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Effect of Probiotic Bacteria on Shrimp Pond Ecosystem and their Influence on Growth and Survival of Litopenaeus Vannamei

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ABSTRACT

The survival and growth of shrimp is key constraints that influence the shrimp aquaculture. Good growth and high survival rate of shrimp endorse 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. The isolated potential probiotic bacterial strains are applied in the shrimp ponds to observe their influence on the water quality variables. To make sure the secure applicability of the isolated probiotics, a preliminary examination was done on Litopenaeus vannamei by screening their average body weight and percentage of survival. Litopenaeus vannamei showed a significant increase in body weight and the survival rate in the probiotic treated ponds than the control ponds. The field experiments proved that the probiotic bacteria treated ponds showed better water quality than the control ponds; this indicated that the isolated probiotic bacteria were capable of inducing a positive effect on the prawn health. it is was concluded that the isolated bacteria were capable of exerting probiotic properties and field studies of the four probiotic bacterial isolates confirmed that they can be safely applied to the prawn culture ponds.

Keywords: Probiotic Bacteria, Shrimp Pond Ecosystem, Aquaculture, Water Quality.

I. INTRODUCTION

Probiotics play a fundamental role on the induction and function of the host immune system. It is reported that the gastrointestinal microbiota of fishes provide a number of functions, such as digestion, angiogenesis, improvement of the mucosal system, and as defense barrier against diseases [1]. Therefore it is very vital to recognize the composition of these microbes in fish and their effects on different factors especially in the case of fish culture with high economical importance. The immune system has greatly evolved as means to uphold the symbiotic association of the host with these highly different and evolving microorganisms. This microbes and immune system alliance permit the initiation of defensive responses to disease causing microorganism [2]. One of the very effective methods for controlling the aquaculture diseases is the application of the probiotics which control the pathogens through different mechanisms. Probiotics enhance the growth of beneficial bacteria and reduce the growth of the pathogens leading to the improved healthier fish or prawn and water quality. The usage of probiotics in aquaculture improves the quality of pond bottom sediment and water, which leads to stress free surroundings for the fishes and improve their health [3].

Identification of probiotic bacteria has usually been very crucial experimental process based on scientific reports. Many of the failures in probiotic research had recorded due to the selection of unsuitable microorganisms. Selection procedures have been defined, but they have to be chosen in accordance for different environments and host species. It is very crucial to recognize the mechanisms of probiotic action and selection process for potential probiotics. Methods of identification, processing of probiotic bacteria, and mode of administration of the probiotic to the host are of very vital steps which make the probiotic application successful [4].

In aquatic animals, the probiotic feed enhances the animal health in terms of size, weight and nutrition. Moreover, the applications of probiotics in the aquatic animals protect them from the virulence of aquaculture diseases caused microbes. Probiotics not only reduce the pathogens but also serve as the good source of nutrients and also have advantageous effect in the digestive processes of aquatic animals. They supply essential vitamins and fatty acids that are necessary to the aquatic animals by synthesizing a complement of enzymes like lipases, proteseas and necessary growth factors that support the digestive process of the aquatic animals. Probiotics have the capability of improving immune response in the aquatic animals. They trigger the non-specific immune response in the aquatic animals. They trigger the non-specific immune response in aquatic animals which is of very important in disease resistance of the aquatic fishes. In the present study, the probiotic bacteria was isolated from the gastrointestinal tract of aquatic fishes and their probiotic ability to show impact on ***Litopenaeus vannamei*** health and pond ecosystem was assessed by conducting field experiments [5].

II. MATERIALS AND METHODS

Sample collection and isolation of gastro intestinal tract from fresh water fishes

Aquatic fishes were selected as source for the isolation of probiotic bacteria. The aquatic fishes selected were two fresh water fishes *Wallago attu* commonly called as Wallago and *Oreochromis mossambicus* commonly called as Tilapia. These two fresh water fishes were collected from the Sangamaheshwaram confluence, where two rivers meet, located around 12 km away from Nandikotkur, Kurnool district of Andhra Pradesh with GPS coordinates of 16^{0}1'11.88272" N and 78^{0}19'46.488" E. Ten fishes from each species were brought to the lab to isolate probiotic bacteria (Figure 3.1). These samples were brought to the lab in live conditions with the help of aerators. Probiotic bacteria normally reside in the gastro intestinal tract (GIT) of the host, hence gastro intestinal tract were chosen for the isolation of probiotic bacteria. The selected fresh water fishes i.e., *Wallago attu* (WA) and *Oreochromis mossambicus* (OM) were dissected in the laboratory under sterile conditions and the GITs were removed aseptically. They were processed immediately for the isolation of bacteria and stored under -20ºC for further usage.

Characterization of isolated probiotic bacteria

The isolated bacterial strains from fresh water fishes were evaluated for their probiotic potential by acid tolerance, bile tolerance and antagonistic activity.

