ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 114-122

www.fisheriesjournal.com
Received: 18-07-2016
Accepted: 19-08-2016

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Enhancement of growth potential in freshwater prawn *Macrobrachium rosenbergii* with usage of water and feed probiotics in the culture operation

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Abstract
In the present investigation, an attempt was made to probe in to the usage of both water and feed probiotics individually and in combination to monitor the growth patterns of freshwater Male and Female prawns *Macrobrachium rosenbergii*. The feed conversion ratio, Protein efficiency ratio and Percent survival rates were considerably increased in the culture operation, where water and feed probiotics were broadcasted. Gut microbial load was decreased and *Lactobacillus* count were significantly increased suggesting that both feed and water probiotics are capable of eliminating the pathogens, thereby promoting growth potentials in prawns. The Anti-oxidant enzyme activities such as SOD, Catalase and GPX were also significantly increased suggesting the elimination of ROS in the prawn species, thereby promoting growth potentials significantly. The results obtained in the present investigation aims to help for the development of adequate technology for the evaluation of the efficiency of microbial agents as Probiotics in Aquaculture in order to improve survival rate and growth to enhance yield and to minimize production cost.

Keywords: *Macrobrachium rosenbergii*; probiotics; anti-oxidant enzymes; growth rates

1. Introduction
Aquaculture has become an important economic activity in many countries. In large scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses. Prevention and control of diseases have led during recent decades to a substantial increase in the use of veterinary medicines. However, the utility of antimicrobial agents as a preventive measure has been questioned, given extensive documentations of the evolution of antimicrobial resistance among pathogenic bacteria. Globally, tones of antibiotics have been distributed in the biosphere during an antibiotic era of only about 60 years duration. Shrimp culture all over the world was affected by disease, inflicting huge loss due to pathogenic microorganisms [12, 14]. For preventing and controlling of diseases, a host of antibiotics and other toxic chemicals are used, possibly creating antibiotic resistant Bacteria. The use of antibiotics, pesticides and other toxic chemicals in aquatic environment for better management are creating human health hazards [25, 27, 31]. Thus, to improve the ecological environment of aquaculture and to evolve better and innovative approaches to mitigate the situation, there is a shift in the focus of attention on the international aquaculture scenario.
In recent years, disease problems caused by *Vibrio* species have emerged as major constraints in Aquaculture production. The application of Antibiotics or other chemicals to culture ponds is expensive and detrimental (Contamination of reared animal, antimicrobial resistance etc.). So therefore to over come this problem certain substances like Probiotics are used. “Probiotics” generally includes Bacteria, Cyanobacteria, Microalgae, and Fungi etc. Probiotic bacteria are generally called the Bacteria which can improve the water quality of Aquaculture and inhibit the Pathogens in water thereby increasing production [10, 14, 16]. Many Researchers attempt to use some kind of Probiotics in Aquaculture water to regulate the Microflora of Aquaculture water, control pathogenic microorganisms, to enhance decomposition of the undesirable organic substances in Aquaculture water, and improve ecological environment of Aquaculture [19]. In addition, the use of Probiotics can increase the population of food organisms, improve the nutrition level of Aquacultural animals and improve immunity of cultured animals to pathogenic microorganisms [8].

The present investigation was aimed to understand the impact of different types of Probiotics i.e Water Probiotics and Feed Probiotics on the growth patterns of Freshwater Prawn *M. rosenbergii*. Water Probiotics namely PROBAC-BC or MICROZYME-BS and Feed Probiotics ZYMETIN were used in Aqua farms.

2. Materials and Methods

The present study was conducted over a culture period of twelve months (From March2014 to January 2015) using earthen ponds located at Ramayapatnam (Latitude 15^0 02^1 55^{11} N, Longitude 80^0 02^1 50^{11} E), Prakasam District of Andhra Pradesh, India. All the culture conditions were maintained as described earlier by Rangappa [43]

The Feed Probiotic ZYMETIN was added to the feed 5 g/1 Kg feed, where as PROBAC-BC or MICROZYME-BS are added as following.

2.1 Dosage in Grams per Hectare

Days	Stocking Density		
	3 - 5	8 - 10	10 - 20
7 Days of Post Stocking	75	125	200
21st Day	150	400	600
35th Day	250	550	900
50th Day	300	650	1050
65th Day	350	750	1200
85th Day	450	1100	1750
100th Day	500	1150	1900
115th Day	600	1350	2200
130th Day	450	1100	1800
180th Day	400	1000	1600
210th Day	300	850	1200
250th Day	300	800	1000
300th Day	250	600	750
350th Day	200	500	600

Food conversion ratio (FCR), Protein efficiency ratio (PER) were determined as follows:

FCR: Feed consumed (g) X Number of prawns / Weight gain (g)

PER: Weight gain / Protein intake

Gut Microbial analysis details were described by Rangappa [43]. Biochemical parameters including assaying and Peroxidase activity [37], Superoxide dismutase (SOD) [23], and Protein content [29] using bovine serum as standard in the different probiotic treated prawn samples collected during the month of December, 2014. The data obtained was subjected to Statistical Analysis by using SPSS 14 version.

