Influence of the isolated probiotic bacteria on the water quality parameters of shrimp pond and their effect on growth and survival of the shrimp

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Abstract— Pond ecosystem is an important parameter influencing the shrimp production. Healthy and hygienic pond parameters promote higher shrimp production rates. As the culture period progress water quality of the pond changes due to the metabolic activities carried out by the animal. All these parameters should be maintained at optimum levels for higher survival and growth rates. Though several pond probiotics has been launched in the market search for isolation of potent probiotic bacteria with suitable pond application is a never ending process. This indicates the importance of pond environment in the animal production. In the present research isolated and identified probiotic bacteria are applied in the shrimp ponds to observe their influence on the water quality variables, growth and survival of the shrimp.

Index Terms— Aquaculture, Molecular characterization, Pond ecosystem, Probiotics, Shrimp,

1 INTRODUCTION

It is well known that the demand for animal protein for human consumption is currently on the rise and is largely supplied with terrestrial farm animals. Aqua farming however is an increasingly important option in animal production. This activity requires high-quality feeds with high protein content which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and favour growth. Some of the most utilized growth-promoting additives include hormones, antibiotics, ionophores and some salts. Though these do promote growth, their improper use can result in adverse effects in the animal and the final consumer as well as lead to resistance in pathogenic bacteria in the case of antibiotics. Hence, probiotics are considered as the best alternatives for antibiotics in the field of aquaculture. On the other hand probiotics were also known to stimulate the immune system of the aquatic animal [1]. In aquaculture, probiotics were used as water and feed probiotics. Water probiotics improve the quality of water and the pond bottom sediment thereby creating a stress free environment for the animal and thus improves its health [2]. Feed probiotics keep the aquatic animals healthy in terms of weight, size and nutrition [2]. In addition, probiotics also protect the aquatic animals from different microorganism and their virulence can also be controlled [3]. The probiotic bacteria exist as the normal flora of the host. The composition of the gut and intestinal flora of fish is utmost important in the aquaculture as it influences the productivity, spoilage of fish and faecal contaminant spread [4]. Besides this the gut and intestinal flora help in digestive function by producing certain digestive enzymes and they also acts as first line defence mechanism against the pathogen attack [5].Though Probiotics are widely used in poultry and swine rearing little has been done to incorporate them into aquaculture. Thus this study was designed to isolate and screen gut microflora of Common carp, *Cyprinus carpio*. The present research paper discusses about the isolation and identification of gut bacteria of *C. carpio* with potent probiotic characteristics and their identification by molecular sequencing and field experiments to ensure the safety application of these isolated probiotic strains in shrimp aquaculture.

2 MATERIALS AND METHODS

2.1 Isolation of gastrointestinal tract bacteria from Cyprinus carpio

Fresh water fish were collected from the fisherman in Nellore in live conditions and were brought to the lab with the help of aerators. The animals were dissected in live and sterile conditions. The gastrointestinal tracts (GIT) were aseptically removed and taken into a pre- sterilized homogenator and was finely ground and collected by adding sterile PBS solution. The microbiological protocol for isolation of the probiotic bacteria was done as described by Lakshmi et al. [1]. Probiotic characterization of the isolated strains was done by Acid tolerance, Bile tolerance, Antagonistic activity (Anti-vibrio activity).

2.2 Probiotic characterization

The isolated strains were assessed for their probiotic potential.

(a) Acid tolerance

Acid tolerance of the bacteria was tested as described by Conway et al. [6]. The PBS solution was adjusted to 2.5 by addition of HCl. The log phase cultures (8 hrs) were taken and 1ml of the culture was added to the PBS at 2.5 pH and incubated in a shaker incubator at 37°C. The viable organisms were enumerated by plating 0.1ml of the culture in regular intervals at 0, 1, 2 and 3 hrs on respective media and incubated at 37°C.

(b) Bile tolerance

The procedure of Klaenhammer and Kleeman [7] was used to

determine the bile tolerance of strains at final concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1.0% and incubated for 3hrs. The viability of strains was observed by plating the samples on the respective media and incubated for 24hrs at 37°C.

(c) Antagonistic activity or Anti-Vibrio activity

The antagonistic activity of probiotic bacteria is mainly due to bacteriocin production. The activity was determined against *Vibrio parahemolyticus* and *V.alginolyticus*. The Tagg and McGiven method of assay for bacteriocin [8] was used to determine the antagonistic activity of bacterial strains. Briefly, the log phase cultures of bacteria were taken for the test and the vibrio test cultures were grown for overnight in T1N1 broth. The log phase culture of the probiotic bacterial strains was added to the well on T1N1 agar and incubated at 37°c for 2hrs, later 0.1ml of the overnight cultures of the vibrio organisms was spread on the T1N1 agar medium and were observed for the presence of inhibition zones.

Finally the strains which showed potent probiotic properties were further confirmed at the molecular level by 16S rRNA sequencing method.