Acid tolerance test

Conway et.al method was followed to assess the acid tolerance test [6]. By using HCL, the pH of the phosphate buffer solution (PBS) was adjusted to 2.5. Later, 1 ml of log phase cultures of the isolated bacterial strains were added to the PBS of 2.5 pH and incubated at 37 ^{0}C in shaker incubator. The colony forming units of the bacterial strains were counted by plating 0.1 ml of the culture broth at regular intervals of 0, 1, 2 and 3 hours on relevant media by incubating at 37 ^{0}C for 24 hours.

Bile tolerance

Klaenhammer and Kleeeman procedure was followed to evaluate the Bile tolerance test [7]. Bile salt solutions was prepared with the concentration of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 % and the log phase cultures of the each bacteria strains were added to different concentration bile salt solutions. Later, the colony forming units of the bacterial strains was checked by plating the samples on the relevant media by incubating at 37 ^{0}C for 24 hours.

Anti-vibrio activity or Antagonistic activity

The bacteriocin produced by the probiotic bacteria is responsible for the antagonistic activity. The antagonistic activity was performed against *V.alginolyticus and Vibrio parahemolyticus.* To find out the antagonistic activity of the bacterial strains, Tagg and Mcgiven method of assay for bacteriocin was followed [8]. The overnight vibrio test cultures and log phase cultures of bacterial strains were taken for the antagonistic activity test. Log phase bacterial cultures were inoculated in the well on T1N1 agar and incubated for 24 hours at 37^{0}C. Later 0.1 ml of the vibrio cultures was plated on the T1N1 agar medium and checked for the existence of the inhibition zones.

Biochemical characterization of the isolated probiotic bacteria

The procedure for biochemical characterization was followed as described by Potter (2008) [9]. The biochemical tests include Gram's staining, catalase test, amylase test, gelatinase test, carbohydrate utilization test, hydrogen sulphide production test, urea hydrolysis and IMViC reaction.

Molecular level characterization and phylogentic analysis of isolated bacterial

The isolated probiotic bacteria were characterized at the molecular level by 16S rRNA sequencing.

Field experiments

The isolated probiotic bacteria were used in the field studies of *Litopenaeus vannamei* culture pond. The investigational studies were carried out for duration of 90 days. The isolated probiotic strains were formulated into a powder form with soy protein and maltodextrin as appropriate carrier and preservative with a range of 20 billion cfu/gm. This probiotic product was used for in the field studies of pond at the range of one kilogram per acre. The ponds of 1.5 acre in size and depth of 7 feet in which 4 feet water was maintained were used as control and experimental ponds throughout the experimental study. The ponds were located located at Bapatla, Andhra Pradesh, India with GPS coordinates of 15^0 53'47.8392" N and 80^0 27'37.5624' E. Total Six ponds were maintained, Control pond without adding any probiotic bacteria, Experimental pond-1, Experimental pond-2, Experimental pond-3, and Experimental pond-4 applied with individual isolated probiotic strains, Experimental pond-5 (Figure 3.10) applied with the combined product of probiotic strains. Standard water quality parameters were also investigated for a period of 90 days to make certain the effect of the usage of probiotic application. To investigate the effect of isolated probiotic bacteria on water quality parameters, water samples were collected from the experimental and control ponds at the regular intervals of 15 days (1, 15, 30, 45, 60, 75 and 90 days). The population of total heterotrophic bacteria (THB) and total virbrio count (TVC) in water was done at regular intervals for microbiological analysis. The survival and growth rate of the *Litopenaeus vannamei* was also investigated in the probiotic treated ponds.

Water quality variables

The standard water quality parameters include, salinity, transparency, pH, temperature, dissolved oxygen, alkalinity, nitrites, nitrates, phosphates and total ammonia nitrogen. Water quality parameters like pH, temperature and salinity were recorded on field using pH meter, thermometer and salinometer (Refractometer). The standard water quality parameters were measured according to the M. A. Stand and H. Fauson standard methods [10].

Estimation of total heterotrophic bacteria (THB)

Harris et al (1986) method was followed to estimate the total heterotrophic bacteria (THB) [11].

Estimation of total Vibrio count (TVC)

Dalmin et al., (2001) was followed to estimate the total vibrio count (TVC) [12].

Influence of isolated probiotic bacteria on the growth and survival rate of the shrimp Estimation of the growth of the shrimp

The influence of probiotics on the growth of *Litopenaeus vannamei* was investigated by estimating the average body weight of the *Litopenaeus vannamei* at regular intervals. Mustafa and Ridzwan 2000 method was followed to calculate the Average body weight of the shrimps [13].

Estimation of the Survival rate of the shrimp

Sambasivam et al 2003 was followed to estimate the survival rate of the *Litopenaeus vannamei* [14].

Statistical analysis

The scientific study data mentioned in the results were the means of triplicates.

III. RESULTS AND DISCUSSION

The main motto of our present scientific investigation is to find out effective probiotic bacteria for shrimp aquaculture. And the experimental design was planned according to the current investigation necessity. The probiotic bacteria isolated from the aquatic fishes adjust much better to the pond conditions when compared to the probiotic bacteria isolated from the other sources [15].