Table 1: Growth rates (in g) of Male Fresh water Prawn M. rosenbergii treated with different Probiotic treatments

Month	Control	With Water probiotics	With Feed Probiotics	With W P + F P
February	0.32	-	-	-
March	1.08 ±0.30 PDC	1.87 ±0.28 (+73.14)	1.9 ±0.41 (+75.92)	2.01 ±0.68 (+86.11)
April	4.12 ±1.12 PDC	7.31 ±1.25 (+77.42)	7.6 ±1.33 (+84.46)	8.01 ±1.24 (+94.41)
May	11.88 ±1.03 PDC	19.87 ±2.00 (+67.85)	20.23 ±2.36 (+70.28)	21.2 ±2.25 (+78.45)
June	19.94 ±1.81 PDC	28.88 ±2.05 (+44.83)	29.36 ±1.89 (+47.24)	30.18 ±2.27 (+51.35)
July	30.12 ±2.44 PDC	41.87 ±2.27 (+39.01)	43.06 ±2.09 (+42.96)	44.07 ±2.17 (+46.31)
August	45.15 ±1.32 PDC	61.32 ±2.47 (+35.81)	62.64 ±1.94 (+38.73)	64.18 ±2.81 (+42.14)
September	60.74 ±2.18 PDC	78.07 ±2.361 (+28.53)	79.49 ±1.68 (+30.869)	80.86 ±2.284 (+33.12)
October	79.95 ±3.38 PDC	101.430 ±2.72 (+26.866)	103.03 ±1.937 (+28.86)	104.68 ±1.63 (+30.93)
November	89.42 ±2.22 PDC	100.170 ±1.77 (+12.02)	101.85 ±2.95 (+13.900)	102.87 ±2.42 (+15.04)
December	93.920 ±1.96 PDC	100.65 ±2.17 (+7.165)	101.8 ±2.73 (+8.390)	102.37 ±1.59 (+8.99)
January	96.040 ±1.71 PDC	99.53 ±2.10 (3.63)	100.47 ±1.23 (+4.612)	101.42 ±1.99 (+5.601)

The values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.
WP + FP: Water Probiotics and Feed Probiotics

Table 2: Growth rates (in g) of Female Fresh water Prawn M. rosenbergii under different Probiotic treatment

Month	Control	With Water probiotics	With Feed Probiotics	With W P + F P
February	0.32	-	-	-
March	1.01 ±0.21 PDC	1.72 ±0.30 (+70.297)	1.79 ±0.37 (+77.227)	1.85 ±0.51 (+83.168)
April	3.120 ±1.47 PDC	5.46 ±0.53 (+75.00)	5.61 ±0.51 (+79.807)	5.77 ±0.31 (+84.935)
May	9.75 ±1.02 PDC	16.7 ±2.21 (+71.282)	17.07 ±1.64 (+75.075)	17.41 ±1.59 (+78.564)
June	16.89 ±2.06 PDC	24.37 ±1.90 (+44.286)	25.01 ±1.78 (+48.075)	25.64 ±2.86 (+51.805)
July	28.93 ±1.91 PDC	42.05 ±2.58 (+45.350)	43.78 ±1.47 (+51.330)	44.35 ±3.07 (+53.301)
August	41.190 ±2.31 PDC	54.64 ±2.01 (+32.653)	56.370 ±1.45 (+36.853)	57.300 ±2.96 (+39.111)
September	49.420 ±1.889 PDC	58.45 ±3.06 (+18.271)	59.17 ±3.32 (+19.728)	59.55 ±3.61 (+20.497)
October	56.34 ±2.21 PDC	63.65 ±1.940 (+12.974)	64.36 ±1.949 (+14.235)	65.22 ±2.254 (+15.761)
November	60.72 ±2.16 PDC	65.84 ±1.90 (+8.432)	66.67 ±2.06 (+9.799)	67.45 ±2.66 (+11.08)
December	63.18 ±1.54 PDC	66.16 ±2.15 (+4.716)	67.07 ±2.40 (+6.157)	67.29 ±3.95 (+6.505)
January	65.32 ±2.24 PDC	68.17 ±4.10 (4.363)	*68.74 ±2.56 (+5.23)*	69.34 ±1.84 (+6.154)

The values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.
WP + FP: Water Probiotics and Feed Probiotics

Table 3: Growth rates (in g) of Male Fresh water Prawn M. rosenbergii under different Probiotic treatments

