

Bacterial Diversity of Giant Freshwater Prawn, *Macrobrachium rosenbergii* and Screening for Probiotic Potential Bacteria

Mujeeb RKM^{1*}, Jesmi Yousuf², Hatha MAA³ and Thomas AP²

¹Department of Aquaculture and Fishery Microbiology, MES Ponnani College, Ponnani, India

²School of Environmental Sciences, Mahatma Gandhi University, Kottayam, India

³School of Marine Sciences, Cochin University of Science and Technology, Cochin, India

Received Date: July 11, 2017, **Accepted Date:** October 31, 2017, **Published Date:** November 09, 2017.

*Corresponding author: Mujeeb Rahiman KM, Department of Aquaculture and Fishery Microbiology, MES Ponnani College, Ponnani, India, E-mail: rkmmujeeb@gmail.com.

Abstract

In an attempt to explore the probiotic potential of bacteria found in the endemic habitat of *Macrobrachium rosenbergii*, bacteriology of the samples associated with the natural environment of *M. rosenbergii* has been studied. A total of 752 isolates were characterized up to genus level. While feed items and the intestine of adult *M. rosenbergii* showed highest total viable count (2.20×10^7 to 7.20×10^8 cfu g⁻¹ and 2.95×10^8 to 1.37×10^9 cfu g⁻¹ respectively), it was relatively low in the water (6.00×10^3 to 1.40×10^4 cfu ml⁻¹) as well as in the larval samples (8.40×10^4 to 6.40×10^5 cfu g⁻¹). Characterisation of the various genera of heterotrophic bacteria revealed good diversity of both gram negative and gram positive genera. Bacterial genera such as *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Vibrio*, *Bacillus*, *Streptococcus* and *Enterobacteriaceae* were identified from all the samples. The screening and probiotic potential study found that *Brevibacillus laterosporus* isolated from the larval sample showed antibacterial activity against fish and prawn pathogens. No adverse effect was noticed when the Post Larvae (PL) of *M. rosenbergii* challenged with the selected probiotic strains and showed good hydrolytic enzyme potential.

Keywords: Heterotrophic bacteria; Natural environment; *M. rosenbergii*; Antibacterial activity; *Brevibacillus laterosporus*

Introduction

Aquaculture is developing very rapidly in recent years and has significant role in the economic development of the nation; also contribute to the world supply of food and food security. Both developed and developing countries practise small scale to large scale aquaculture systems and have important contribution to food supply, income generation and trade. Approximately 90% of global aquaculture production is based in Asia [1,2]. *Macrobrachium rosenbergii*, popularly known as Giant freshwater prawn has a great export market worldwide and is an excellent candidate for freshwater aquaculture. Being the largest species, *M. rosenbergii* is commercially exploited from Vembanad Lake, Kerala, India with a peak fishing season during monsoon and post monsoon. The health of aquatic animals has greatly influenced by the environment which they live and their health status is directly influenced by the presence of microorganisms when compared to the health status of terrestrial animals or humans [3].

Disease outbreak is promoted by intensification and represents one of the biggest causes of loss in aquaculture [4–6]. Since conventional disease management strategy, such as treatment with antibiotics is leading to unfavourable consequences like emergence of drug resistant bacteria, researchers are encouraged to find out alternative strategies such as vaccination, use of immunostimulant and probiotics for the health management of aquatic animal is being tried out. The regulation of antibiotics by European Union [7] and the demand of alternative products against antibiotics [5], open the way to use environment friendly products. The World Bank invested

US\$ 275 million during 1996–2010 for disease related research in shrimp aquaculture [8]. Use of probiotics as an alternative source instead of antibiotics is proving to be an environment friendly mode of health management and capable of modulating the immune system [9]. Recently, the study using biofloc technology combined with the addition of probiotics showed the enhancement of disease resistance and nonspecific immune responses in *M. rosenbergii* [10].

The research for beneficial probiotic bacterial cultures are reported in recent years for the culture of commercially important aquaculture organisms [11,12]. The selection and development of probiotics for different cultured species in India assumes greater significance considering the rejection of farm raised shrimp by EU, citing presence of trace levels of antibiotics in the shrimp. *M. rosenbergii* is emerging as a popular species for aquaculture in India owing to many favourable attributes. Cruz et al. [5] strongly suggested the importance of microbial ecology study and the relationship of microbes with the cultured organism and the importance of phylogenetic identification of probiotic microorganisms. Taking this into consideration an attempt has been made to study bacteriology associated with the natural environment of *M. rosenbergii* and evaluate the probiotic potential of these bacterial isolate to use in the hatchery and culture system of *M. rosenbergii*.