Isolation of probiotic bacteria from Gastro intestinal tracts of *Wallago attu* (WA), *Oreochromis mossambicus* (OM)

14 colonies were selected from the each fresh water fish *Wallago attu* (WA1) and *Wallago attu* (WA2) and 10 colonies were selected from each of the other fresh water fishes *Oreochromis mossambicus* (OM1) and *Oreochromis mossambicus* (OM2). All the bacterial isolates may not hold effective probiotic properties. Therefore selection of the potent probiotic bacteria was done by preliminary screening. The bacteria isolates which are proficient of overcoming all these screening tests were chosen for further research and molecular investigation. The fourteen isolates from each fish Wallago *attu* (WA1) and *Wallago attu* (WA2) were given names as WA1-1 to WA1-14 and WA2-1 to WA2-14. The ten isolates from the each fish *Oreochromis mossambicus* (OM1) and *Oreochromis mossambicus* (OM2) were given names as OM1-1 to OM1-10 and OM2-1 to OM2-10.

Probiotic characterization of isolated bacteria strains

Acid tolerance test

The first line of screening the probiotic potential of bacteria is acid tolerance test. During digestion, the pH condition of the stomach is 2.5 as it supress the growth of pathogenic microorganisms in the host system. Generally, the probiotic bacteria which settle in the gut and intestine should survive in the acidic pH. For this reason, the acid tolerance is regarded as one of the main feature of probiotic strain [16]. From the fourteen bacterial isolates obtained from Wallago attu (WA1), the isolate WA1-1 showed 8 cfu (Colony forming units) at 0 hour, 7 cfu at 1 hour, 7 cfu at 2 hour and 5 cfu at 3 hour. Among all the isolates of Wallago attu (WA1), the isolate WA1-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates Wallago attu (WA1). From the ten bacterial isolates obtained from Wallago attu (WA2), the isolate WA2-1 showed 10 cfu (Colony forming units) at 0 hour, 8 cfu at 1 hour, 5 cfu at 2 hour and 5 cfu at 3 hour. Among all the isolates of Wallago attu (WA2), the isolate WA2-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Wallago attu (WA2). Among ten bacterial isolates obtained from Oreochromis mossambicus (OM1), the isolate OM1-1 showed 8 cfu (Colony forming units) at 0 hour, 7 cfu at 1 hour, 7 cfu at 2 hour and 5 cfu at 3 hour. Among all the isolates of Oreochromis mossambicus (OM1), the isolate OM1-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Oreochromis mossambicus (OM1). From ten bacterial isolates obtained from Oreochromis mossambicus (OM2), the isolate OM2-1 showed 12 cfu (Colony forming units) at 0 hour, 6 cfu at 1 hour, 3cfu at 2 hour and 0 cfu at 3 hour. Among all the isolates of Oreochromis mossambicus (OM2), the isolate OM2-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Oreochromis mossambicus (OM2). The four isolates WA1-1, WA2-1, OM1-1 and OM2-1 were showing the cfu ranging between 10 to 5 cfu/0.1ml, this indicate among all the screened bacteria these four isolates were considered to be acid tolerant.

Bile tolerance test

Pathogens are suppressed by the production of bile salts by the host as defense mechanism. One of the essential feature of the probiotic bacteria is to resist the bile salt inhibition. The feed supplements of the shrimp aquaculture generally consist of bile salts as source of sterols which are necessary for synthesis of moulting hormone. But bile salts are usually bacterial growth suppressors. Therefore the bacterial strain that is to be used as probiotic product should resist the inhibition of bile salts. The four isolates that were acid tolerant were able to exhibit the bile salt tolerance, hence in the present study they were selected as the bile salt tolerant isolates [17, 18].

From the fourteen bacterial isolates obtained from Wallago attu (WA1), the isolate WA1-1 showed 10 cfu (Colony forming units) at 0.2 %, 8 cfu at 0.4%, 7 cfu at 0.6%, 5 cfu at 0.8% and 5 cfu at 1.0 % . Among all the isolates of Wallago attu (WA1), the isolate WA1-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates Wallago attu (WA1). From the ten bacterial isolates obtained from Wallago attu (WA2), the isolate WA2-1 showed 10 cfu (Colony forming units) at 0.2 %, 9 cfu at 0.4%, 8 cfu at 0.6%, 7 cfu at 0.8% and 7 cfu at 1.0 %. Among all the isolates of Wallago attu (WA2), the isolate WA2-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Wallago attu (WA2).

Among ten bacterial isolates obtained from Oreochromis mossambicus (OM1), the isolate OM1-1 showed 10 cfu (Colony forming units) at 0.2 %, 5 cfu at 0.4%, 5 cfu at 0.6%, 3 cfu at 0.8% and 3 cfu at 1.0 %. Among all the isolates of Oreochromis mossambicus (OM1), the isolate OM1-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Oreochromis mossambicus (OM1). From ten bacterial isolates obtained from Oreochromis mossambicus (OM2), the isolate OM2-1 showed 8 cfu (Colony forming units) at 0.2 %, 6 cfu at 0.4%, 4 cfu at 0.6%, 2 cfu at 0.8% and 2 cfu at 1.0 %. Among all the isolates of Oreochromis mossambicus (OM2), the isolate OM2-1 was selected for the present study as it showed more acid tolerance when compared with the other isolates of Oreochromis mossambicus (OM2). The four isolates WA1-1, WA2-1, OM1-1 and OM2-1 were showing the cfu ranging between 10 to 2 cfu/0.1ml, this indicate among all the screened bacteria these four isolates were considered to be acid tolerant.