	With Water Probiotics				With Feed Probiotics			With Water Probiotics + Feed Probiotics		
Month	**Control (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**
March	1.08 ±0.304 PDC	1.87 ±0.282 (+73.14)	0.79 ±0.423	0.026 ±0.014	1.9 ±0.412 (+75.92)	0.82 ±0.515	0.027 ±0.0172	2.01 ±0.688 (+86.11)	0.93 ±0.894	0.031 ±0.029
April	4.12 ±11.122 PDC	7.31 ±1.252 (+77.42)	3.19 ±1.232	0.106 ±0.041	7.6 ±1.335 (+84.46)	3.48 ±1.65	0.11 ±0.055	8.01 ±1.248 (+94.41)	3.89 ±1.391	0.129 ±0.046
May	11.88 ±1.031 PDC	19.87 ±2.006 (+67.25)	7.99 ±2.390	0.266 ±0.079	20.23 ±2.366 (+70.28)	8.35 ±2.923	0.278 ±0.097	21.2 ± 2.256 (+78.45)	9.32 ±1.900	0.310 ±0.063
June	19.94 ±1.814 PDC	28.88 ±2.058 +(44.83)	8.94 ±2.780	0.298 ±0.092	29.36 ±1.89 (+47.24)	9.42 ±2.744	0.314 ±0.0915	30.18 ±2.274 (+51.34)	10.24 ±2.14	0.341 ±0.071
July	30.12 ±2.443 PDC	41.87 ±2.27 (+39.01)	11.75 ± 4.196	0.391 ±0.139	43.06 ±2.09 (+42.96)	12.94 ±3.52	0.431 ±0.117	44.07 ±2.177 (+46.31)	13.95 ±4.151	0.465 ±0.138
August	45.15 ±1.325 PDC	61.32 ± 2.47 (+35.81)	16.17 ±3.113	0.539 ±0.103	62.64 ±1.947 (+38.73)	17.49 ±1.51	0.583 ±0.050	64.18 ±2.816 (+ 42.14)	19.03 ± 2.275	0.634 ±0.075
September	60.74 ±2.183 PDC	78.07 ±2.361 (+28.53)	17.33 ±1.544	0.577 ±0.051	79.49 ±1.68 (30.86)	18.75 ±2.19	0.62 ±0.073	80.86 ±2.284 (+33.12)	20.12 ±3.22	0.670 ±0.107
October	79.95 ±3.387 PDC	101.43 ±2.728 (+26.86)	21.48 ±3.299	0.716 ±0.11	103.03 ± 1.937 (+28.86)	23.08 ±3.82	0.769 ±0.127	104.68 ±1.630 (+30.93)	24.73 ±3.41	0.824 ±0.113
November	89.42 ±2.229 PDC	100.17 ±1.77 (+12.021)	10.75 ±3.20	0.358 0.106	101.85 2.953 (+13.90)	12.43 ±4.201	0.414 ±0.140	102.87 ±2.421 (+15.04)	13.45 ±3.622	0.448 ±0.120
December	93.92 ±1.957 PDC	100.65 ±2.177 (+7.16)	6.73 ±2.683	0.224 ±0.089	7101.8 ±2.739 (+8.390)	7.88 ±3.719	0.262 ±0.124	102.37 ±1.593 (+8.99)	8.45 ±3.29	0.281 ±0.109
January	96.04 ±1.717 PDC	99.53 ±2.109 (+3.633)	3.49 ±3.259	0.116 ±0.108	100.47 ± 1.232 (+4.612)	4.43 ±2.44	0.14 ±0.081	101.42 ±1.993 (+5.60)	5.38 ±3.392	0..179 ±0.113

All values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.

Table 4: Growth rates (in g) of Female Fresh water Prawn M. rosenbergii under different Probiotics treatments

	With Water Probiotics				With Feed Probiotics			With Water Probiotics + Feed Probiotics		
Months	**Control (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**	**Experimental (g)**	**Increment (g)**	**Daily Growth rate (g)**
March	1.01 ±0.216 PDC	1.72 ±0.308 (+70.29)	0.71 ±0.488	0.023 ±0.016	1.79 ±0.370 (+77.22)	0.78 ±0.345	0.026 ±0.0115	1.85 ±0.509 (+83.16)	0.84 ±0.473	0.028 ±0.015
April	3.12 ±1.470 PDC	5.46 0.535 (+75.00)	2.34 ±1.487	0.078 ±0.049	5.61 ±0.513 (+79.808)	2.49 ±1.533	0.083 ±0.0511	5.77 ±0.398 84.93	2.65 ±1.435	0.088 ±0.047
May	9.75 ±1.020 PDC	16.7 ±2.214 (+71.28)	6.95 ±2.384	0.231 ±0.079	17.07 ±1.640 (+75.07)	7.32 ±1.881	0.244 ±0.062	17.41 ± 1.592 (+78.56)	7.66 ±1.943	0.255 ±0.064
June	16.89 ±2.064 PDC	24.37 ±1.907 (+44.286)	7.48 ±2.313	0.249 ±0.077	25.01 ±1.785 (+48.07)	8.12 ±2.543	0.270 ±0.084	25.64 ±2.857 (+51.80)	8.75 ± 4.050	0.291 ±0.135
July	28.93 ±1.913 PDC	42.05 ±2.580 (+45.35)	13.12 ± 3.821	0.437 ±0.127	43.78 ±1.472 (+51.33)	14.85 ±2.554	0.495 ±0.085	44.350 ±3.068 (+53.30)	15.42 ±3.840	0.514 ±0.128
August	41.19 ±2.316 PDC	54.64 ±2.014 (+ 32.653)	13.45 ±2.796	0.448 ± 0.093	56.37 ±1.453 (+36.85)	15.18 ±2.189	0.506 ±0.073	57.30 ±2.958 39.11	16.11 ± 4.415	0.537 ±0.147
September	49.42 ±1.889 PDC	58.45 ±3.061 (+18.27)	9.03 ±4.485	0.301 ±0.149	59.17 ±3.324 (19.72)	9.75 ±4.71	0.325 ±0.157	59.55 ±3.61 (+20.49)	10.13 ±4.96	0.337 ±0.165
October	56.34 ±2.212 PDC	63.65 ±1.940 (+12.97)	7.31 ±3.37	0.243 ±0.112	64.36 ±1.949 (+14.23)	8.02 ±2.728	0.267 ±0.091	65.22 ±2.25 (+15.76)	8.88 ±3.712	0.296 ±0.123
November	60.72 ±2.164 PDC	65.84 ±1.901 (+8.43)	5.12 ±2.981	0.170 ±0.099	66.67 ±2.059 (+9.799)	5.95 ±1.486	0.198 ±0.049	67.45 ±2.663 (+ 11.08)	6.73 ± 1.31	0.224 ±0.044
December	63.18 ±1.543 PDC	66.16 ±2.156 (+4.71)	2.98 ±2.16	0.099 ±0.072	67.07 ±2.405 (+6.15)	3.89 ±2.693	0.129 ±0.089	67.29 ±3.948 (+6.505)	4.11 ±2.967	0.137 ±0.098
January	65.32 ±2.249 PDC	68.17 ±4.106 (+4.363)	2.85 ±2.598	0.095 ±0.086	68.74 ±2.56 (+5.23)	3.42 ±2.00	0.114 ±0.066	69.34 ±1.844 (+6.154)	4.02 ±2.321	0.134 ±0.077

All values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.