2.3 Molecular identification of the isolated bacteria and phylogenetic analysis

The isolated bacteria were identified at the molecular level by 16S rRNA sequencing and their phylogenetic relation was studied by constructing phylogenetic tree.

2.4 Field Experiments

The above isolated and in vitro tested probiotic strains were further taken for field studies in *Litopenaeus vannamei* culture pond. The experimental study was done for 90 days duration. The strains were formulated into a powder form with an appropriate carrier and preservative with a range of 20 billion cfu/gm. This probiotic product was applied to the pond at the rate of 1kg/acre. Inorder to ensure their field applicability and effect on pond ecosystem Standard water quality variables were studied for a culture period of 90 days. The ponds were maintained as control and experimental ponds throughout the experimental study. Total three ponds were maintained, control pond, Experimental pond-1 and 2 applied with individual isolated strains. Samples were collected for Water quality variable analysis at regular intervals of 1, 15, 30, 45, 60, 75 and 90 days. Microbiological analysis of water was also done by observing the population of Total heterotrophic bacteria (THB) and Total Vibrio count (TVC) at regular intervals of 30, 60 and 90 days, the growth and survival rate of the animal was also observed at respective intervals.

2.4.1. Water quality variables

The standard water quality parameters include temperature, salinity, transparency, pH, dissolved oxygen (D.O), alkalinity, total ammonia nitrogen, nitrites, nitrates and phosphates. These water quality variables were recorded by standard measurement techniques [9].

2.4.2. Microbiological analysis

As mentioned above the population of THB and TVC was observed by plating the samples on Zobell's marine agar medium and TCBS medium respectively.

2.4.3. Effect on growth and survival of animal

To ensure the safe applicability of the isolated probiotics, a preliminary observation was done on animals by monitoring their average body weight and percentage of survival for every 30 days interval.

Average body weight of the shrimps was calculated by following formula:

$$\text{Average body weight (ABW) (g)} = \frac{\text{Total wet weight of shrimps (g)}}{\text{Total number of shrimps}}$$

The estimation of the survival rate of the animal was calculated by the following formula:

$$\text{Survival Rate (\%)} = \frac{\text{No. Of approximate animals present in culture pond}}{\text{No. Of seed stocked}} \times 100$$

2.4.4. Statistical analysis

All the experimental data given in the results were means of triplicates.

3 RESULTS AND DISCUSSION

The ultimate aim of our research is to design probiotic bacteria for shrimp aquaculture; hence the experimental design is setup as per the requirement. Application of probiotics in aquaculture from various sources is being successfully reported but usually the probiotic bacteria isolated from aquatic sources adopt much better to the pond conditions when compared to the other sources of isolation [1].

3.1. Isolation and Screening of Probiotic bacteria from the gastrointestinal tracts of *Cyprinus carpio*

The fish is collected from the fisherman in live condition from its natural habitat. In its natural habitat fish need to adopt different defence mechanisms in order to cope up with the changes in the environment and survive. The bacterial composition of the aquatic system influences the gut and intestinal flora and they constantly face different pathogens [3]. In order to withstand such competition GIT flora tends to produce antimicrobial molecules, lytic enzymes and bacteriocins where they effectively inhibit the pathogen and promote the survival of the host [10], [11], [12], [13]. Thus the bacteria isolated from the gut and intestine of the fish will surely exhibit the probiotic properties.

As a part of our experiment we have selected *C. carpio* as one of the sources for isolation of probiotic bacteria. Previous reports also suggest that bacteria isolated from *C. carpio* posses potential probiotic properties and are capable of inhibiting some aquaculture pathogens [14]. A total of 14 microbial strains were isolated from the intestine of *C. carpio* and for convenience the isolated strains were given names as CC-1 to CC-14 where CC stands for *C.carpio*. These 14 strains were evaluated for their probiotic properties and finally two strains CC-1 and CC-2 with potent probiotic ability were considered

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and were designated as DVRSG-1 and DVRSG- 2.

3.2. Acid tolerance

2.5 pH is known to be the condition of pH observed during digestion and it is significant as it inhibits the proliferation of pathogenic microbes in the host system. Usually probiotic bacteria need to colonize and survive in the intestinal and gut regions where the acidic pH exists hence tolerance to acidic conditions was considered to be one of the major characteristic features of a probiotic strain and was taken as a determining factor. Among the 14 isolated strains, DVRSG-1 and DVRSG-2 significantly differ in their acid tolerance ability (Table.1).

TABLE 1
ACID TOLERANCE OF THE ISOLATED BACTERIAL STRAINS

Strains	Acid tolerance at pH 2.5 (Log Cfu/0.1ml)			
	Cfu at 0hr	Cfu at 1 hr	Cfu at 2hrs	Cfu at 3hrs
CC-1	**8**	**7**	**7**	**5**
CC-2	**10**	**8**	**5**	**5**
CC- 3	6	3	3	2
CC- 4	6	3	2	2
CC- 5	5	3	2	2
CC-6	6	5	2	0
CC-7	5	4	2	0
CC-8	4	2	2	0
CC-9	4	3	2	2
CC-10	5	3	3	2
CC-11	6	5	4	2
CC-12	7	5	3	1
CC-13	6	5	3	2
CC-14	5	3	2	0

Values are the means of triplicates.