Antagonistic activity

V. alginolyticus and *V. parahemolyticus* are one of the severe pathogens that make huge loss to fish and shrimp aquaculture throughout the globe. Bacteriocins reduce the pathogenic microorgansims by permeabilizing the cell membrane causing outflow of cellular substances and finally cell death [19,20]. So for the present experiment these two vibrios were selected to assess the antagonistic activity of the four isolates that withstand the acid and bile tolerance test. In the present study, the four isolates that were acid and bile tolerant were able to inhibit the growth of the *V. alginolyticus* and *V. parahemolyticus* pathogens. The figure 1.A , figure 1.B, figure 1.C and figure 1.D shows the results of antagonistic activity of isolated probiotic strains on *V. alginolyticus* and *V. parahemolyticus.* The bacterial strains (WA1-1, WA2-1, OM1-1 and OM2-1) with effective probiotic capability (acid tolerant, bile tolerant and antagonistic activity from the above mentioned isolates were designated as VVRANU -1, VVRANU -2, VVRANU - 3 and VVRANU -4 (VVRAU = v.venkata ratnamma acharya nagarjuna university). List of the finalized probiotic isolates and designation of the finalized probiotic isolates were mentioned in the table 1 and table 2.

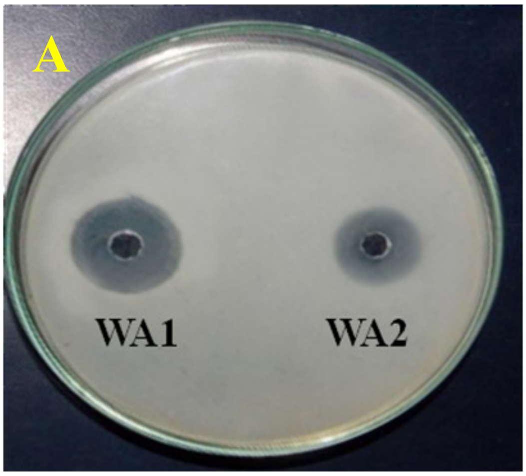

Figure 1.A

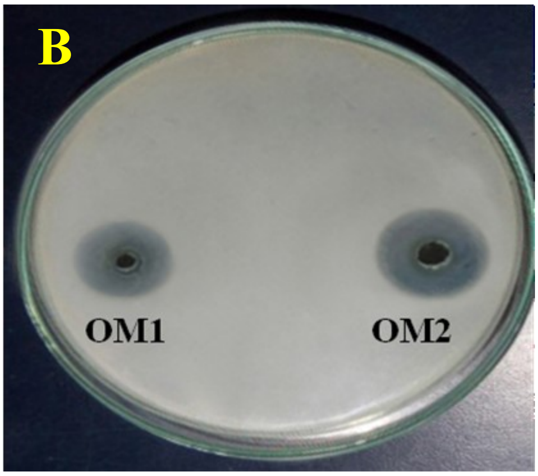

Figure 1.B

Figure 1. A and Figure 1. B illustrates Antagonistic activity towards *Vibrio alginolyticus*

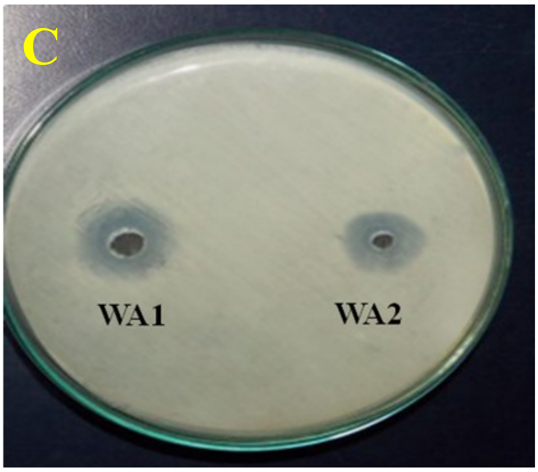

Figure 1.C

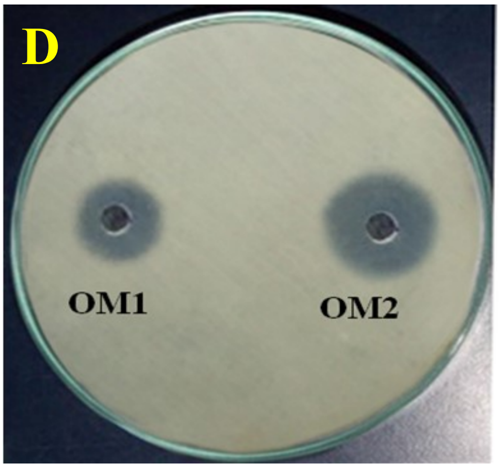

Figure 1.D

Figure 1. C and Figure 1. D shows Antagonistic activity towards *Vibrio parahemolyticus.*

Biochemical characterization of the isolated probiotic bacteria

The results of the biochemical characterization of the isolated probiotic bacteria were listed in the table 3.