Table 5: Food Conversion Ratio (FCR), Percent survival and Protein efficiency ratio (PER) of Male Fresh water prawn M. rosenbergii under different probiotics treatments

Parameter	Control	With Water probiotics	With Feed Probiotics	With WP + FP
FCR	2.06 ±0.06 PDC	1.94 ±0.07 (-5.82)	1.92 ±0.51 (-6.79)	1.82 ±0.05 (-11.65)
% Survival	85.0 ±3.28 PDC	89.1 ±1.53 (+4.82)	90.0 ±2.67 (+5.88)	92.0 ±3.31 (+8.23)
PER	1.34 ±0.05 PDC	1.31 ±0.04 (-2.24)	1.29 ±0.06 (- 3.73)	1.27 ±0.04 (-5.22)

The values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.
WP + FP: Water probiotics and Feed probiotics

Table 6: Food conversion ratio (FCR), Percent survival and Protein efficiency ratio (PER) of Female Fresh water prawn M. rosenbergii under different probiotics treatments

Parameter	Control	With Water probiotics	With Feed Probiotics	With WP + FP
FCR	2.01 ±0.08 PDC	1.90 ±0.07 (-5.47)	1.88 ±0.05 (-6.46)	1.79 ±0.03 (-10.94)
% Survival	84.0 ±3.65 PDC	88.4 ±1.83 (+5.23)	88.0 ±1.41 (+4.75)	91.2 ±2.17 (+8.56)
PER	1.28 ±0.035 PDC	1.23 ±0.032 (-3.90)	1.22 ±0.035 (-4.68)	1.20 ±0.027 (-6.25)

The values are Mean ± SD of Six individual observations.
PDC: Percent deviation over Control.
WP + FP: Water Probiotics and Feed Probiotics

Table 7: Gut microbial load of Male Fresh water prawn M. rosenbergii under Feed probiotics

Micro organism	Control	WP + FP used ponds 120 DOC	WP + FP used ponds 240 DOC
Total plate count	5.6×10^8	2.5×10^8	2.2×10^8
Total coliforms	1586	1113	1034
Total fecal coli forms	345	148	140
Facultative anaerobes	6.8×10^7	3.1×10^7	3.0×10^7
Lactobacillus sp	1.7×10^6	4.6×10^7	4.9×10^7

Table 8: Gut microbial load of Female Fresh water prawn M. rosenbergii under Feed probiotics

Micro organism	Control	WP + FP used ponds 120 DOC	WP + FP used ponds 240 DOC
Total plate count	5.3×10^8	2.3×10^8	2.1×10^8
Total coliforms	1579	1109	950
Total fecal coli forms	341	146	138
Facultative anaerobes	6.5×10^7	2.9×10^7	2.7×10^7
Lactobacillus sp	1.58×10^6	4.52×10^7	4.7×10^7

All values are Mean ± SD of six individual observations
Values are expressed as CFU/g

Table 9: Anti-oxidant enzymes in Midgut gland tissue of male Fresh water prawn M. rosenbergii under different Probiotic treatments

	Control	Final		
		With Water probiotics	With Feed probiotics	With WP+FP
SOD	20.4 ±2.201 PDC	24.3 ±1.972 (+19.117)	25.4 ±1.673 (+24.50)	26.2 ±2.207 (+28.431)
Catalase	40.18 ±2.158 PDC	44.2 ±2.465 (+10.004)	45.26 ±2.183 (+12.643)	50.28 ±2.163 (+25.139)
GPX	6.14 ±1.377 PDC	8.14 ±1.209 (+31.714)	8.85 ±1.710 (+43.203)	9.84 ±1.72 (+59.196)

Table 10: Anti-oxidant enzymes in Midgut gland tissue of Female Fresh water prawn M. rosenbergii under different Probiotic treatments

	Control	Final		
		With Water probiotics	With Feed probiotics	With WP+FP
SOD	19.2 ±1.730 PDC	22.5 ±2.295 (+17.187)	23.9 ±1.704 (+24.479)	24.6 ±1.452 (+28.125)
Catalase	37.16 ±1.769 PDC	41.51 ±2.057 (+11.706)	43.18 ±2.139 (+16.200)	47.13 ±2.355 (26.829)
GPX	4.66 ±1.497 PDC	6.37 ±1.338 (+36.69)	7.38 ±1.367 (+58.369)	8.54 ±2.007 (+83.261)

All values are Mean ± SD of six individual observations
All values are statistically significant at $P<0.001$
PDC: Percent deviation over control
Values are expressed as Units/g wet weight of tissue/hr

3. Results and Discussion

The present chapter dealt in with the impact of Probiotics i.e. Water Probiotics and Feed Probiotics on the growth patterns of Fresh water prawn *M. rosenbergii* during culture activity. In the present investigation the growth patterns of Male and Female *M. rosenbergii* were monitored in different conditions i.e. Control condition, where only feed was provided, in another condition along with normal broadcasting of feed with water probiotics were used to keep the water environment clean, in another condition along with normal feed with feed probiotics were mixed broadcasted and in the Fourth condition both feed probiotics and water probiotics were introduced in the culture environment.