Materials and Methods

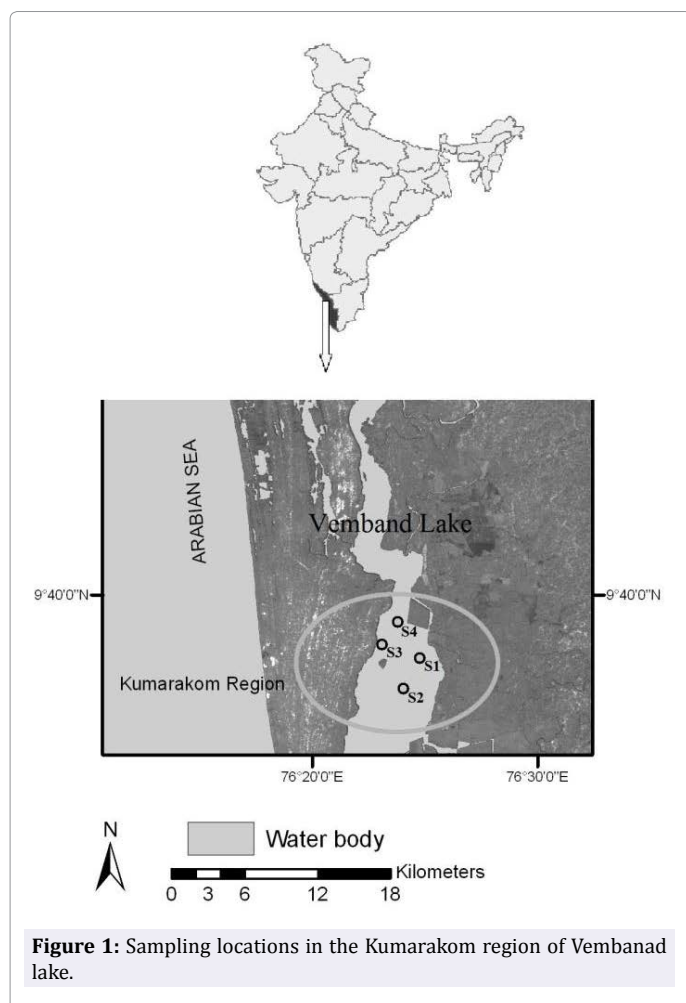
Description of the Study Area

The Vembanad estuary is one of the Ramsaar site in India and it is one of the largest tropical wetland with mangroves. It is located between 9° 29' and 10° 10' North latitude and 76° 13' and 76° 31' East longitude, extending a stretch of 60 Km from Cochin bar mouth in the north to Alleppey in the south with an estimated area of 21050 ha. Kumarakom region of Vembanad estuary was chosen as the sampling area and this region is a part of Kuttanad known as the home ground of *M. rosenbergii* [13].

Collection of Water Samples

Water, sediment and adult *M. rosenbergii* samples were collected from four different stations (Figure 1) and necessary precautions were taken to minimize the contamination of the sample. Water and sediment samples were collected in sterile bottles and sterile jars respectively. The adult *M. rosenbergii* were collected by fisherman in live condition and brought to the laboratory for analysis. The larvae and Post Larvae (PL) were collected by using 500 µm plankton net and the collected larvae were identified into different stages of growth by using the manual for the culture of *M. rosenbergii* [14].

Two samples of larval and PL feed items were collected using a



500 μm plankton net and adult feed items from the bottom of the lake with the help of fisherman. The planktonic feed items of larvae and PL mainly consisted of zooplankton, small worms, larval stages of invertebrates and small amounts of phytoplankton. Crustacean, mollusc, filamentous algae, plants and remnants of plants, etc. were identified as adult feed of *M. rosenbergii*. All the samples except the adult *M. rosenbergii* were kept in an icebox and immediately brought to the laboratory for analysis.

Analysis of the Physio-Chemical Parameters

Physico-chemical parameters of water samples such as temperature were measured *in situ* using centigrade thermometer, salinity by salinity refractometer (Atago, Japan) and pH by digital pH meter (Eutech, Singapore). Dissolved oxygen was estimated by the Winkler method [15]. The pH of sediment was measured using the method described by Sharmila et al. [16].

Bacteriological Analysis

Preparation of samples: Water and sediment samples were serially diluted aseptically to 10^{-3} and 10^{-5} . Larvae and PL were washed in 0.1% benzalkonium chloride and washed in sterile water and the water adhered to it was removed by sterile blotting paper before weighing. The samples were homogenized aseptically by using glass homogeniser and diluted to 10^{-5} . The intestine of *M. rosenbergii* was removed aseptically, weighed and homogenized in glass homogeniser and serially diluted to 10^{-6} . The food samples were washed three times with sterile water, and homogenized aseptically in glass homogeniser after weighing and diluted to 10^{-6} .

Estimation of total bacterial load: Estimation of bacterial load was done by spread plate method by using tryptone soya agar (TSA) and $\frac{1}{2}$ strength Zobell's marine agar ($\frac{1}{2}$ ZMA). Total viable count (TVC) of bacteria were enumerated and selected for isolation of bacteria after incubating the plates at 30°C for 24–48 hours plates with 30 to 300 colony-forming units (cfu).

Isolation and identification of bacterial isolates: All bacterial colonies were purified before identification and a total of 752 bacterial isolates from water (131 isolates), sediment (114 isolates), intestine of adult *M. rosenbergii* (155 isolates), larvae and PL (206 isolates) and food samples (146 isolates) were characterized to the genus level using the taxonomic keys [17–20].

Probiotic Potential Study

Pathogenic bacteria used: Fish, prawn and human pathogens such as *Aeromonas hydrophila* (MTCC 646), *Vibrio parahaemolyticus* (MTCC 451), *Vibrio harveyi* (CUSAT, Kerala), *Vibrio vulnificus* (MTCC 146), *Escherichia coli*, *Salmonella newport* and *Salmonella typhi* (isolated from Vembanad lake) were used as pathogens to determine the antibacterial activity of the probiotic strains against pathogenic bacteria.

Determination of antibacterial activity of isolates by well diffusion and cross streak method: For the determination of antibacterial activity by well diffusion method, 2 ml of a young culture (16–18 hour in TSB) of *A. hydrophila*, *V. vulnificus*, *V. harveyi*, *V. parahaemolyticus*, *S. typhi*, *E. coli*, and *S. newport* were prepared and poured over the TSA medium and incubated at 30°C for 15 minutes. Sterile gel puncher was used to punch three millimetre diameter wells in the plates and 30 microliters of 18 hours bacterial culture in TSB was pipette into the wells and incubated at 30°C for 24 hours. Clear zone around the wells was noted as the presence of antibacterial activity.

In cross-streak method, an 18 hours bacterial culture in TSB that showed positive results in well diffusion method was streaked on TSA plate as a thick band with 2 cm in width on the centre of the TSA plate. The growth was scrapped after incubation for 24 hours at 30°C , and treated by chloroform for 15 minutes. After air drying for ten min to remove any residual chloroform, the 18 hours old cultures of pathogenic bacteria were streaked vertical to bacterial band and incubated for 24 hours at 30°C . A linear clear zone was noted as the presence of antibacterial activity.

In vivo experiment for the safety of probiotic strain to post larvae of *M. rosenbergii*: For testing the safety of probiotic strain to post larvae, 18 hours culture of potential probiotic bacteria were added to 1 L beaker containing 500 ml sterilised filtered freshwater to obtain 10^5 cells ml^{-1} . To each beaker, 25 numbers of PL (PL-20) of *M. rosenbergii* were introduced and any signs of disease or mortality up to 96 hours were monitored. The experiment was duplicated and the control was maintained without any bacterial inoculum.