Molecular identification and Phylogenetic analysis of the isolated probiotic bacteria

The complete nucleotide sequence of the four isolated probiotic bacteria was obtained. The sequences of VVRANU-1, VVRANU-2, VVRANU-3, and VVRANU-4 were deposited at the NCBI GenBank under the accession numbers KY496934, MF112025, MF112026 and MF112028 respectively.

Table 1. List of finalized probiotic isolates

Source of Isolation	No. of isolates screened	No. of isolates selected
Wallago attu fish-1	14	1 (WA1-1)
Wallago attu fish-2	10	1 (WA2-1)
Oreochromis mossambicus fish-1	10	1 (OW1-1)
Oreochromis mossambicus fish-2	10	1 (OW2-1)

Table 2. Designation of finalized probiotic isolates

Finalized isolates	Designation
WA1-1	VVRANU-1
WA1-2	VVRANU-2
OM1-1	VVRANU-3
OM2-1	VVRANU-4

Table 3. Results of biochemical tests of finalized probiotic isolates

Biochemical tests	Results of the finalized isolates			
	VVRANU-1	VVRANU - 2	VVRANU - 3	VVRANU - 4
Gram staining	+ve	+ve	+ve	+ve
Catalase test	+ve	+ve	-ve	-ve
Amylase test	+ve	+ve	-ve	-ve
Gelatinase test	+ve	+ve	-ve	-ve
Sugar utilization test				
Glucose	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve
D-Maltose	+ve	+ve	+ve	+ve
Galactose	+ve	-ve	+ve	+ve
Fructose	+ve	+ve	+ve	+ve
Xyloses	+ve	+ve	-ve	-ve
Hydrogen sulphide production test	-ve	-ve	-ve	-ve
Urease test	-ve	-ve	-ve	-ve
IMViC tests				
Indole production test	-ve	-ve	-ve	-ve
Methyl red reduction test	-ve	-ve	-ve	-ve
Voges Proskeur test	+ve	+ve	-ve	-ve
Citrate utilization test	+ve	-ve	-ve	-ve

Table 4. Identified list of isolated probiotic bacteria

Isolated Probiotic bacteria	Identification at molecular level
VVRANU-1	*Bacillus subtilis*
VVRANU -2	*Bacillus amyloliquefacicus*
VVRANU -3	*Lactobacillus acidophilus*
VVRANU -4	*Lactobacillus rhamnosus*

The list of the identified probiotic bacteria is represented in table 4.

Field Experiment

Water Quality Variables

Temperature

The temperature of experimental and control ponds were recorded at regular time period. The results of the temperature showed no considerable variation in the pond water temperature in both control and probiotic treated ponds. As the culture period goes on the temperature was found to be increasing but the increase was not very significant. From the results it was concluded that the probiotics do not have any influence on the pond water temperature. In aquaculture, the temperature and salinity are very important parameters which are considered, so generally probiotic bacteria which doesnot influence the salinity and temperature are to be selected. Figure 2 illustrates the results of effect of probiotics on the temperature on control and experimental ponds.

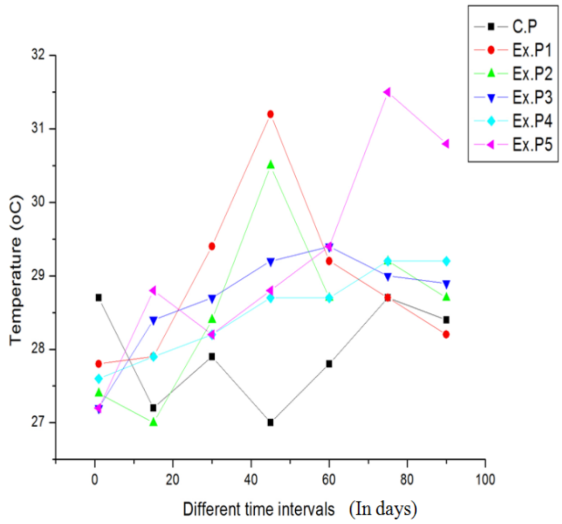

Figure 2

pH

The pH reading for all the experimental and control ponds were taken at the experimental timings. pH was recorded to be higher in the probiotic treated ponds than the control ponds, but the raise in pH among the probiotic treated ponds was not exhibiting any significant dissimilarity. The link between the pH readings and the circumstances existing in the pond at particular pH should be clearly studied in order to know the effect of probiotics on ponds. From the graph, it is clear that pH is comparatively stable in probiotic treated ponds than the control ponds. From 30th day there is drastic fall in the pH (low than 7 pH) of the control pond.