The growth patterns of Male and Female *M. rosenbergii* were monitored in the above conditions mentioned and presented in Tables.1 to 3. The growth patterns were recorded for one year on Monthly basis, starting from March to January of next year. The growth patterns recorded for control Male *M. rosenbergii* clearly demonstrates that, the animals obtained 96 g maximum weight during the 360 day culture period (Table. 1). In the control, the culture conditions include, the normal feeding activity without any addition of either Water Probiotics or Feed Probiotics. In the second condition, where Water Probiotics are regularly used clean the water environment along with normal feeding activity, the growth patterns were also showed relatively good progress obtaining 99.53 g of average weight at the end of culture activity. So the maximum weight was raised to 99.53 g compared to the control value of 96 g. In another set of experiment, where Feed Probiotics are mixed with Feed and subsequently broadcasted in the culture environment, the prawns showing better growth rates and attaining 100.47 g of average weight at the end of culture activity compared to the control weight of 96 g. In the last set of experiment in which, both Water probiotics and Feed Probiotics were used and the growth patterns are recorded to be maximum and the prawns attained an average weight of 101.42 g compared to control weight of 96 g. Similarly the growth pattern of Female *M. rosenbergii*

was recorded in the above Four different conditions and presented in Table. 2. In the Control conditions the maximum weight obtained was 65.32 g compared to 68.17 g obtained in Water Probiotics used ponds, 68.74 g in the Feed Probiotics used ponds and 69.34 g in the feed and Water Probiotics used ponds. Month wise daily growth rates for Male and Female *M. rosenbergii* under different experimental conditions were monitored and presented in Table.3. The Food Conversion Ratio (FCR), Percent Survival (% Survival) and Protein Efficiency Ratio (PER) values were recorded and presented in Tables.4 and 5 The FCR values for Male *M. rosenbergii* were recorded as 2.06 in the control and was relatively high compared to 1.94 obtained for Water Probiotics used pond, 1.92 obtained for Feed Probiotics used pond and 1.82 obtained for both Water and Feed Probiotics used ponds (Table.4). The percent survival values observed for Male *M. rosenbergii* was ranged between 85 to 92%. The PER values for Male *M. rosenbergii* were recorded and range of 1.27 to 1.34. Similarly the Food Conversion Ratio (FCR), Percent survival and Protein Efficiency Ratio (PER) values were recorded for Female *M. rosenbergii* and presented in Table. 5. The total viable counts (cells/g) observed in the gut of both male and female fresh water prawns *M. rosenbergii* were presented in Tables. 6 and 7. The total plate count was reduced in feed probiotic treated prawns. In case of Control the Total plate count was recorded to be 5.6×10^8 whereas in experimental prawns it is 2.5×10^8. In case of Total coliforms, Total fecal coliforms and facultative anaerobes similar kind of result obtained. The beneficial bacteria i.e. *Lactobacillus sps.* was increased in probiotic treated animals. The Antioxidant enzymes including Superoxide Dismutase (SOD), Catalase and Glutathione Peroxidase (GPX) were estimated in both Male and Female *M. rosenbergii* and presented in Tables. 8 & 9. All the Three selected enzyme activities showed an elevation in the experimental conditions compared to controls. In the last decade, the consumption of Aquatic products has been increased substantially, but the World Fishery production was decreased and hence the production of Aquatic products through controlled conditions have been came in to lime light. The reduction in the capture fisheries was partly compensated for the fast growth of Aquaculture industry. The need for enhanced disease resistance, feed efficiency, and growth performance of cultured organisms is substantial for various sectors of this industry. If growth and feed efficiency are increased in commercial aquaculture, the cost production was likely to be reduced. It is also observed if Aquatic organisms show higher resistance to diseases and maximum survival rates, the cost of medication and overall production costs will be reduced to minimum. Hormones, antibiotics, ionospheres and some salt compounds have been used to some extent to prevent disease and act as growth promoters. However, their inadequate application can produce adverse effects such as hormonal imbalance, poisoning and predisposition to disease development. In search of new options, several studies have been carried out to test new compounds, from which the Aquaculture industry has developed the concept of "Functional additives". Among these additives, the most important addition of microorganisms to the diet is named as "Probiotics" [14, 17]. These Probiotics has shown to improve the energy expenditure derived from other sources such as Carbohydrates and increase the incorporation of protein for growth patterns, increase the immunity and disease resistance of the host organisms. The common Probiotics used in pond management are live Bacterial inocula (non-pathogenic organisms) rich in extracellular enzymes claims about the potential benefits of Probiotics in Aquaculture ponds include: Enhancing decomposition of Organic Matter, Reduction in Nitrogen and Phosphorous concentrations, Better algal growth, Greater availability of Dissolved Oxygen, Reduction in Blue-Green algae, Control of Ammonia, Control of Nitrate and Hydrogen sulfide, Lower incidence of disease and greater survival and Better Shrimp/Prawn/Fish production. The addition of Probiotics to Aquaculture ponds should not result in any damage to the fish and shrimp crop or to the environment. The sustainability and the success of Aquaculture depend on the quality of Soil, Water, Seed selected and Feed used. A good quality of Soil, Water, Seed and Feed plays an important role in the successful yield under skillful management practices. The ponds often accumulate with uneaten feed materials, excreta, molted shells, dead algae and surface run off organic matter carried by wind and water. When all the above mentioned materials remain un-degraded or partially degraded in reduced oxygen condition and toxic gases such as H_2S and NH_3 will be produced. These gases give rise to stress to the cultured organisms resulting in the loss of appetite, sluggishness, gulping for Oxygen etc, and ultimately results in the reduction of growth patterns. It has been postulated that the primary principle for accelerated organic matter decomposition by Probiotics (especially particulate matter that settles to create black sludge) is a function of C: N ratio management by beneficial heterotrophic bacteria [4, 17]. In the present investigation Ammonia, H_2S and CO_2 contents were decreased consequent upon the usage of Water Probiotics in the culture ponds, whereas the Dissolved oxygen (DO) content of the water was increased. Inoculation of probiotics enhances the domination of heterotrophic bacteria in the environment. The Microbial preparations used in Aquaculture may be broadly divided into three types i.e. Bio control agents, Probiotics and Bioremediation agents. Bio control agents are those methods of treatment using the antagonism among microbes to kill or reduce the number of pathogens in the Aquaculture environment. Those bacterial treatments which improve the water quality and thus indirectly the production were termed as bioremediation agents. The bioremediation agents have also been termed as Bio-augmentation agents or water additives [31] and Probiotics. Porubcan [38] reported that the increase in yield and survival of *Penaeus monodon* by using bio-filters Pre-inoculated with nitrifying bacteria which helped to decrease the amount of ammonia and nitrate in the rearing water. Porubcan [39] further reported that the introduction of *Bacillus sps*. in proximity to pond aerators reduced the chemical oxygen demand and increased the yields. Recently several commercial products have sought to exploit the idea that bacteria, which improve the water quality, may be beneficial to animal health. Among the shrimp farmers in India, these products are known as "Water Probiotics" and most of them contain nitrifying bacteria and/or *Bacillus sps*. The nitrifying bacteria have strict ecological niches and they have not been detected in the gastrointestinal tract of animals [17]. *Bacillus sps*. are not autochthonous in the gastrointestinal tract, but they have been isolated from fish [25, 50], Crustaceans [48], bivalves [51] and shrimp larval rearing medium [45]. Many of these *Bacillus sps*. strains have antibiotic properties and may be active during intestinal transit. At present the definition and classification brought forth by Gatesoupe [17] serve the purpose and can be applied without confusion in shrimp culture. The commercial

