



Original Research Article

Populations of *Escherichia coli* and *Pseudomonas aeruginosa* in the gut of farmed giant freshwater prawn *Macrobrachium rosenbergii*

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ABSTRACT

In this study, *Escherichia coli* and *Pseudomonas aeruginosa* populations in the gut of the giant freshwater prawn, *Macrobrachium rosenbergii* cultured in an Aqua farm at Nallepully, Palakkad, Kerala, India, was investigated to understand the quarantine and food safety. The physico-chemical characteristics of pond water was as follow: temperature, $24.2 \pm 0.6^\circ\text{C}$; pH, 6.7 ± 0.3 ; DO, 6.5 ± 0.4 ; TDS, 1200 ± 27 ; BOD, 42 ± 3.9 ; COD, 140 ± 7.2 and ammonia, 0.24 ± 0.03 mg/l. These parameters fall within the recommended range prescribed for aquaculture of *M. rosenbergii*. The digestive tract was dissected out, homogenized with phosphate buffered saline (pH, 7.2) under aseptic condition, the homogenate was serially diluted and the aliquot was seeded over the surface of nutrient agar plates and incubated. *E. coli* was cultured on Trypticase soy agar and MacConkey agar, and *P. aeruginosa* was cultured *Pseudomonas* isolation agar medium, and they were identified and confirmed by routine biochemical tests. Among the two bacterial species, *E. coli* was the most abundant at 7.6×10^5 cfu/g than *P. aeruginosa*, 2.4×10^5 cfu/g. In this study, though *E. coli* is a beneficial bacterium but its population was at risk, whereas *P. aeruginosa* is a harmful bacterium and its population should be limited under the check in the culture pond to maintain quality production of *M. rosenbergii*.

Keywords

Macrobrachium rosenbergii,
Escherichia coli,
Pseudomonas aeruginosa.

Introduction

The culture practices of freshwater prawn, particularly, the giant river prawn, *Macrobrachium rosenbergii* known as “scampi” is rapidly expanding in many countries. In India, *M. rosenbergii* is one of the important commercial crustaceans as it has good demand in both domestic and export markets (MPEDA, 2010; FAO, 2013). Production of healthy and quality seeds has been a major obstacle in expansion of the culture of *M. rosenbergii*.

A complex of environmental factors, microbiological profiles and management practices influence the success of the production cycle. In rearing *M. rosenbergii*, survival rates are dependent on several factors such as stocking densities (Marques *et al.*, 2000), water volume/ surface area (D’Abramo *et al.*, 2000), pH level of water (Chen and Chen, 2003), and food supplement (Barros and Valenti, 2003). Probiotics, *Lactobacillus thermophilus*,

Lactobacillus halveticus, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Lactobacillus rhamnosus* and *Streptococcus lactics* and *Vibrio alginolyticus* are used as water additives to maintain the water quality (Salim *et al.*, 2005; Talpur *et al.*, 2012). Probiotics products: KKU02, KKU03, Biogen[®], Binifit[™], and Probiotics: *Bacillus subtilis*, *Bacillus licheniformis*, *Enterococcus faecium*, *Lactobacillus sporogenes*, *Lactobacillus ceremoris*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae* are as feed supplements to improve growth, survival and health including promotion of nutrient metabolism and innate immune response of *M. rosenbergii* (Suralikar and Sahu, 2001; Venkat *et al.*, 2004; Keysami *et al.*, 2007; Deeseenthum *et al.*, 2007; Shinde *et al.*, 2008; Saad *et al.*, 2009; Rinisha *et al.*, 2010; Seenivasan *et al.*, 2011, 2012a-d).

Several reasons are attributed to the low production in freshwater prawn culture. For example, poor feeding management and microorganisms particularly bacteria both beneficial (nutrients recycling, organic matter degrading etc.) and harmful (as parasites) are play vital roles in the pond ecosystem (Phatarpeker *et al.*, 2002; Lalitha *et al.*, 2010). Biological contaminants such as viruses, bacteria, fungi, protozoa and helminthes constitute the major cause for *M. rosenbergii* (Oanh *et al.*, 2001; Rodriguez *et al.*, 2001; Sahul Hameed *et al.*, 2004; Lalitha *et al.*, 2010). Microorganisms have been implicated in many disease conditions, viral diseases such as hepatopancreatic parvovirus-type virus (HPV-type) (Anderson *et al.*, 1990; Gangnonngiw *et al.*, 2009), *Macrobrachium* muscle virus (MMV) (Tung *et al.*, 1999), and nodavirus (MrNV) and extra small virus (XSV) (Sahul Hameed *et al.*, 2004) is associated with *Macrobrachium rosenbergii*