The results obtained in the current experiment also support that the isolated bacteria were capable of exhibiting probiotic properties. In a study by Zhou et al., [15] the probiotic bacteria at pH-2 with cfu 7.7±0.04 to 7.88±0.08 were reported as acid tolerant bacteria. Similar reports were given by Lin et al., [16], 5.83±0.06 cfu was recorded at pH-2, 3hr exposure for probiotic bacteria. The isolated bacteria showed cfu ranging from 5.35±0.03 to 7.72±0.02. These values of cfu refer that the isolated bacteria can survive (better than the remaining isolates) even in the low pH conditions that prevail in the host intestine. However the gastric pH of the intestine will decrease in the presence of feed and feed ingredients which support the viability and survival of the probiotic bacteria [17], [6], [18], [19].

3.3. Bile tolerance

Bile tolerance was the second important feature attributed by a probiotic strain. Bile salts play a very important role in lipid digestion and absorption [20]. Bile salts are usually given in feed supplements where they serve as a source of sterols which are an essential nutritional requirement. Prawns require steroids for the production of moulting hormone, to allow the rapid passage through their different larval growth phases. Steroids must be present in their diet for them to reach normal size. The bile salts were given in concentrations from 0.1% to 0.5%. Bile salts were considered as natural bacterial growth inhibitors, hence the strain used as a probiotic supplement needs to be tolerant to these concentrations of bile salts.

The isolated strains were tested at 0.2% to 1% bile salt concentrations. DVRSG-1 and DVRSG-2 showed good tolerance when compared to the remaining (Table-2).

TABLE 2
BILE TOLERANCE OF THE ISOLATED STRAINS

	Bile tolerance at different concentrations (Log Cfu/0.1ml)				
Strains	Cfu at 0.2%	Cfu at 0.4%	Cfu at 0.6%	Cfu at 0.8%	Cfu at 1.0%
CC-1	**10**	**8**	**7**	**5**	**5**
CC-2	**10**	**9**	**8**	**7**	**7**
CC-3	5	6	6	6	5
CC-4	6	5	5	4	0
CC-5	6	5	5	3	1
CC-6	6	4	4	4	0
CC-7	5	3	2	2	0
CC-8	5	5	3	3	0
CC-9	6	5	3	2	0
CC-10	5	4	3	2	0
CC-11	6	4	3	0	0
CC-12	5	4	3	2	1
CC-13	0	0	0	0	0
CC-14	0	0	0	0	0

Values are the means of triplicates.

According to Gilliland et al [21], 0.3% of bile salts concentration is the critical concentration used to screen the bile tolerant bacteria. The bile tolerance capacity of probiotics was attributed by the Bile salt hydrolase enzymes. The enzymes act by deconjugating the bile salts by hydrolysing the amide bond and liberate the glycine/taurine moiety from the steroid core. The resulting acids are termed as unconjugated or deconjugated bile acids [22]. In the present experiment among the isolated bacterial strains showed cfu 8.53±0.02 for DVRSG-1and 8.65±0.04 for DVRSG-2 which are significant for bile tolerant probiotic bacteria. Similar results were reported by Zhou et al., [23], [15] in which 8.34 to 8.75 as acceptable cfu for bile toler-

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ant bacteria.

3.4. Antagonistic activity or Anti-Vibrio activity

Inhibition zones were observed only for DVRSG-1 and DVRSG- 2 strains after incubation period, these two strains inhibited the growth of both *V. alginolyticus* and *V. parahemolyticus* (Fig. 1 and 2). These two species of vibrios were considered to be serious pathogens causing severe outbreaks of Vibriosis in different parts of world leading to major economic loss in the aquaculture industry (shrimp and fish as well). Hence we have chosen these pathogens for the evaluation of the bacteriocin assay. In our experiment DVRSG-1 and DVRSG-2 were able to inhibit the growth of the pathogens hence these two strains were positive for bacteriocin assay.

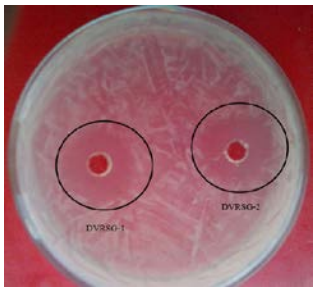

Fig 1. Antagonistic activity of Probiotic strains against *Vibrio parahemolyticus*

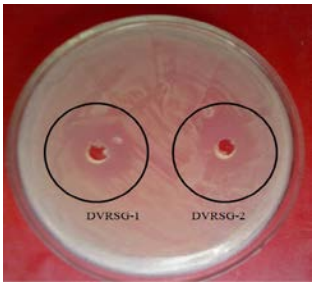

Fig 2. Antagonistic activity of Probiotic strains against *Vibrio alginolyticus*

Bacteriocins inhibit the pathogenic bacteria by permeabilizing the cell membrane leading to leakage of cellular solutes and eventually cell death [24], [25]. Previous reports suggest that bacteriocins were capable of effectively inhibiting gram positive bacteria where teichoic acids and lipoteichoic acids in the bacterial membrane serves as receptors for the bacteriocin indicating their narrow target specificity [26].