PCR Amplification of the 16S rDNA, Sequencing and Phylogenetic Analysis of Probiotic Bacteria

DNA extraction was done by Bacterial Genomic DNA (prep) Kit (Chromous Biotech, India) and 16S rDNA genes were amplified with the universal primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTACCTTGTTACGACTT-3') by using the polymerase chain reaction (PCR) [21].

The nearest taxa of the 16S rRNA gene sequence (1418–1542 bases) was identified by BLAST sequence similarity search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The CLUSTAL W software was used to align 16S rRNA gene sequences and Maximum Likelihood

(ML) and Neighbour-Joining (NJ) methods with MEGA version 5 [22] were used to construct the phylogenetic tree.

Hydrolytic Enzyme Activity

The ability of *B. laterosporus* to utilize different substrate was done by using the BBL CRYSTAL™ Identification (ID) system for Gram-Positive (GP) bacteria. A total of 20 different substrates were used to check the hydrolytic enzyme activity.

Result and Discussion

Physico-Chemical Parameters

Physico-chemical parameters of water and sediment samples collected from the sampling sites revealed that temperature, pH and DO of water ranged between 28.5°–31.0°C, 5.8–6.7 and 6.9–7.2 mg L⁻¹ respectively. The salinity of water samples were within the range of 0–4 ppt. The pH of sediment samples ranged between 5.48–6.46. Similar pH values were reported by Nandan and Unnithan [23] from Vembanad Lake and the physico-chemical parameters analysed were within the optimal range for growth and survival of *M. rosenbergii* in their natural environment [14]. Low salinity and the slightly acidic nature of water and sediment samples recorded in the present study are in agreement with the results [24] from the Kuttanad region of Vembanad Lake. Thanneermukam barrage constructed across the Vembanad Lake to prevent the ingress of saline water into the rice fields of this area cause the low salinity of water.

Bacteriological Results

Bacteriology of Water, Sediment and Intestine: The TVC load of water samples ranged from 6.00 × 10³ to 1.40 × 10⁴ cfu ml⁻¹ and that of sediment samples ranged from 8.24 × 10⁵ to 1.42 × 10⁶ cfu g⁻¹ were in concurrence with those of Sharmila et al. [16] and Harish et al. [25] from shrimp farm. TVC load from the intestine of adult *M. rosenbergii* samples ranged from 2.95 × 10⁸ to 1.37 × 10⁹ cfu g⁻¹. The TVC load of the intestinal tract of *M. rosenbergii* were

comparable to those reported by Phatarpekar et al. [26] in digestive tract of *M. rosenbergii*, as well as in the gut samples of *P. indicus* from backwaters and *P. monodon* from seawater [27,28]. However, Al-Harbi [29] reported the bacterial count of 3.40 × 10⁵ to 8.70 × 10⁶ cfu g⁻¹ from the digestive tract of *M. rosenbergii* cultured in concrete tanks.

Out of 16 genera identified, 13 genera were present both in water and sediment samples and 10 genera were present in all the samples (Table 1, Figure 2). Cavallo et al. [30] reported that most common bacteria in water and sediment samples were Gram negative rods. In contrast, the study [31] reported higher proportions of Gram positive bacteria from sediment. Out of 16 genera isolated from the present study 12 genera were previously reported [26,32]. Results revealed that bacterial genera of water were more similar to genera encountered in the sediment sample which is supported by observation of Austin and Allen [33] from freshwater reservoir fishery. All the bacterial genera found in the

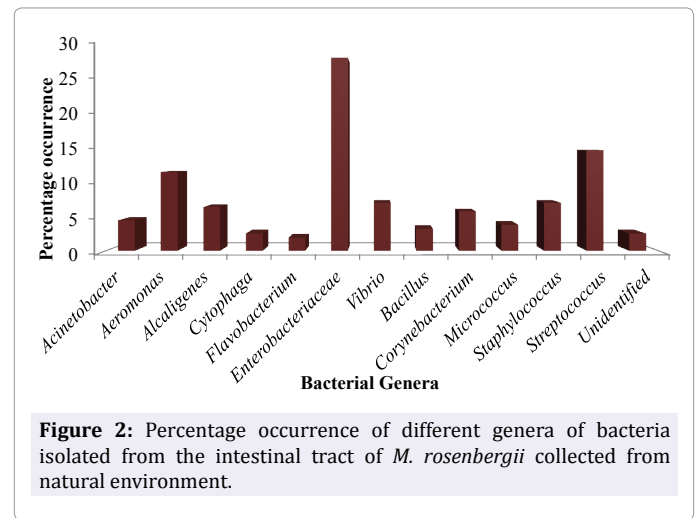


Figure 2: Percentage occurrence of different genera of bacteria isolated from the intestinal tract of *M. rosenbergii* collected from natural environment.

Genera	Percentage Occurrence				
	Site 1 28* (23)#	Site 2 (44) (35)	Site 3 (32) (25)	Site 4 (27) (31)	Total (131) (114)
Gram Negative					
<i>Acinetobacter</i>	28.57 (--)	15.91 (25.71)	15.61 (--)	-- (25.81)	15.27 (14.91)
<i>Aeromonas</i>	-- (4.35)	15.91 (--)	-- (--)	14.82 (--)	8.40 (0.88)
<i>Alcaligenes</i>	7.14 (--)	9.09 (--)	21.87 (12.00)	3.70 (9.68)	10.69 (5.26)
<i>Cytophaga</i>	14.29 (--)	4.55 (2.86)	-- (4.00)	-- (3.23)	4.58 (2.63)
<i>Flavobacterium</i>	3.57 (8.70)	-- (8.57)	-- (--)	-- (--)	0.76 (4.39)
Enterobacteriaceae	-- (--)	11.37 (22.86)	18.75 (--)	25.92 (29.02)	13.74 (14.91)
<i>Moraxella</i>	-- (4.35)	11.36 (--)	-- (8.00)	-- (--)	3.82 (2.63)
<i>Pseudomonas</i>	10.72 (4.35)	-- (--)	3.13 (--)	3.70 (--)	3.82 (0.88)
<i>Vibrio</i>	3.57 (13.04)	-- (--)	21.87 (16.00)	14.82 (--)	9.16 (6.14)
Gram Positive					
<i>Bacillus</i>	-- (39.13)	4.55 (28.57)	3.13 (40.00)	11.11 (25.81)	4.58 (32.46)
<i>Corynebacterium</i>	3.57 (26.08)	4.55 (8.57)	-- (--)	-- (--)	2.29 (7.89)
<i>Kurthia</i>	-- (--)	-- (2.86)	-- (--)	-- (--)	-- (0.88)
<i>Listeria</i>	-- (--)	-- (--)	-- (8.00)	14.82 (6.45)	3.05 (3.51)
<i>Micrococcus</i>	7.14 (--)	-- (--)	3.13 (4.00)	-- (--)	2.29 (0.88)
<i>Staphylococcus</i>	21.43 (--)	2.27 (--)	3.13 (--)	3.70 (--)	6.87 (--)
<i>Streptococcus</i>	-- (--)	18.17 (--)	6.25 (--)	-- (--)	7.63 (--)
Unidentified	-- (--)	2.27 (--)	3.13 (8.00)	7.41 (--)	3.05 (1.75)
Total	100.00	100.00	100.00	100.00	100.00

