier in freshwater fish (González et al., 2001). A variety of tacterial species such as Aeromonas sp, Pseudomonas sp and Benekea spp. producing extracellular lipases or proteases have teen implicated in shell disease (Tonguthai, 1995). Vibrio and Aeromonas were isolated from juveniles and adults of Macrobrachium sp. with tlack spot tacterial necrosis/disease (Lomtardi & Latao, 1991; Brady & Lasso de la Vega, 1992). Chen et al. (2003) reported an epizootic yeast and E. faecium co-infection in M. rosenbergii in Taiwan causing a cumulative mortality of 25%.

Table 3. Characteristics of bacteria isolated from Macrobrachium rosenbergii intestine.

Genera	Lipase	Amylase	Gelatinase
Aeromonas spp.	+	+	+
Enterobacteriaceae*	+	-	+
Shewanella	+	+	+
Flavobacterium	+	-	+
Moraxella/Acinetobacter	+	-	-
Pseudomonas/Xanthomonas	+	+	+
Bacillus	+	+	+

* Enterobacteriaceae were identified as Enterobacter cloacae, Citrobacter freundii.

The present study revealed that farmed freshwater prawn inhatit consideratle populations of tacteria in their gastrointestinal tract. Contamination of editle portions of prawn could originate from gastrointestinal sources. The presence of potential human pathogens such as Aeromonas hydrophila, Aeromonas veronii tiovar sobria, Aeromonas veronii tiovar veronii, Enterobacter faecium, Enterobacter faecalis and Enterobacter cloacae

suggests that handling and cross contamination may cause diseases in susceptitle individuals. The study indicated high level of pathogens and indicator tacteria such as faecal coliforms and enterococci in the intestine of prawns. Good farm management practices need to te adopted to improve hygienic status of farm reared prawns.

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