These pH and bile tolerant and bacteriocin assay positive strains, DVRSG-1 and DVRSG-2 were finalized as two potent probiotic strains. These were further identified by 16S rRNA sequencing and were analyzed for their identity with other species by phylogenetic tree construction.

3.5.16S rRNA gene sequencing and phylogenetic analysis

BLAST analysis of the partial 16S rRNA gene sequencing indicated the identity of the isolates as strains of *Staphylococcus* genera.

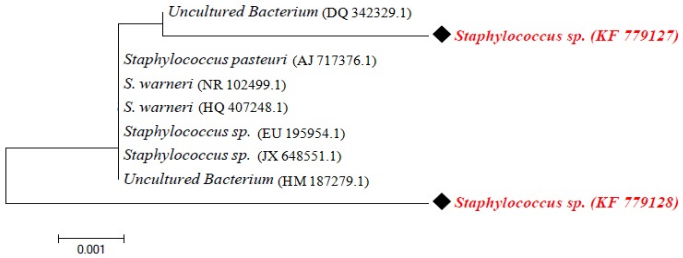

Fig 3. Phylogenetic analysis of isolated probiotic bacteria atch

sequence identity with *Staphylococcus pasteuri* strain and *S. warneri.* These strains were deposited to NCBI and they were assigned the accession numbers KF779127 for DVRSG-1 strain and KF779128 for DVRSG-2. Phylogenetic analysis confirmed the isolates identity as a strain of *Staphylococcus* sp. (Fig.3).

3.6. Field Experiments

Once the isolated bacteria were tested invitro for their probiotic ability they were formulated into a finished product and applied to the shrimp (*Litopenaeus vannamei*) ponds in order to evaluate their applicability and sustainability in the pond ecosystem. The results obtained support the safe applicability and fine existence of the isolated probiotic bacteria in the pond ecosystem.

3.6.1. Water quality variables

The temperature did not differ significantly on each sampling day and there were no noticeable change in control and probiotic treated ponds. The temperature was recorded to be to 27.8 ºC for control and 27 ºC for experimental ponds (Table.3). Same results were observed for pH (Table.3) and salinity (Table.3) which varied between 7.6 to 8.0 for pH and 15 to 20 ppt for salinity, which are observed to be an optimum range for shrimp ponds. Alkalinity also remained constant throughout experimental period in both control and experimental pond (Table.3). Transparencies between 45-55 cm were recorded in control ponds and 35-45cm in probiotic treated ponds (Table.3) and the D.O. levels were higher in probiotic treated ponds (5.3) than the control (4.0) (Table.3).

The values for transparency varied significantly at the end of the experiment. The recommended values for transparency are 30-40cm [27] and the optimum range of secchi disc reading was reported by Soundarapandian et al., [28] between 25-40cm. In the present study transparencies between 45-55cm were recorded in control ponds and 35-45cm in probiotic treated ponds, low transparency readings were observed for probiotic treated ponds than control. Similar results were reported by Hossain et al., [29]. It indicates the high penetration of light into the pond resulting in the optimal growth of phytoplankton thereby inhibiting the growth of odour producing and pathogenic bacteria. This statement is also supported by the reports given by Rajinikanth et al., [30].

Dissolved oxygen plays an important role in the culture ponds; it keeps the pond ecosystem healthy and promotes the growth of aerobic bacteria and a low level of D.O. hampers the metabolic activities and reduces the shrimp growth [31]. In the present experiment D.O. levels were higher in probiotic treated ponds (5.3) than the control (4.0). It reflects the higher microbial load in the control ponds. The variations in the transparency and D.O. levels during the culture period were shown in the Table.3.