Table 1: Percentage occurrence of different genera of bacteria, in water and sediment samples from the natural environment of *M. Rosenbergi*.

gastrointestinal tract of *M. rosenbergii* were also detected in the samples from its natural environment revealed the importance of keeping good microbial quality of their environment in the culture system.

Acinetobacter and members of the family Enterobacteriaceae were predominant ones in water, while *Bacillus*, *Acinetobacter* and members of the Enterobacteriaceae family were predominant genera from sediment samples. *Staphylococcus* and *Streptococcus* were identified only from sediment samples and *Kurthia* from water samples. Percentage occurrence of different genera of bacteria from the intestinal tract of *M. rosenbergii* (Figure 2) revealed the predominance of members of the family Enterobacteriaceae, *Streptococcus* and *Aeromonas*. The opportunistic pathogens like *Vibrio* and *Aeromonas* were isolated from water, sediment and intestinal samples. New MB [34] reported that the *Vibrio* are not primary pathogens and exist in and around crustaceans in marine or brackish water environments as part of their normal microflora and researchers frequently observed the presence of *Vibrio*, and *Aeromonas* from freshwater and marine water culture system [35–37]. *Listeria* in water and sediment samples observed less frequently and it is observed by previous researchers from marine water, sediment, *P. monodon* and various seafoods [38–40]. Several researchers reported the association of *Streptococcus* with the mucosa of the gastrointestinal tract [41–43]. *Streptococcus* is one of the lactic acid producing bacteria and they can survive in the environment and able to adhere to the exposed surface of the epithelial cell [44]. The results [29,37] suggested that aeromonads are indigenous in *M. rosenbergii* and fish intestine, water and sediments. Lalitha and Surendran [45] also reported the presence of Gram positive genera such as *Micrococcus*, *Bacillus* and coryneforms from *M. rosenbergii*'s gastrointestinal tract that are similar to the present findings.

It was reported that in the culture system, the decline in *M. rosenbergii* production has been mostly due to disease outbreaks [46,47]. The aquatic organisms are attached by variety of bacteria on their surfaces, tissues and body fluids and all aquatic organisms are exposed to various microflora that harbour multiple pathogens and immunological factors, food and animal physiology are some of the factors which affect the balance of intestinal microbiota [48]. The higher number in the digestive tract than the surface water representing a favourable place for the bacterial species [45,49].

Bacteriology of *M. rosenbergii* Larvae and PL: The TVC load from various larval and PL stages ranged from 8.40×10^4 to 6.40×10^5 cfu g⁻¹. Miyamoto et al. [50] and Sahul Hameed [36] also noted similar bacterial counts larvae during rearing as in the present study. Twelve genera were isolated from larval and PL samples of *M. rosenbergii* (Table 2) with predominance of *Vibrio*, *Moraxella*, *Acinetobacter*, *Alcaligenes* and *Bacillus*. All the genera encountered in the water samples and more than 80% of genera from intestinal samples were also isolated from larvae and PL of *M. rosenbergii* corresponding to the environmental samples. Anderson et al. [32] also reported close similarity of bacteria between washed larval tissue slurries and hatchery water. The findings of present study differs with the observations of Phatarpekar et al. [26] who studied microflora of freshwater prawn hatchery and reported the predominance of *Alcaligenes*, *Pseudomonas*, *Streptococcus* and members of the family Enterobacteriaceae from hatchery reared larvae. One of the reasons for this may be the disinfection process of water that is used for the hatchery operation which may help the dominance of certain bacteria.

Bacteriology of food items of *M. rosenbergii*: Load of TVC was significantly high ($p < 0.05$) in adult feed samples than larval feed samples. The TVC load in larval and adult feed samples ranged

Genera	Percentage Occurrence				
	Sample 1# (52)*	Sample 2# (56)	Sample 3# (52)	PL (46)	Total (206)
Gram Negative					
<i>Acinetobacter</i>	19.23	10.73	7.69	21.74	14.56
<i>Aeromonas</i>	3.85	--	3.85	4.35	2.91
<i>Alcaligenes</i>	7.69	7.14	3.85	8.70	6.80
<i>Flavobacterium</i>	--	3.57	3.85	4.35	2.91
Enterobacteriaceae	3.85	7.14	3.85	--	3.88
<i>Moraxella</i>	19.23	25.00	19.22	26.07	22.33
<i>Pseudomonas</i>	--	--	3.85	4.35	1.94
<i>Vibrio</i>	19.23	32.14	30.76	13.04	24.27
Gram Positive					
<i>Bacillus</i>	11.53	7.14	3.85	4.35	6.80
<i>Corynebacterium</i>	3.85	3.57	3.85	--	2.91
<i>Micrococcus</i>	--	3.57	3.85	4.35	2.91
<i>Streptococcus</i>	7.69	--	--	--	1.94
Unidentified	3.85	--	11.53	8.70	5.84
Total	100.00	100.00	100.00	100.00	100.00

Table 2: Percentage occurrence of different genera of bacteria recovered from various larval stages and post larvae collected from natural environment of *M. rosenbergii*.