white tail disease (WTD) or white muscle disease (WMD). Bacterial diseases, bacterial necrosis (Aquacop, 1977), larvae mid-cycle disease (Anderson *et al.*, 1989) due to *Alcaligenes* spp., and *Enterobacter* spp., *Spiroplasma* disease due to a novel pathogen *Spiroplasma* MR-1008 (Liang *et al.*, 2011), and black spot, brown spot and shell disease are caused by *Vibrio* spp., *Pseudomonas* spp., and *Aeromonas* spp., (FAO, 2014). Fungal agents, *Fusarium solani*, *Debaryomyces hansenii* and *Metschnikowia bicuspidate* caused idiopathic Muscle Necrosis (IMN) in larvae (FAO, 2014). Outbreak of the viral, bacterial and fungal diseases increases risk, deterring investment and commercial development of intensive larval culture and grow out systems of freshwater prawn species, *M. rosenbergii*, *Macrobrachium nipponense*, *Macrobrachium malcolmsonii* and *Macrobrachium amazonicum* owing to the large-scale mortality.

In view of the interest in microbial safety of aquaculture products, the collection of prawn microflora information is imperative (Reilly and Kaferstein, 1997). Thus, microbiota settlement is a key component to promote health due to competitive mechanisms and enhance development and maturation of the immune system. Therefore in order to understand the health status and consumer safety of *M. rosenbergii* cultured in an aquaculture farm at Nallepully, Palakkad, Kerala, India, the present study was carried out to determine whether the gut of *M. rosenbergii* have *E. coli* (a beneficial bacterium) and *P. aeruginosa* (a harmful bacterium).

Materials and Methods

M. rosenbergii adults were taken from artificial earthen pond at the freshwater prawn culture station located in Nallepully,

Palakkad, Kerala, India. The pond was completely dependent upon the supply of groundwater as a water source throughout the year. Water was added in the pond to compensate for the loss from evaporation and seepage. Fertilizers together with artificial feed were used. The pond water physico-chemical parameters, such as dissolved oxygen (DO), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD) and ammonia (NH₃) was measured using the method of APHA (2005). The pH was measured by electronic digital pH meter (Model-Century, CP901). The surface water temperature was checked with digital thermometer.

For the isolation and identification of bacteria from prawn's gut, the procured prawns were immediately deactivated by keeping them in freezer at -20°C for 10 minutes. Then the surface was sterilized with 50 ppm formalin for 30 seconds in order to remove the external flora. Then the digestive tract was dissected out individually and homogenized with phosphate buffered saline (pH, 7.2) under aseptic condition. Afterwards the homogenate were serially diluted up to 10⁻⁵ dilution individually. From this 0.5 ml of aliquots was taken and seeded over the surface of freshly prepared nutrient agar plates and incubated at 37°C for 24 h. The loop-full culture was taken from agar plates and mixed with nutrient broth for 24 h at 35°C. This culture was used to further study. This isolates were inoculated over Trypticase Soy Agar and MacConkey agar for *E. coli*, and *Pseudomonas* Isolation Agar for *P. aeruginosa* and they were incubated at 37°C for 24 h. The bacterial colonies were enumerated with the formula, Bacterial count (CFU/g) = Number of colonies × Dilution factor/ Volume of sample (g). The presence of these bacteria were confirmed through routine biochemical tests, such as Gram's staining, motility test, indole test,

methyl red test, Voges-Proskauer test, citrate utilization test, triple sugar iron test, urease test, oxidase test and catalase test following the criteria described in the Bergey's Manual of Systematic Bacteriology (Holt *et al.*, 1996).