TABLE 3.
INFLUENCE OF ISOLATED PROBIOTIC BACTERIA ON PHYSICAL WATER QUALITY VARIABLES IN CONTROL (UN-

TREATED) AND EXPERIMENTAL PONDS (PROBIOTIC TREATED PONDS) AT REGULAR TIME INTERVALS

Water quality variable	Pond	Culture time in days						
		1	15	30	45	60	75	90
Temperature (ºC)	C.P	26.4	26.9	27.0	27.3	27.5	28.0	27.8
	Ex.P-1	26.4	26.5	28.2	30.0	28.0	27.9	27.0
	Ex.P-2	26.4	26.0	27.0	29.5	27.5	28.0	27.5
pH	C.P.	7.8	8.0	7.9	7.5	7.7	7.8	7.6
	Ex.P-1	7.8	8.0	7.8	7.5	7.2	7.5	7.3
	Ex.P-2	7.5	7.8	7.6	7.8	7.6	7.5	7.5
Salinity (ppt)	C.P.	15.4	18.6	16.5	15.8	20.2	20.5	15.7
	Ex.P-1	15.5	16.2	17.4	16.8	18.4	17.5	15.0
	Ex.P-2	16.3	17.5	17.4	16.6	18.2	16.7	16.5
Alkalinity (mg/l)	C.P.	145.3	155.4	155.5	180.2	220.2	200.2	220.0
	Ex.P-1	150.2	150.5	155.4	175.3	190.3	200.0	200.2
	Ex.P-2	155.5	155.4	160.3	175.4	195.4	200.1	200.1
Transparency (cms)	C.P.	54.2	49.1	50.0	48.3	50.3	47.1	44.2
	Ex.P-1	44.4	44.5	42.2	43.1	39.4	39.0	36.2
	Ex.P-2	44.5	45.0	43.2	43.3	40.5	38.2	37.4
D.O (mg/l)	C.P.	4.0	4.5	4.2	4.2	4.2	4.0	4.0
	Ex.P-1	4.5	4.8	5.2	5.0	5.3	5.4	5.3
	Ex.P-2	4.5	4.7	4.9	5.0	5.2	5.3	5.3

Values are the means of triplicates

The other water quality variables, Total Ammonia-Nitrogen, Nitrites, Nitrates, Phosphates concentrations were reported to be significantly lower than the control and the fluctuations during the culture period were shown in the Figures (fig.4, 5, 6, 7).

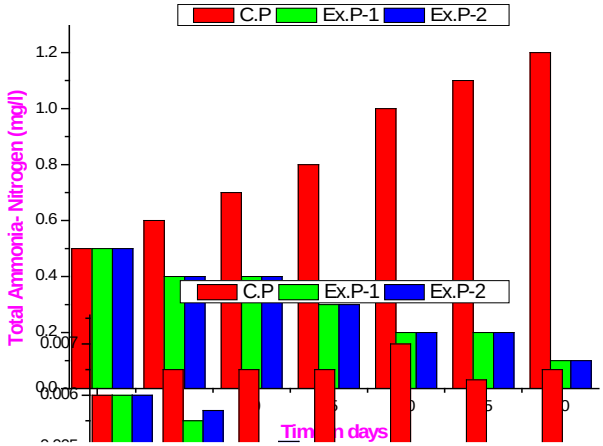

Fig 4. Influence of isolated probiotic bacteria on Total Ammonia Nitrogen (TAN) in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

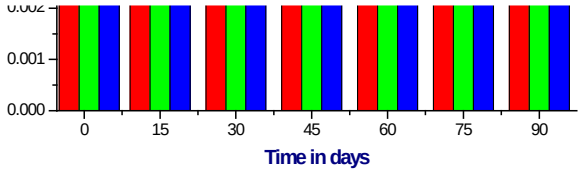

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Fig 5. Influence of isolated probiotic bacteria on Nitrites in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

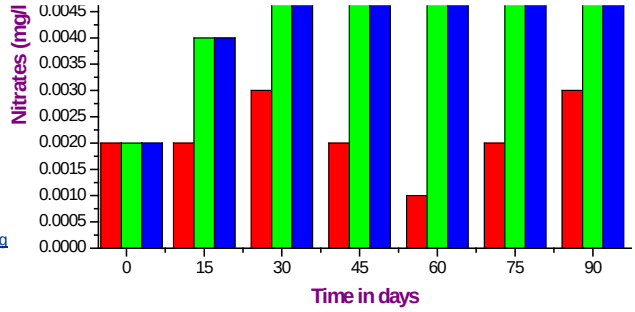

Fig 6. Influence of isolated probiotic bacteria on Nitrates in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

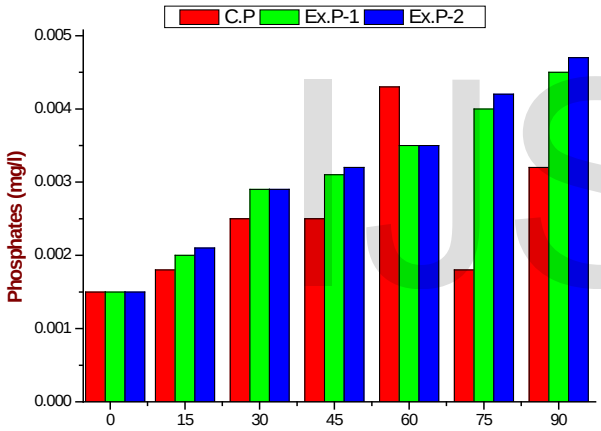

Fig 7. Influence of isolated probiotic bacteria on Phosphates in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

The readings obtained in the present experiment explains that added probiotic bacteria supported the beneficial microbial communities in the pond ecosystem thereby enhancing their activities to decompose the organic matter and reducing the nitrogen and phosphorous concentrations in the probiotic treated ponds when compared to the control pond. Similar observations were recorded by the studies done by Wang et al., [32] when the ponds were treated with commercial probiotics; a report given by Rajinikanth et al., [30] when treated with commercial probiotics also referred similar results. The readings of the present experimental study support the in vitro bacteriocin assay results of the selected probiotic strains. When applied to the field besides the bacteriocin activity another mechanism, competitive exclusion is to be considered, competitive exclusion mechanism is an important mode of defence exhibited by the probiotic bacteria to encounter the pathogen attacks [33], probiotic bacteria compete with the pathogenic bacteria for nutrients and existence and suppress the growth of pathogens thereby decreasing their population.