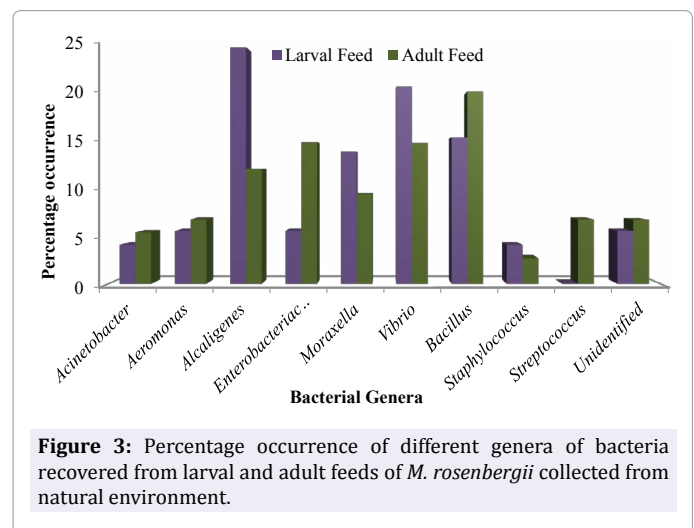


Figure 3: Percentage occurrence of different genera of bacteria recovered from larval and adult feeds of *M. rosenbergii* collected from natural environment.

from 2.20×10^7 to 7.20×10^8 cfu g⁻¹. Feed samples from natural environment revealed high TVC as the feed items contained fish remains, algal and plant matter etc. that are mostly in decayed state. Most of the adult food items were in contact with sediment and was in partially decomposed state, which could have high bacterial load. While the bacterial flora of the larval feed items of *M. rosenbergii* in natural environment (Figure 3) was dominated by of *Alcaligenes*, *Bacillus* was found to be the predominant bacterial genera in adult feed items. *Vibrio* was found to be the second most common genera from both larval and adult feed items. The predominant genera from larval and adult feed items were also found to be abundant in larvae and in the intestine of *M. rosenbergii*. These observations were supported by the findings of Moriarty [51]. Most of the adult feed is seen in bottom sediment surfaces which could have resulted in the dominance of *Bacillus* in the adult feed sample as in sediment sample.

Probiotic Potential Study

A total of 752 bacterial isolates were preliminary screened for the selection of potential probiotic strains. Based on antibacterial

activity against tested pathogens, a *Bacillus* sp. (characterised by phenotypically) isolated from the larval samples of *M. rosenbergii* showed high antibacterial activity against *A. hydrophila*, *V. harveyi*, *V. parahaemolyticus*, *V. vulnificus*, *Salmonella typhi* and *E. coli*. Aquatic candidate probiotics for larviculture have been isolated from adults [52–54] and healthy larvae [55–57] have been reported previously. It was reported that beneficial bacterial preparations that are species-specific probiotics show specific benefits and greater effectiveness in prevention of disease and maintain a healthy intestinal balance and immune response [58,59]. The partial genomic sequencing of the 16S rRNA of *Bacillus* sp. and the blasting of the sequence revealed the identity of this potential probiotic bacterium as *Brevibacillus laterosporus*. The gene sequence of 16S rRNA of the bacterial strain was deposited in GenBank with accession number KF111726. The nearest phylogenetic bacteria similar to *Brevibacillus laterosporus* are shown in figure 4. *Brevibacillus laterosporus* (previously *Bacillus laterosporus*) was first isolated from water [60] and it was reclassified from *Bacillus brevis* cluster, with *Brevibacillus brevis* as the type strain. *Brevibacillus laterosporus* is an important species as a biological control agent and because of its uniqueness in its spore formation and physiological activities [61,62]. *Brevibacillus* sps is recently reported as a probiotic in aquaculture [63], however *Brevibacillus*

laterosporus as prophylactic and health food supplements or novel foods in human was reported years back [64]. The genus *Bacillus*, *Paenibacillus* and *Brevibacillus* are single endospore formers and represent a special case among the bacteria used as probiotics. The use of spore formers as probiotics have some advantages like the shelf life period, resistance to adverse environmental conditions and low cost of production etc.

Before going for the experimental trial, it should be confirm that the probiotic bacteria should not show any pathogenic or adverse effect on host [65] and the probiotic bacteria are then selected according to the antagonistic property against the pathogens and by *in vitro* testing [66–70]. The results of pathogenicity effect of the *Brevibacillus laterosporus* on post larvae of *M. rosenbergii* (Figure 5) showed that the bacteria has no pathogenic effect on the post larvae and the survival was higher with that of control ($p < 0.01$). Hydrolytic enzyme activity of *B. laterosporus* (Table 3) shown that the bacteria were capable of utilising 15 out of 20 substrates used in the BBL crystal GP ID kit. This result shows the high ability of bacteria to produce varieties of different hydrolytic enzymes and their potential to act as a food supplement through feed to help in the digestion of food materials. The antibacterial compounds and varieties of enzymes produced by the *Bacillus* help to control the proliferation of pathogenic bacteria.