Result and Discussion

Table 1 depicts the physico-chemical parameters, such as temperature (24.2±0.6° C), pH (6.7±0.3), DO (6.5±0.4 mg/l), TDS (1200±27 mg/l), BOD (42±3.9 mg/l), COD (140±7.2 mg/l) and ammonia (0.24±0.03 mg/l) of the culture pond water. The ideal range of physico-chemical parameters for culture of *M. rosenbergii* have been reported by many authors and they fall as given below: temperature, 21.9-33.5° C; pH, 6.7-8.5; DO, >7.0 mg/l; TDS, 76.5-128 mg/l; BOD, 2.3-8.2 mg/l; COD, 5.3-17.6 mg/l; NH₃, 0.07-0.14 mg/l; nitrate-N 0.01-0.08 mg/l; Nitrite-N, 0.001-0.006 mg/l; PO₄, 0.07-0.1 mg/l (Fair and Foftner, 1981; Sandifer *et al.*, 1983; Boyd and Tucker, 1998; Boyd, 2000; New, 2002; Correia *et al.*, 2002; Lalitha and Surendran, 2004; Yathavamoorthi *et al.*, 2010; Prasad *et al.*, 2012). In the present study, the pH of pond water was in ideal state, the DO was slightly lower from the recommended value, whereas, TDS, BOD, COD and ammonia was much higher than the recommended value. The recorded values of TDS, BOD, COD and ammonia indicate the fact that the pond water may be polluted. However, the prawns were thrived in the environment, but we do not know that how long it would continue. Therefore, it requires dilution by addition of freshwater/ ground water.

The bottom layer of pond water, where prawns spend most of the time, may become hypoxic or even anoxic, due to organism's respiration and decomposition of accumulated organic matter of unconsumed food, and faeces, particularly at night time,

and these hypoxic conditions can lead to poor immune responses in culture organism and susceptible to pathogenic infections, which would certainly threaten prawn's life. Hence, DO often been considered an important environmental factor determining the success of prawn culture, and DO value >5 mg/l have always been recommended for intensive culture practice of *M. rosenbergii* (Cheng and Chen, 2002; Cheng *et al.*, 2002a; Cheng *et al.*, 2003). BOD range of 2 to 4 mg/l does not show pollution while levels beyond 5 mg/l are indicative of serious pollution (Clerk, 1986). BOD level between 3.0-6.0 ppm is optimum for normal activities of fishes; 6.0-12.0 ppm is sub-lethal to fishes and >12.0 ppm can usually cause fish kill due to suffocation (Bhatnagar *et al.*, 2004). Santhosh and Singh (2007) recommended optimum BOD level for aquaculture should be less than 10 mg/l but the water with BOD less than 10-15 mg/l can be considered for fish culture. The COD of water represents the amount of oxygen required to oxidize all organic matter, biodegradable and nonbiodegradable by a strong chemical oxidant. This is an indication of both sewage and industrial pollution. The ideal value of COD should be less than 50 mg/l for fish culture (According to guidelines for Water Quality Management for fish culture in Tripura). COD value between 6.0-20.0 mg/l is reported for fish culture (Bhavimani and Puttaiah, 2014). These water quality variables were also reported suitable for bacterial proliferation.

In the present study, the presence of typical faecal coliform bacterium, *E. coli*, and the pathogenic bacterium, *P. aeruginosa* was identified in the gut of *M. rosenbergii* through culture morphology and biochemical confirmation tests (Figs.1, 2 and 3; Tables 2 and 3). Among these two bacteria, *E. coli* was found to be present at 7.6×10^5 cfu/g, whereas, *P. aeruginosa* was found to be at 2.4×10^5 cfu/g, and at these

colony levels the prawns were thrived in the culture pond. Usually, faecal contamination in any water body raises coliform pathogens (Geldrieck, 1990; Harish *et al.*, 2003; Lalitha and Surendran, 2004; Prakash and Karmagam, 2013). The ideal colony numbers need to be evaluated both in water and prawn's gut to assess the pathogenesis of these bacteria. However, the numbers seems to be alarming. Therefore, proper regulatory measures suggested to limits the *E. coli* population in the culture pond. The Environment Protection Agency (EPA) recommends *E. coli* as the best indicator of health risk from water contact in recreational waters. Lalitha *et al.* (2010) reported that Faecal coliform, *E. coli* was found to be $3.39-5.04 \log_{10}$ cfu/g from *M. rosenbergii* gut. It was not the normal flora of bacteria in prawn. The numbers of aerobic heterotrophic bacteria from *M. rosenbergii* eggs, larvae and post larvae, which were found to vary from 7.9×10^4 to 8.2×10^6 , 0.8×10^5 to 81.1×10^5 and 38.3×10^5 to 10.9×10^6 cfu/g respectively (Sahul Hameed *et al.*, 2003). It has been reported that *E. coli*, *Pseudomonas* spp., *Enterobacter* spp., *Vibrio* spp. *Aeromonas* spp. and *Staphylococcus aureus* were isolated from the gut of pond cultured *M. rosenbergii* (Prakash and Karmagam, 2013).