availability of Probiotics and bioremediation agents on shrimp culture and its wide spread usage in India has spawned separate terminologies among shrimp farmers. The strict probiotic agents are known as gut-probiotics and the bioremediation agents are known as Water Probiotics.

In the present investigation, the month wise analysis of Water parameters in the ponds clearly demonstrate that due to the usage of water probiotics, the Ammonia content, H_2S and CO_2 contents were reduced and Oxygen content was increased. It has been already established that, the microbial communities play an important role in Aquaculture system and pond productivity. Bioremediation agents serve to modify or manipulate the microbial communities in water and sediment such that they reduce or eliminate selected Pathogenic Microbes and generally improve growth and survival of the targeted species. There are various ways through which Bioremediation agents could act in Aquaculture systems. These include competitive exclusion of Pathogens, enhancing digestion through the supply of essential enzymes, moderating and promoting the direct uptake of dissolved organic materials, active promotion of Pathogen inhibiting substances and other possible mechanisms [22]. According to Bratwold *et al.* [5] the specific ecological applications of microbial ecology management in shrimp ponds include the following : Optimizing nitrification rates to keep low Ammonia concentrations, optimizing de-nitrification rates to eliminate excess nitrogen from ponds as nitrogen gas, maximizing carbon mineralization to Carbon dioxide to minimize sludge accumulation, maximizing primary productivity that stimulate shrimp production and also secondary crops and maintaining a diverse and stable pond community where undesirable species do not become dominant. Boyd [6] and Boyd & Gross [7] found that Bacterial *Bacillus sps*. additions in the pond did not improve the water quality as expected. They also observed higher survival rates of fish in ponds treated frequently with 3 species of live *Bacillus*. The mode of action was unknown because water quality was not measurably improved. Pond studies also showed that applications of an enzyme preparation tended to enhance microbial mineralization of organic matter, but no effect on fish production was observed. Boyd and Gross [7] concluded that too little is known about the modes of action these bioremediation agents, the conditions under which they may be effective, their application rates and methods for several recommendations of their use. Most of such studies show that the addition of probiotics has no effect on the water quality of cultured shrimps [47]. Mcintosh *et al* [30] hypothesized that the outcome of microbial supplement addition may not be profound in Aquaculture facilities where waste water is flushed daily out of the systems. Hence they conducted a study to evaluate it routine use of a commercially produced bacterial supplement could improve water and sludge quality and *Litopenaeus vannamei* under zero water discharge with a low protein diet and high stocking density. There are few reports [32, 34, 40] of bioremediation agents working well in shrimp Aquaculture system. Moriarty[32] compared luminescent *Vibrio sps*. counts and shrimp production in ponds in which a *Bacillus sps*. based bioremediation (Pond Pro-VC^{Tn}) was used and those on which it were not used. The phototrophic bacteria are known to utilize any commonly found toxic substances including Ammonia, Nitrates, Hydrogen sulphide in a waste system [36]. The principle chemical reactions are carbon decomposition via both Respiration and Fermentation, Nitrification/De-nitrification and sulphate reduction [4]. The available literature suggests the beneficial effects of Water Probiotics, in the management of water quality. In the present study Ammonia and Hydrogen sulphide were reduced in Water Probiotics treated culture system. Water quality plays an important role in Aquaculture production. A complete understanding of the relationship between water quality and aquatic productivity is absolutely essential for optimum growth and production. Water temperature is probably the most important environmental variables in prawn culture activity, because it directly affects metabolism, oxygen consumption, growth and molting and survival. Any general change in temperature affects the animal immune system. The optimum range of temperature for the shrimp rearing is between 28^0-32^0C [24]. The temperature in the present study was ranges between from 27 ºC to 29 ºC. There was no marked difference in temperature between control and Probiotics treated tanks in the present study. pH of the culture medium is also playing an important role in the metabolism and controls several physiological processes of the culture organisms. The pH of the culture environment changes with the accumulation of residual feed, dead algae and excreta. The pH values are known to influence the levels of Ammonia, Nitrate and Hydrogen sulphide [24, 26]. Dissolved Oxygen in the Aquatic medium is an important factor not only for the respiration of aquatic organisms but also to maintain favorable chemical and hygienic environment of the aquatic body. When the Dissolved Oxygen (DO) levels are relatively low and anaerobic conditions exist, nitrate is reduced into Ammonia, which is toxic. Low Dissolved Oxygen (DO) levels increases pH of the aquatic environment and low tension also hampers metabolic performance in shrimp and can reduce growth and molting and causing high mortality [18]. Oxygen level in the culture medium was maintained in the desired range by aeration. Intermittent aeration was done in the present study and therefore the oxygen level did not vary significantly.