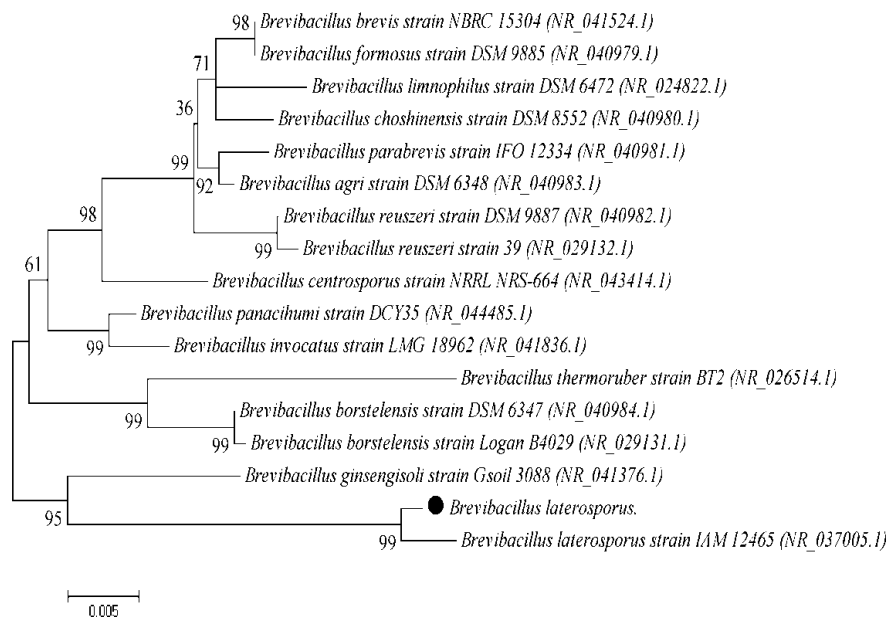


Figure 4: Phylogenetic trees based on 16S rDNA gene sequences showing the relationship of *Brevibacillus laterosporus* with their nearest phylogenetic relatives.

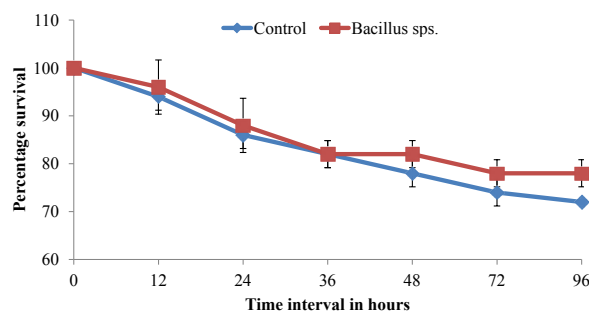


Figure 5: Effect of potential probiotic stain on the survival of the post larvae of *M. Rosenbergii*.

Substrate	Enzymatic Reaction
4MU- β -D-glucoside	Positive
L-valine-AMC	Negative
L-phenylalanine-AMC	Positive
4MU- α -D-glucoside	Positive
L-pyroglutamic acid-AMC	Positive
L-tryptophan-AMC	Positive
L-arginine-AMC	Positive
4MU-N-acetyl- β -D-glucosaminide	Positive
4MU-phosphate	Positive
4MU- β -D-glucuronide	Negative
L-isoleucine-AMC	Negative
p-nitrophenyl- β -D-glucoside	Positive
p-nitrophenyl- β -D-cellobioside	Positive
Proline & Leucine-p-nitroanilide	Positive
p-nitrophenyl-phosphate	Negative
p-nitrophenyl- α -D-maltoside	Positive
o-nitrophenyl- β -D-galactoside (ONPG) & p-nitrophenyl- α -D-galactoside	Positive
Urea	Negative
Esculin	Negative
Arginine	Positive

Table 3: Result showing the enzymatic hydrolysis of different substrates by *Brevibacillus laterosporus* sps.

Conclusion

In the study, an attempt was made to find out the bacterial diversity associated with various life stages of giant freshwater prawn, and screening for probiotic potential among these bacteria. The study strengthens the microbial ecology and correlation of microbial communities (microorganisms on water, sediment, gut and larvae). The screening and probiotic potential study found that *Brevibacillus laterosporus* showed antibacterial activity against fish and prawn pathogens. No adverse effect was noticed when the PL of *M. rosenbergii* challenged with the selected probiotic strains. The present study suggested *Brevibacillus laterosporus* as a promising probiotic candidate for hatchery and culture operations of *M. rosenbergii*. However, thorough studies are suggested with detail evaluation on the effect in vivo with these bacteria.

Acknowledgements

The authors wish to express their gratitude to the University Grants Commission (UGC), Govt. of India, for the financial assistance (F.3-37/2002).

Conflict of Interest

Authors declared that they have no conflict of interest.

References

- Feldhusen F. The role of seafood in bacterial foodborne diseases. *Microbes Infect.* 2000;2(13):1651–60.
- New MB. History and global status of freshwater prawn farming. In: New MB, Valenti WC, editors. *Freshwater Prawn Culture: The Farming of Macrobrachium rosenbergii*. Malden, MA, USA:Blackwell; 2008. p. 1-11.
- Ringo E, Lovmo L, Kristiansen M, Bakken Y, Salinas I, Myklebust R, Olsen ER, et al. Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: a review. *Aquac Res.* 2010;41(4):451–67.
- Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ER, de la Pena LD. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 1990;91(1-2):1–13.
- Martínez Cruz P, Ibáñez AL, Monroy Hermsillo OA, Ramírez Saad HC. Use of Probiotics in Aquaculture. *ISRN Microbiol.* 2012;2012:916845. doi: 10.5402/2012/916845.
- Lakshmi B, Viswanath B, Sai Gopal DVR. Probiotics as antiviral agents in shrimp aquaculture. *J Pathog.* 2013;2013:424123. doi: 10.1155/2013/424123.
- Ronson PJ, Medina-Reyna CE. Probióticos en la Acuicultura. *Ciencia Y Mar.* 2002; 454-49. Spanish.
- Lundin CG. Global attempts to address shrimp disease, Marine/Environmental Paper No. 4 Land, Water and Natural Habitats Division, Environment Department. The World Bank 1996.p. 45.
- Goutam B, Arun KR. The advancement of probiotic research and its application in fish farming industries. *Res Vet Sci.* 2017;115:66–77. doi: 10.1016/j.rvsc.2017.01.016.
- Shuyan M, Jinyu Z, Chenze Z, Longsheng S, Xiaojun Z, Guohong C. Effects of C/N ratio control combined with probiotics on the immune response, disease resistance, intestinal microbiota and morphology of giant freshwater prawn (*Macrobrachium rosenbergii*). *Aquaculture* 2017;476:125–33.
- Hauville M, Zambonino JL, Gordon B, Migaud H, Main K. Effects of a mix of *Bacillus* sp. as a potential probiotic for Florida pompano, common snook and red drum larvae performances and digestive enzyme activities. *Aquacult Nutr.* 2016;22(1):51–60.
- Susmita D, Kausik M, Salma H. A review on application of probiotic, prebiotic and synbiotic for sustainable development of Aquaculture. *J Entomol Zool Stud.* 2017;5(2):422–29.
- Kurup BM, Harikrishnan M. Reviving the *Macrobrachium rosenbergii* (de Man) fishery in Vembanad Lake, India. *NAGA ICLARM Q.* 2000; 23(2):4–9.
- New MB. Farming freshwater prawns: A manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). FAO Fisheries Technical Paper 2002; 428:212.
- APHA. "Standard methods for the examination of water and waste water," American Public Health Association, Washington DC; 1998.
- Sharmila R, Abraham TJ, Sundararaj V. Bacterial flora of semi- intensive pond reared *Penaeus indicus* and the environment. *J Aquacult Trop.* 1996;11:193-202.
- Muroga K, Higashi M, Keetoku H. The isolation of intestinal microflora of farmed red seabream (*Pagrus major*) and black seabream (*Acanthopagrus schlegelii*) at larval and juvenile stages. *Aquaculture* 1987;65:79–88.
- Barrow GI, Feltham RKA. Cowan and Steel's manual for the identification of medical bacteria. Cambridge University press; 1993.
- Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergy's manual of determinative Bacteriology. Lipponcott Williams & Wilkins; 2000.
- Harley JP, Prescott LM. Laboratory exercise in Microbiology, 5th Edn. Mc Graw Hill Companies, Inc; 2004.
- Bosshard PP, Santini Y, Gruter D, Stettler R, Bachofen R. Bacterial diversity and community composition in the chemocline of the meromictic alpine Lake Cadagno as revealed by 16S rDNA analysis. *FEMS Microbiol Ecol.* 2000;31(2):173–182.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol.* 2011 Oct;28(10):2731–9. doi: 10.1093/molbev/msr121.
- Nandan SB, Unnithan. Ecology of Vembanad lake with special reference to *Macrobrachium rosenbergii*," In: *Freshwater Prawns-2003*. Nambudiri DD, Susheela J, Jayachandran KV, Sankaran TM, Nair CM editors. p. 151. International Symposium Abstract, College of Fisheries, Panangad, India, 2003.
- Sing ISB, Rosamma P, Maqbool TK, Ramesh S, Harikrishnan P, Menon NR. Bacteria associated with epizootic ulcerative syndrome in fishes of inland waters of Kerala. *Indian J Microbiol.* 1994;34(3):233-40.