3.6.2. Microbial analysis

The microbial count for the TVC (Yellow colonies- *Vibrio alginolyticus*; Green colonies- *V.parahemolyticus*) was significantly lower in the probiotic treated ponds than the control (Table. 4). The periodic plating of the samples showed a decrease in the vibrio count which was an important property to be considered for probiotic bacteria. THB represent the beneficial and pathogenic microbial communities that exist in a pond ecosystem. In the present experiment the THB count does not vary significantly in control and experimental ponds.

The microbial composition of the pond depends upon the organic matter deposited irrespective of the other environmental factors [34]. The pond water parameters like temperature, salinity, pH, D.O. greatly influence the microbial distribution and their existence [35]. THB represent the beneficial and pathogenic microbial communities that exist in a pond ecosystem. In the present experiment the THB count does not vary significantly in control and experimental ponds (Table 4). This can be inferred as the rise in the THB of the control represent the pathogenic microbial communities whereas the THB count in the probiotic treated ponds stands for the increase in the beneficial bacterial communities. This statement can be supported from the results of the TVC in the probiotic treated ponds.

TABLE 4
MICROBIAL ANALYSIS IN CONTROL (UNTREATED) AND EXPERIMENTAL PONDS (PROBIOTIC TREATED PONDS) AT REGULAR TIME INTERVALS.

Type of Micro organisms	Pond	Culture time in days		
		30	**60**	**90**
Total Heterotrophic bacteria (THB) Cfu (x10^7/ml)	C.P	8.7	9.4	9.6
	Ex.P-1	8.5	9.1	9.7
	Ex.P-2	8.3	9.0	9.5
Total Vibrio count (TVC)Cfu (x10^7/ml) Yellow colonies	C.P	1.9	2.0	2.1
	Ex.P-1	1.5	1.2	1.0
	Ex.P-2	1.7	1.1	1.0
Green colonies	C.P	1.4	2.0	2.8
	Ex.P-1	1.0	0.9	0.5
	Ex.P-2	1.0	0.7	0.2

Values are the means of triplicates.

3.6.3. Effect on Growth and Survival of the animal

The average body weight of the animal did not differ significantly but the probiotic treated ponds showed mild increase in the animal body weight for which the reasons are unknown (Fig. 8). Survival percentage showed a significant variation in the probiotic treated ponds to control pond (Fig. 9). This can be supported by the above results which show that the isolated probiotic bacteria plays a significant role in maintaining

good water quality parameters which promotes the high survival and growth rate of the shrimp.

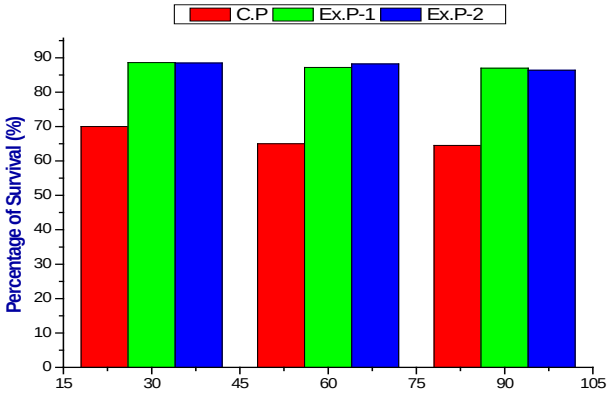

Fig 8. Influence of isolated probiotic bacteria on Average body weight in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

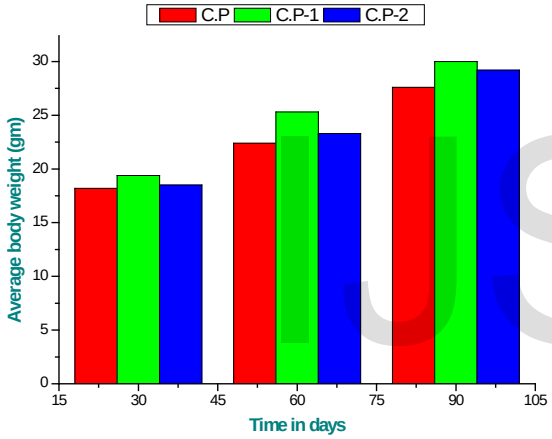

Fig 9. Influence of isolated probiotic bacteria on Percentage of survival in Control (untreated) and Experimental ponds (Probiotic treated ponds) at regular time intervals. Values are the means of triplicates.