Bacterial antagonism is a common phenomenon in nature; therefore, microbial interactions play a major role in the equilibrium between competing beneficial and potentially pathogenic microorganism. The microbiota in the gastrointestinal tract of aquatic animals can be modified, for example, by ingestion of other microorganisms. Therefore, microbial manipulation constitutes a viable tool to reduce (or) eliminate the incidence of opportunistic pathogens [9, 10]. Organism's life depends upon Oxygen as the final acceptor of Electrons in Mitochondrial Electron Transport, but the process also generates toxic metabolites, Reactive Oxygen Species (ROS), leak from Mitochondria into the Cytoplasm where they cause Cellular damage by oxidizing a variety of biologically important molecules, including DNA, Proteins, Lipids and Carbohydrates. Aerobic organisms possess a baseline status of antioxidant system, involved in a variety of detoxification reactions, to assure the maintenance of a balance between production and removal of reactive oxygen species (ROS) and other Pro-oxidants. These ROS include superoxide anion radical (O_2) hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH). As a consequence of the reactivity of ROS and their potential to damage cells and tissues, marine and other organisms balance the production of these radical with a wide variety of cellular antioxidant defenses. Prominent among these antioxidants are the enzymes super oxide dismutase (SOD, EC.1.15.1.1), Catalase (CAT; EC.1.11.1.6), Glutathione peroxides (GPX; EC.1.11.1.9) and Glutathione reductase (GSSGR).

A. Superoxide dismutase (SOD) Catalyses the conversion of Superoxide anion radical to H_2O_2.
B. Catalase (CAT) reduces H_2O_2 to Water $2H_2O_2 \rightarrow 2H_2O + O_2$
C. Glutathione Peroxidase (GSH-Px) acts on conjunction with other enzymes to H_2O_2 and to terminate lipid peroxidation.

Despite the relative scarcity information the relationship between age and oxidative stress in Aquatic organisms, the general definition of ageing as "The progressive accumulation of changes that are responsible for decreased ability of organisms to maintain Physiological homeostasis, which may eventually lead to functional impairment and even death. Further, Dandepat *et al* [11] found that the antioxidant defenses play an important role in providing protection to the developing larvae from oxidative assault. Production of ROS during larval development is likely to depend upon metabolic status of the cell and the ambient oxygen tension. Although a definite role of the ROS and antioxidants is established in various cellular processes such as development, differentiation, regeneration and regression [1] knowledge on the role of ROS and antioxidants during the embryonic and larval development of aquatic animals in general and Crustaceans in particular is Scanty [2]. A number of studies have demonstrated potential for ROS generation, antioxidant enzyme and free radical scavenges responses and oxidative damage in species of Invertebrates, mainly in mollusks [27, 28]. As a consequence of the reactivity of ROS and their potential to damage cells and tissues, and other organisms balance the production of these radicals with a wide variety of cellular antioxidant defenses. Prominent among these antioxidants are the enzymes Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX). Antioxidant enzymes can be induces by various environmental pro-oxidant conditions i.e. increased ROS generation. In normal metabolism i.e. without the influence of stress conditions, a balance exists between the generation of ROS and other pro-oxidants and their detoxification and removal by antioxidant defense mechanisms [55]. However, either an increase in ROS production above the level that can be removed by antioxidant defenses or a decrease in the capacity of the antioxidant defenses, could result in oxidative damage to key molecules including DNA, Protein and Lipids (Lipid peroxidation) [19]. A number of studies have demonstrated potential for ROS generation, antioxidant enzyme and free radical scavenging responses, and oxidative damage in species of Invertebrate including mollusks [27, 28]. However, few studies have been undertaken on Crustaceans and little is known about such mechanisms. Generally pro-oxidant/antioxidant balance and detoxification of potentially damaging ROS is crucial for cellular homeostasis [27]. In the present investigation CAT, SOD and GPX activities, the key antioxidant enzymes were found to indicate the involvement of detoxification of ROS species. The ROS are continuously produced as unwanted by-products of normal oxidative metabolism, principally from mitochondrial respiration [49]. Therefore, intrinsic changes in oxygen consumption over an animal's lifetime can be important factor affecting age and possibly sex dependent differences in the antioxidant status of Aquatic organisms. For example, during the course of egg and embryonic development, a gradual increase in oxygen uptake was seen in the prawn *Macrobrachium malcolmsonii* that appeared to be counteracted by an increase in CAT, SOD and GPX activities [2]. In the present investigation all the antioxidant enzymes are playing key role in the detoxification of ROS species and inducing the growth potentials. So the present investigation demonstrates that *M. rosenbergii* was found to possess the key antioxidant enzyme activities CAT, SOD and GPX, similar to a wide variety of other aquatic Invertebrates species including Echinoderms, Molluscs, Annelids, and other Crustaceans.