Members of the genus *Pseudomonas* are a ubiquitous group of Gram-negative, rod-shaped and motile bacteria showing metabolic versatility. They can survive in environments hostile to many other bacteria. This is one of the most diverse bacterial genera, containing over 60 validly described species (Jensins *et al.*, 2004). Recently, *P. aeruginosa* was identified as harmful to *M. rosenbergii* (Ramalingam and Ramarani, 2007). Prakash and Karmagam (2013) pointed that *P. aeruginosa* was found to be 18.3% and 30% from farm cultured *M. rosenbergii*.

Table.1 Physicochemical characteristics of culture pond water

Parameters	Values (mg/l)
Temperature (°C)	24.2±0.6
pH	6.7±0.3
DO	6.5±0.4
TDS	1200±27
BOD	42±3.9
COD	140±7.2
Ammonia	0.24±0.03

All values are mean ± SD (n=3).

DO, Dissolved Oxygen, TDS, Total Dissolved Solids;

BOD, Biochemical Oxygen Demand; COD, Chemical Oxygen Demand.

Table.2 Population of *E. coli* and *P. aeruginosa* in the gut of farmed *M. rosenbergii*

Bacterial Species	Population (cfu/g)
<i>E. coli</i>	7.6×10^5
<i>P. aeruginosa</i>	2.4×10^5

Table.3 Biochemical characterization of *E. coli* and *P. aeruginosa* culture from the gut of farmed *M. rosenbergii*

Characteristics	<i>E. coli</i>	<i>P. aeruginosa</i>
Cell Morphology	rods	rods
Gram's Staining	-	-
Motility Test	+	+
Indole Test	+	+
Methyl Red Test	+	-
Voges-Proskauer Test	-	+
Citrate Utilization Test	-	+
Urea Test	-	+
Catalase Test	+	+
Oxidase Test	-	+
Triple Sugar Iron Test	Acid + H ₂ S	Acid + H ₂ S

Fig.1 *E. coli* on Trypticase soy agar medium

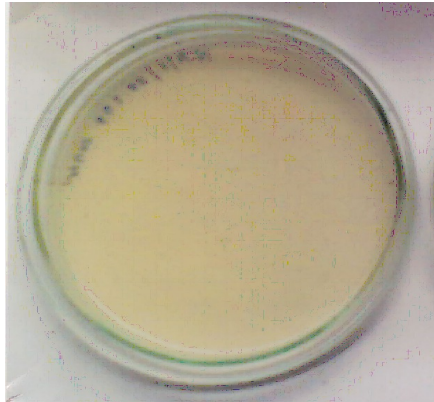


Fig.2 *E. coli* on MacConkey agar medium

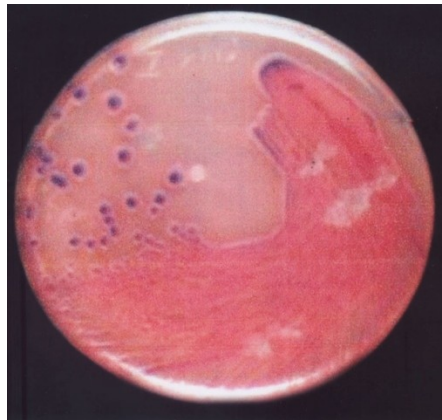
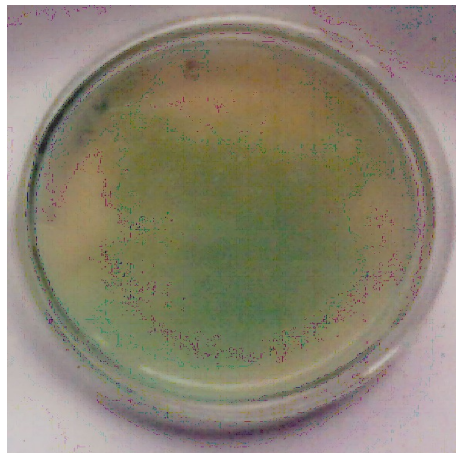


Fig.3 *P. aeruginosa* on *Pseudomonas* isolation agar medium



It has been reported that *Pseudomonas* spp. has also been reported in the digestive tract of *M. rosenbergii* cultured in concrete tank (Al-Harbi, 2003). In aquaculture, *P. aeruginosa* and *Pseudomonas fluorescens* especially are the most frequently isolated opportunistic pathogenic species (Shiose *et al.*, 1974; Alderman and Polglase, 1988).

This study concludes that though *E. coli* is a beneficial bacterium its population was seemed to be at risk in the gut of *M. rosenbergii*. *P. aeruginosa* is a harmful bacterium and its population should be checked and maintained under the limit in the culture pond to quarantine the quality production of *M. rosenbergii*.

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