25. Harish R, Nisha KS, Hatha AAM. Prevalence of opportunistic pathogens in paddy cum shrimp farms adjoining Vembanad Lake, Kerala, India. *Asian Fish Sci.* 2003;16(3):185-94.
26. Phatarpekar PV, Kenkre VD, Sreepada RA, U.M.Desai, C.T.Achuthankutty. Desai UM, Achuthankutty CT. Bacterial flora associated with the larval rearing of the giant fresh water prawn *M. rosenbergii*. *Aquaculture* 2002;203(3-4):279-91.
27. ICAR, Indian Council of Agricultural Research project report, 4(II), ASR-I; 1983.
28. Surendran PK, Nirmala T, Nambiar VN. Comparative microbial ecology of fresh water and brackish water prawn farms. *Fish Technol.* 2000;37(1):25-30.
29. Al-Harbi AH. Bacterial flora of freshwater prawn, *Macrobrachium rosenbergii* (de Man), cultured in concrete tanks in Saudi Arabia. *J Appl Aquacult.* 2003;14:113-124.
30. Cavallo RA, Rizzi C, Vozza T, Stabilli L. Viable heterotrophic bacteria in water and sediments in Mar Piccolo of Taranto (Ionian Sea, Italy). *J Appl Microbiol.* 1999;86(6):906-16.
31. Okpokwasili GC, Alapiki AM. Bacterial flora associated with a Nigerian freshwater fish culture. *J Aquacult Trop.* 1990;5:87-90.
32. Anderson IG, Shamsudin MN, Nash G. A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrobrachium rosenbergii* hatcheries in Malaysia. *Aquaculture* 1989;81(3-4):213-23.
33. Austin B, Allen DA. Microbial quality of water in intensive fish rearing. *J Appl Bacteriol Symp Supp.* 1985;59(s14):207S-26S.
34. Michael B New. Freshwater Prawn culture: a review. *Aquaculture* 1990;88(2):99-143.
35. Bright Singh IS, Lakshmanaperumalsamy P, Chandramohan D. Studies on the bacteria associated with *Penaeus indicus* in a culture system. PhD Thesis. Cochin University of Science & Technology, Cochin, India; 1986.
36. Sahul Hameed AS. A study of the aerobic heterotrophic bacterial flora of hatchery reared eggs, larvae and post-larvae of *Penaeus indicus*. *Aquaculture* 1993;117(3-4):195-204.
37. Sugita H, Tanaka K, Yoshinami M, Deguchi Y. Distribution of *Aeromonas* species in the intestinal tracts of river fish. *Appl Environ Microbiol.* 1995;61(11):4128-30.
38. Bhaskar N, Setty TM, Mondal S, Joseph MA, Raju CV, Raghunath BS, et al. Prevalence of bacteria of public health significance in the cultured shrimp (*Penaeus monodon*). *Food microbial.* 1998;15(5):511-19.
39. Howgate P. Review of the public health safety of products from aquaculture. *Int J Food Sci Technol.* 1998;33(2):99-125.
40. Varma PRG. *Listeria monocytogenes* in marine products. In: Sea Food quality Assurance. Mukundan MK, Krishna S, Ravindran K, editors. 89-93. Central Institute of Fisheries Technology, India; 2000.
41. Trust TJ, Sparrow RAH. The bacterial flora in the alimentary tract of freshwater and salmonid fishes. *Can J Microbiol.* 1974;20(9):1219-28.
42. Sugita H, Tokuyama K, Deguchi Y. The intestinal microflora of carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella* and Tilapia *Sarotherodon niloticus*. *B Jpn Soc Sci Fish.* 1985;51:1325-29.
43. Ringo E, Storm E. Microflora of Arctic charr, *Salvelinus alpinus* (L.): gastrointestinal microflora of free-living fish, and effect of diet and salinity on the intestinal microflora. *Aquacult Fish Manage.* 1994;25(6):623-29.
44. Ringo E, Gatesoupe FJ. Lactic acid bacteria in fish: a review. *Aquaculture* 1998;160(3-4):177-203.
45. Lalitha KV, Surendran PK. Bacterial microflora associated with farmed freshwater prawn *Macrobrachium rosenbergii* (de Man) and the aquaculture environment. *Aquac Res.* 2004;35(7):629-35.
46. Kutty MN. Towards sustainable freshwater prawn aquaculture - lessons from shrimp farming, with special reference to India. *Aquac Res.* 2005;36(3):255-63.
47. FAO. Cultured Aquatic Species Information Programme, *Macrobrachium rosenbergii* (De Man, 1879), FAO, Rome, Italy; 2008.
48. Sweetman JW, Torrecillas S, Dimitroglou A, Rider S, Davies SJ, Izquierdo MS. Enhancing the natural defences and barrier protection of aquaculture species. *Aquac Res.* 2010;41(3):345-55.
49. Sakata T. Microbiology in the digestive tract of fish and shellfish. In: Lesel R editor. *Microbiology of poecilotherms*. Amsterdam: Elsevier Science Publishers; 1990.
50. Miyamoto G, Brock J, Nakamura R, Nakagawa L, Shimojo R, Sato V, et al. A preliminary microbiological and water quality survey of two Hawaiian prawn (*Macrobrachium rosenbergii*) hatcheries. In: Rogers GL, editor. *First International Conference on Warm water Aquaculture-Crustacea*. Hawaii: Brigham Young University; 1983.
51. Moriarty DJW. Interactions of microorganisms and aquatic animals, particularly the nutritional role of the gut flora. In: *Microbiology of poecilotherms*. Lesel R editor. Elsevier Science Publishers, Amsterdam; 1990.
52. Riquelme C, Araya R, Escribano R. Selective incorporation of bacteria by *Argopecten purpuratus* larvae: implications for the use of probiotics in culturing systems of the Chilean scallop. *Aquaculture* 2000;181(1-2):25-36.
53. Rengpipat S, Rukpratanporn S, Piyatiratitivorakul S, Menasaveta P. Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). *Aquaculture* 2000;191(4):271-88.
54. Gullian M, Thompson F, Rodriguez J. Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. *Aquaculture* 2004;233(1-4):1-14.
55. Gatesoupe FJ, Zambonino-Infante JL, Cahu C, Quazuguel P. Early weaning of seabass larvae, *Dicentrarchus labrax*: the effect on microbiota, with particular attention to iron supply and exoenzymes. *Aquaculture* 1997;158(1-2):117-127.
56. Gatesoupe FJ. Siderophore production and probiotic effect of *Vibrio* sp. associated with turbot larvae, *Scophthalmus maximus*. *Aqua Liv Resour.* 1997;10(4):239-46.
57. Ringo E, Vadstein O. Colonization of *Vibrio pelagicus* and *Aeromonas caviae* in early developing turbot (*Scophthalmus maximus* L.) larvae. *J Appl Microbiol.* 1998;84(2):227-33.
58. Pandiyan P, Balaraman D, Thirunavukkarasu R, Jothi George EG, Subramaniyan K, Manikkam S, et al. Probiotics in aquaculture. *Drug Invent Today* 2013;5(1):55-59.
59. Verschuere L, Rombaut G, Sorgeloos P, Verstraete W. Probiotic bacteria as biological control agents in aquaculture. *Microbiol Mol Biol R.* 2000;64(4):655-71.
60. Favret ME, Yousten AA. Insecticidal activity of *Bacillus laterosporus*. *J Invertebr Pathol.* 1985;45(2):195-203.
61. Shida O, Takagi H, Kadowaki K, Komagata K. Proposal for two new genera. *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. *Int J Syst Bacteriol.* 1996;46:939-46.
62. Zahner V, Rabinovitch L, Suffys P, Momen H. Genotypic diversity among *Brevibacillus laterosporus* strains. *Appl Environ Microbiol.* 1999;65(11):5182-5.
63. Mahdhi A, Kamoun F, Messina C, Santulli A, Bakhrouf A. Probiotic properties of *Brevibacillus brevis* and its influence on sea bass (*Dicentrarchus labrax*) larval rearing. *Afr J Microbiol Res.* 2012;6(35):6487-95.
64. Hong HA, Duc LH, Cutting SM. The use of bacterial spore formers as probiotics. *FEMS Microbiol Rev.* 2005;29(4):813-35.
65. Chythanya R, Karunasagar I, Karunasagar I. Inhibition of shrimp pathogenic vibrios by a marine *Pseudomonas* I-2 strain. *Aquaculture* 2002;208(1-2):1-10.
66. Balcazar JB, de Blas I, Zarzuela IR, Cunningham D, Vendrell D, Muzquiz JL. The role of probiotics in aquaculture. *Vet Microbiol.* 2006;114(3-4):173-86.