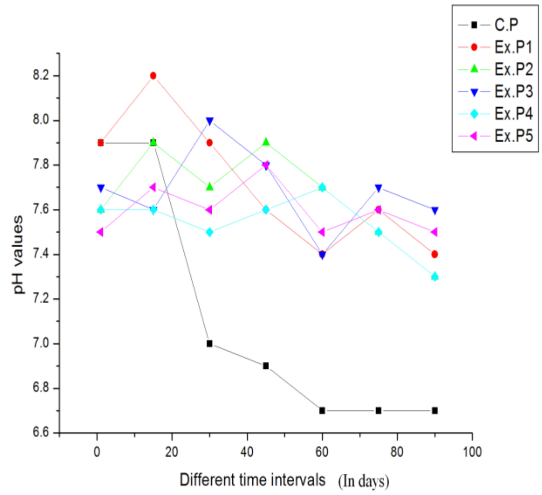

Figure 3

The ponds treated with the probiotic bacteria of VVRANU-1, VVRANU-2, VVRANU-3 and VVRANU-4 showed very little fluctuations in the pH. Figure 3 exemplifies the results of effect of probiotics on the pH on control and experimental ponds.

Salinity

Salinity is a different and significant water quality constraint which manipulates the growth of the shrimp in the pond. Salinity of the water is due to the ions that are present in the water and the concentration of the ions change with the concentrations of the ammonia and several other toxic substances like H_2S, thus the shrimp farmers recurrently change the water of the shrimps ponds to reduce the poisonous effects and uphold an optimal level of the salinity. In the present study, salinity was increasing in both control and experimental ponds with significant increase in the control ponds, but it did not varied noticeably among the probiotic treated ponds. The elevated salinity levels in the control ponds can be endorsed to the above mentioned factors where in the levels of deadly toxic substances might have increased which exhausted the pond water quality and resulted in higher salinity levels while the probiotic bacteria might have reduced these undesirable effects and resulted in low salinity. Figure 4 demonstrates the results of effect of probiotics on the salinity on control and experimental ponds.

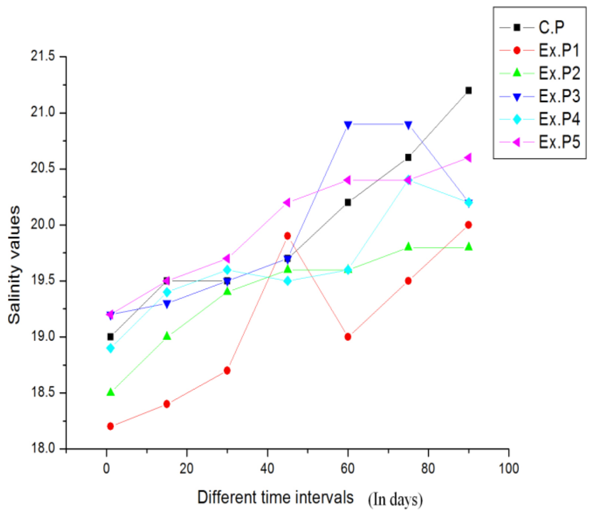

Figure 4

Transparency

Transparency is the deepness to which the sunlight travel into the pond and plays a crucial task in the growth of the aquatic organisms. The transparency of the pond water is effected by the suspended clay particles, dissolved organic particles and microscopic algae. As the time goes on during the culture period the zooplankton, microalgae, and other dissolved particles might have diminished because of exhausted nutrient levels which resulted in better transparency whereas in the probiotic treated ponds the probiotic bacteria might have supplied the required nutrients to promote the growth of the zooplankton and microalgae which showed reduced transparency [21, 22]. The transparency was found to decline as the culture period progress but it appreciably decreased in probiotic treated ponds when compared to the control pond. Among the probiotic treated pond the sixth pond which was applied with a combined product of all isolated probiotic bacteria was showing significant decrease in the transparency than the individual probiotic treated ponds. Figure 5 exemplifies the results of effect of probiotics on the transparency on control and experimental ponds.

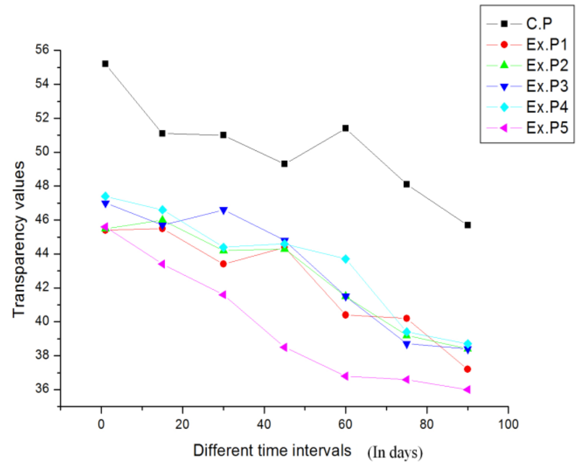

Figure 5

Dissolved Oxygen

The results showed appreciably elevated levels of dissolved oxygen in probiotic treated ponds when compared to the control ponds. The dissolved oxygen levels in the control ponds were good but much lower than experimental ponds. Oxygen in the water of ponds is presented in dissolved state for the aquatic organisms, oxygen essential to perform physiological process like the respiration and oxidation of food material. It is also compulsory to keep hygiene environment in the ponds. If the dissolved oxygen levels were declined the shrimp experiences pressure leading to reduced feed consumption, retarded growth, and body discoloration due to hypoxic conditions and finally it leads to the death of the animal, hence maintaining dissolved oxygen levels in shrimp ponds is considered as a very important management practice.