4 Conclusion

In essence, the fish gastrointestinal tract is a tube-like structure that varies in complexity depending on the various feeding habits of the species. From the above research the isolated strains DVRSG-1 and DVRSG-2 were known to posses probiotic potential. As these were isolated from healthy fish gastrointestinal tracts, these can be safely applied for aquaculture practices. From the in vitro analysis these two strains can be considered as probiotic bacteria and in vivo analysis like field trials ensure their significant role in pond ecosystem. Besides this the preliminary observation on the growth and survival of the animal reflect their positive influence on the animal. From this experimental study it can be concluded that the isolated bacterial strains can be considered as potent probiotic strains for safe application in the shrimp aquaculture. Further research on supplementation of these probiotic bacteria in feed and their effect on the animal productivity, immunity index are under process. We can certainly concluded that a microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part at least, by improving the microbial balance of the fish.
Place the actual footnote at the bottom of the column in which it is cited; do not put footnotes in the reference list (endnotes). Use letters for table footnotes (see Table 1). Please do not include footnotes in the abstract and avoid using a footnote in the first column of the article. This will cause it to appear of the affiliation box, making the layout look confusing.

Acknowledgment

Authors are highly thankful to Macrogen, South Korea for their help in sequencing the isolated organisms. Also thankful to authorities of Sri Venkateswara University, Tirupati, India for their encouragement and support.

References

[1] B. Lakshmi, B. Viswanath, and D.V.R. Sai Gopal, "Probiotics as Antiviral Agents in Shrimp Aquaculture", *Journal of Pathogens*, http://dx.doi.org/10.1155/2013/424123, 2013.

[2] D. J. W. Moriarty, O. Decamp, P. Lavens, "Probiotics in aquaculture September/October", *AQUA culture Asia Pacific magazine*, 2005.

[3] [3] L. Verschuere, G. Rombaut, P. Sorgeloos, and W. Verstraete, "Probiotic bacteria as biological control agents in aquaculture", *Microbiology and Molecular Biology Review,* vol. 64, pp. 655-671, 2000.

[4] [4] N. Uddin, and H. Ahmed, "Bacterial flora of polycultured common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*)", *International Aquatic Research*, vol. 4, pp. 10, 2012.

[5] [5] J. W. Sissons, "Potential of probiotic organisms to prevent diarrhoea and promote digestion in farm animals–a review", *Journal of the Science of Food and Agriculture,* vol. 49, pp. 1–13, 1989.

[6] [6] P. Conway, S. L. Gorbach, and B. R. Goldin, "Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells", *Journal of Dairy Science,* vol. 70, pp.1-12, 1987.

[7] [7] T. R. Klaenhammer and E. G. Kleeman, "Growth characteristics, bile sensitivity and freeze damage in colonial variants of *Lb.acidophilus*", *Applied and Environmental Microbiology,* vol. 41, pp.1461-1467, 1981.

[8] [8] J. R. Tagg, and A. R. Mc Given, "Assay system for Bacteriocins", *Applied Microbiology,* vol. 21, pp. 943, 1971.

[9] [9] M. A. Stand and H. Fauson, Standard methods for the examination of water and waste water edited by (American Public Health Association, Washington DC, USA), APHA, 1995.

[10] [10] C. Dunne, L. Mahony, L. Murphy, G. Thornton, D. Morrissey, S. Halloran, M. Feeney, S. Flynn , G. Fitzgerald, C. Daly, B. Kiely, G. C. Sullivan, F. Shanahan, and J. K. Collins, "In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings", *American Journal of Clinical Nutrition,* vol. 73, pp. 386S-392S, 2001.

[11] [11] D. E. Chang, D. J. Smally, D. L. Tucjer, M. P. Leatham, W. E. Norris, S. J. Stevenson, A. B. Anderson, J. E. Grissom, D. C. Laux, P. S. Cohen, and T. Conway, "Carbon nutrition of Escherichia coli in the mouse intestine", *Proceedings of the National Academy of Sciences,* vol. 101, pp.7427- 7432, 2004.

[12] [12] M. A. Riley and D. M. Gordon, "The ecological role of bacteriocins in bacterial competition", *Trends in Microbiology*, vol. 7, pp. 129-133, 1999.

[13] [13] V. H. Smith and R. D. Holt, "Resource competition and within-host disease dynamics", *Trends in Ecology and Evolution,* vol. 11, pp. 386-389, 1996.

[14] [14] T. Hagi, and T. Hoshino, "Screening and Characterization of Potential probiotic Lactic acid Bacteria form cultured commo.n carp intestine", *Bioscience, Biotechnology and Biochemistry*, vol. 73, pp.1479-1483, 2009.

[15] [15] T. Zhou, B. Li, C. Peng, B. P. Ji, G. Chen, and Y. L. Ren, "Assessment of the Sequential Simulated Gastrointestinal Tolerance of Lactic Acid Bacteria from Kefir Grains by Response Surface Methodology", *Journal Of Food Science*, vol. 74, no. 6, 2009.