67. Aly SM, Abd-El-Rahman AM, John G, Mohamed MF. Characterization of some bacteria isolated from *Oreochromis niloticus* and their potential use as probiotics. *Aquaculture* 2008;277(1-2):1-6.
68. Capkin E, Altinok I. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis disease. *J Appl Microbiol.* 2009;106(4):1147-53. doi: 10.1111/j.1365-2672.2008.04080.x.
69. Strom-Bestor M, Wiklund T. Inhibitory activity of *Pseudomonas* sp. on *Flavobacterium psychrophilum*, in vitro. *J Fish Dis.* 2011;34(4):255-64.
70. Irianto A, Austin B. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J Fish Dis.* 2002;25(6):333-42.

***Corresponding author:** Mujeeb Rahiman KM, Department of Aquaculture and Fishery Microbiology, MES Ponnani College, Ponnani, India, E-mail: rkmmujeeb@gmail.com.

Received Date: July 11, 2017, **Accepted Date:** October 31, 2017, **Published Date:** November 09, 2017.

Copyright: © 2017 Mujeeb RKM, et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Mujeeb RKM, Yousuf J, Hatha MAA, Thomas AP (2017) Bacterial Diversity of Giant Freshwater Prawn, *Macrobrachium rosenbergii* and Screening for Probiotic Potential Bacteria. *J Aqua Mar Sci* 1(1): 101.