FAO has now designated the use of Probiotics as a major means for the improvement of aquatic environmental quality [52]. Most studies on the effects of Probiotics on cultured aquatic animals have emphasized a reduction in mortality (or) the improved resistance against putative pathogens [20]. Several studies on Probiotics have been published during the last decade. However, the methodological and ethical limitations of animal studies make it difficult to understand the mechanism of action of Probiotics, and only partial explanations are available. Nevertheless, some possible benefits linked to the administering of Probiotics have already been suggested as competitive exclusion of pathogenic bacteria [15, 53]; source of nutrients and enzymatic contribution to digestion direct up take of dissolved organic material mediated by the bacteria [15, 34] and others are still being investigated as enhancement of the immune response against pathogenic microorganisms [3, 8, 9, 44], antiviral effects [12]. These may be the cause for growth promoting effects of Probiotics. Jagan Mohan and Prasad [21] reported the effects of Probiotics on the Growth and Survival of penaeid prawn *Penaeus monodon*. Literature is available on the use and selection of Probiotic bacteria for use in the culture of larval aquatic organisms including Penaeid shrimps *Penaeus monodon, Litopenaeus vannamei*, *Fenneropenaeus indicus* and Freshwater prawns *Macrobrachium rosenbergii* and other Crustaceans [14, 16, 33, 35, 42].

Some researchers have suggested that microorganisms have beneficial effects in the digestive process of aquatic animals. In fish it has been reported that Bacteroids and *Clostridium* sp have contributed to the host's nutrition, especially by supplying fatty acids and vitamins [50]. Some microorganisms such as a *Agrobacterium sps*, *Pseudomonas sps, Brevibacterium sps* may contribute to nutritional process in arctic gharr *Salvelinus alpines*. Prieur *et al*., [41] reported that probiotics involved in digestion process of bivalves by producing extra cellular enzymes such as Proteases, Lipases, as well as providing necessary growth factors. In the present study the probiotics increasing the digestion process there by promote the growth rate of animals. Finding of Wang *et al.* [54] also support my study he reported that the microbial flora of adult penaeid shrimp *Penaeus chinensis*, where a complement of enzymes for digestion and synthesize compounds that are assimilated by the animal. Dall and Moriaty [13] have been reported that Microbiota may serve as a supplementary source of food and microbial activity in the track digestive may be a source of vitamins and essential amino acids. Other hand Probiotics enhances the immune response of the host organisms. It has been demonstrated that oral administration of *Clostridium butyricum* bacteria rainbow trout enhanced the resistance of fish to Vibriosis, by increasing the photolytic activity of leucocytes [46]. Rengpipat *et al.* [44] mentioned that the use of *Bacillus sps* provided disease protection by activating both cellular and humoral immune difference in tiger shrimp *P. monodon*. Balcazar [9] demonstrated that the administration of a mixture of bacterial strains *Bacillus* and *Vabrious sps*. positively influenced the growth and survival of

juvenile of white shrimp and presented a protective effect against the pathogen *Vibrio harveyi* and White spot syndrome virus. This protection was due to a stimulation of the immune system, by increasing phagocytosis and antibacterial activity.

Overall, the Research activity of Probiotics in Aquaculture in India is still in its infancy and not much of commercial Probiotics products were licensed in India so far. It is essential to understand the mechanism of action in order to define selection criteria for potential probiotics. Therefore more information on the host/microbe interactions in vivo and development of monitoring tools are still needed for better understanding of the composition and functions of the indigenous micro biota as well as of microbial cultures of 'Probiotics'. The process of screening of new probiotic strains form local Aquaculture rearing units to suit the specific requirement in India was already initiated. In the near future, Probiotics will gain more popularity in Aquaculture of India and the application areas will be expanded. With the increased use of molecular methods for the definitive analysis of the bacterial components of the Probiotic products and for *in vivo* validation, it is expected that both the probiotics quality and functional properties can be significantly be improved. This type of Research can aid for the development of adequate technology for the evaluation of the efficiency of microbial agents as Probiotics in Aquaculture. Therefore, it is important that the sustainability of this industry is maintained by improved Aquaculture practices coupled with the more effective use of scientifically approved disinfectants and sanitizers as feed supplements along with other biological agents in order to improve survival rate and growth to enhance yield and to minimize production cost.

4. References

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