[16] [16] W. H. Lin, C. F. Hwang, L. W. Chen, and H. Y. Tsen, "Viable counts, characteristic evaluation for commercial lactic acid bacteria products", *Food Microbiology*, vol. 23, pp. 74–81, 2006.

[17] [17] W. P. Charteris, P. M. Kelly, L. Morelli, and J. K. Collins, "Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract", *Journal of Applied Microbiology*, vol. 84, no. 5, pp. 759-768, 1998.

[18] [18] Y. Huang and M. C. Adams, "In vitro assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria", *International Journal of Food Microbiology*, vol. 91, no. 3, pp. 253-260, 2004.

[19] [19] G. Zarate, A. Perez-Chaia, S. Gonzalez and G. Oliver, "Viability and β-galactosidase activity of dairy propionibacteria subjected to digestion by artificial gastric and intestinal fluids", *Journal of Food Protection*, vol. 63, no. 9, pp. 1214-1221, 2000.

[20] [20] M. Begley, C. G. M. Gahan, and C. Hill, "The interaction between bacteria and bile", *FEMS Microbiology Reviews,* vol. 29, pp. 625-651, 2005.

[21] [21] S. E. Gilliland, T. E. Staley and L. J. Bush, "Importance in bile tolerance of Lactobacillus acidophilus used as a diatery adjunct", *Journal of Dairy Science*, vol. 67, no. 12, pp. 3045-3051, 1984.

[22] [22] M. Begley, H. Colin, and C. G. M. Gahan, "Bile Salt Hydrolase Activity in Probiotics", *Applied and Environmental Microbiology*, vol. 72, pp. 1729–1738, 2006.

[23] [23] X. Zhou, Y. Pan, Y. Wang and W. Li, "In vitro assessment of gastrointestinal viability of two photosynthetic bacteria, Rhodopseudomonas palustris and Rhodobacter sphaeroides", *Journal of Zhejiang University SCIENCE B,* vol. 8, no. 9, pp. 686-692, 2007.

[24] [24] Y. Hechard and H. G. Sahl, "Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria", *Biochimie*, vol. 84, pp. 545-557, 2002.

[25] [25] G. N. Moll, W. N. Konings, and A. J. Driessen, "Bacteriocins: mechanism of membrane insertion and pore formation", Antonie *Leeuwenhoek*, vol. 76, pp.185–198, 1999.

[26] [26] M. Van Belkum, J. Kok, G. Venema, H. Holo, I. F. Nes, W. N. Konings, and T. Abee, "The bacteriocin lactococcin A specifically increases permeability of lactococcal cytoplasmic membranes in a voltage-independent, protein mediated manner", *Journal of Bacteriology*, vol. 173, pp. 7934–7941, 1991.

[27] [27] MPEDA, Media campaign on "Welfare schemes of Central Government", Gopichettipalayam on 27-28 January, 2006.

[28] [28] P. Soundarapandian, V. Ramanan and G. K. Dinakaran, "Effect of probiotics on the growth and survival of Penaeus monodon (Fabricius)", *Current Research Journal of Social Sciences,* vol. 2, no. 2, pp. 51-57, 2010.

[29] [29] M. I. Hossain, M. M. Kamal, M. A. Mannan, M. A. B. Bhuyain, and M. I. Hossain, "Effects of Probiotics on Growth and Survival of Shrimp (Penaeus monodon) in Coastal Pond at Khulna, Bangladesh", *Journal of Scientific Research*, vol. 5, no. 2, pp. 363-370, 2013.

[30] [30] T. Rajinikanth, P. Ramasamy and V. Ravi, "Efficacy of Probiotics, Growth Promotors and Disinfectants in Shrimp Grow out Farms", American-Eurasian Journal of Agriculture & Environment Science, vol. 7, no. 3, pp. 347-354, 2010.

[31] [31] L. M. Gilles, "Environmental factors affect immune response and resistance in Crustaceans", *The Advocate*, pp: 18, 2001.

[32] [32] Y. B.Wang , Z. R. Xu and M. S. Xia, "The effectiveness of commercial probiotics in northern white shrimp Penaeus vannamei ponds", *Fisheries Science,*vol. 71, pp. 1036–1041, 2005.

[33] [33] R. Fuller, "Probiotic the scientific basis", 1st edition Chapman & Hall London UK, 1992.

[34] [34] R. Sharmila, T. J. Abraham and V. Sundararaj, "Bacterial flora of semi-intensive pond-reared Penaeus indicus (H. Milne Edwards) and the environment", *Journal of Aquaculture in the Tropics*, vol. 11, pp. 193-203, 1996.

[35] [35] R. Palaniappan, "Studies on the microflora of the prawn *Penaeus indicus*, Milne Edwards (crustacea, decopodes, penaedae) with reference to its digestive system. Ph.D Thesis", Annamalai University, India, 1982.

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