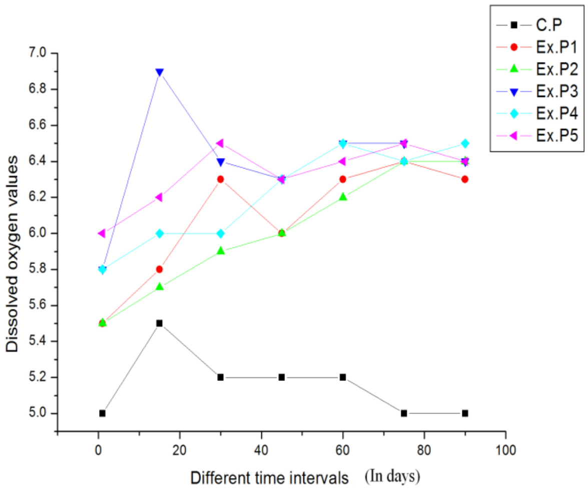

Figure 6

The results obtained suggest that the probiotics in the pond had promoted the growth of phytoplankton and photosynthetic bacteria which increase the photosynthesis in the pond adding oxygen to the pond water which showed higher dissolved oxygen levels than the control pond. Figure 6 shows the results of effect of probiotics on the dissolved oxygen concentration on control and experimental ponds.

Alkalinity

The alkalinity of water is a measure of amount of acid it can counterbalance. If any alterations are made to the water that could elevate or decrease the pH value, alkalinity do something as buffer, defending the water and its life forms from unexpected change in pH. The deviation in the alkalinity has been noted in both experimental and control ponds.

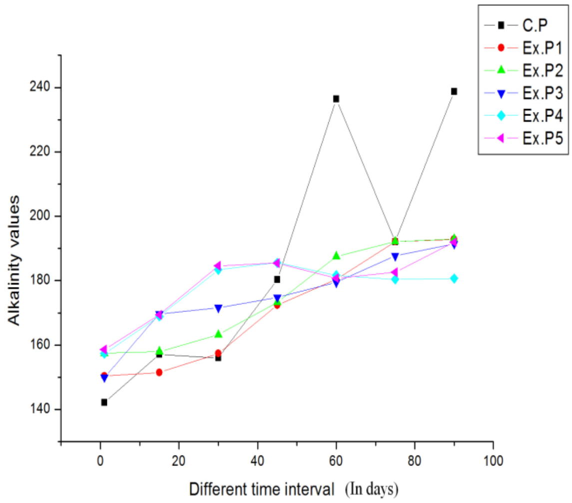

Figure 7

From the results we can see that in the initial time intervals the alkalinity levels were high in the probiotic treated ponds which decreased considerably with the culture time and recognized to be lower than the control at the conclusion of the experiment. Figure 7 shows the results of effect of probiotics on the alkalinity on control and experimental ponds.

Total Ammonia Nitrogen (TAN)

Ammonia released into shrimp ponds as a result of shrimp metabolisms and as a byproduct of decomposition of organic substances. Total ammonia nitrogen comprises of the two types of ammonia as ammonium ion and as unionized ammonia. The concentration of ammonia must be in low concentrations in the ponds as ammonia is extremely toxic to shrimps.

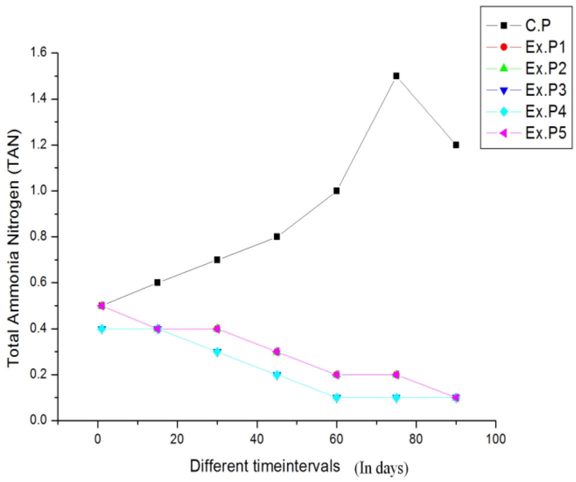

Figure 8

The Total Ammonia Nitrogen was found to be significantly decreased in the probiotic treated ponds while it was higher in the control pond [23]. The results attained showed that the probiotics by preserving the water quality constraints would have supported the growth of the nitrifying and nitrobacteria bacteria which convert Total ammonia nitrogen into nitrate and nitrite. But in the case of control ponds as the culture duration progresses due to the exhausted quality of the pond water parameters the useful nitrogen bacterial count would have been declined which resulted in the addition of ammonia. Figure 8 illustrates the results of effect of probiotics on the Total Ammonia Nitrogen on control and experimental ponds.

Nitrite nitrogen and Nitrate nitrogen

Nitrite concentration was checked in both experimental and control ponds, the nitrite levels were recognized to be high in the control pond when compared to the probiotic treated pond. Generally the ammonia was transformed to nitrite and then to nitrate, the results demonstrated that the Ammonia to nitrite conversion was performed at a slow rate which exhibited higher levels of nitrites in the control pond but the results were reversed in the nitrate concentration levels.

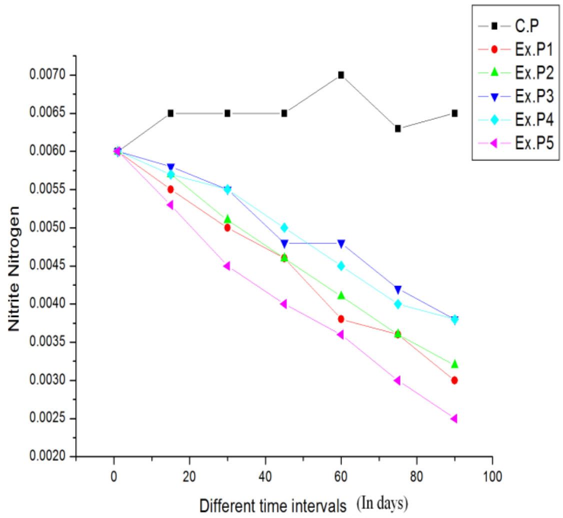

Figure 9

The nitrite form of the ammonia was transformed to the non-toxic nitrate form at elevated rate in the experimental ponds. This specifies that the probiotic bacteria suppoorted the growth of nitrogen bacteria like *Nitrobacter* and *Nitrosomonas* and which played a major task in maintaining nitrite-nitrate concentrations in the shrimp pond. The figure 9 and 10 illustrate the results of effect of probiotics on the Nitrite nitrogen and Nitrate nitrogen on control and experimental ponds.

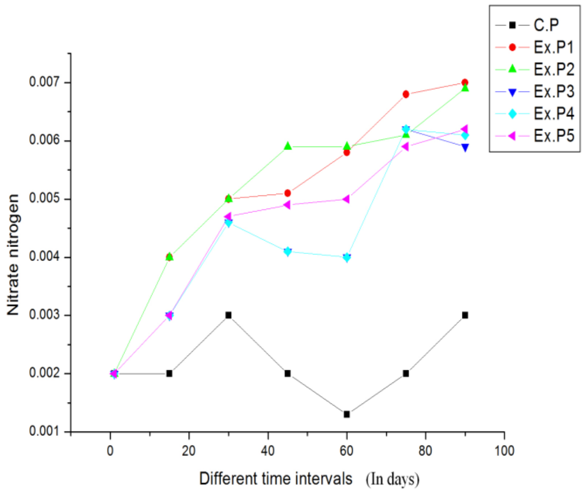

Figure 10

Phosphates

In addition to nitrogen, phosphorous is as one of the vital nutrients in the shrimp ponds. Phosphorous is well known to support the development of phytoplankton, the phytoplankton take in the inorganic phosphorous from the accessible sources in the pond. Bacteria present in the shrimp pond change the organic phosphorous into inorganic form and make them available for the phytoplankton [24]. Concentrations of Phosphates was recorded in both probiotic and control ponds, the levels of phosphates was found to be more in probiotic treated ponds when compared to the control, although the phosphate levels were found to increase in both experimental and control ponds it was extensively observed to be elevated in experimental ponds.

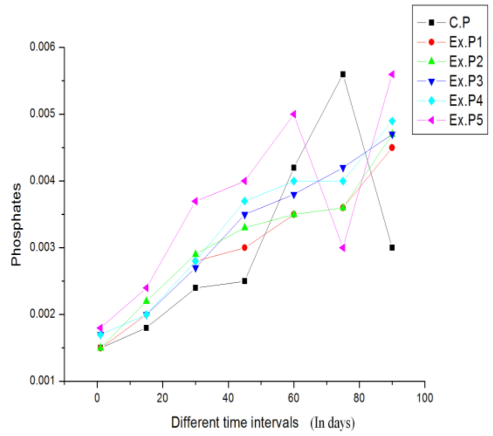

Figure 11

The results obtained propose that the probiotic bacteria enhanced the release of the inorganic phosphorous from the organic form which showed higher concentrations of phosphates in the experimental ponds when compared to the control ponds. Figure 11 illustrates the results of effect of probiotics on phosphates of control and experimental ponds.

Estimation of total heterotrophic bacteria

Total heterotrophic bacteria include both pathogenic and beneficial bacteria, their population depends upon the water quality parameters. The Total heterotrophic bacteria count was recorded in both probiotic treated and control ponds, the Total heterotrophic bacteria count was observed to be increased in both control and experimental ponds but appreciably high in probiotic treated ponds.

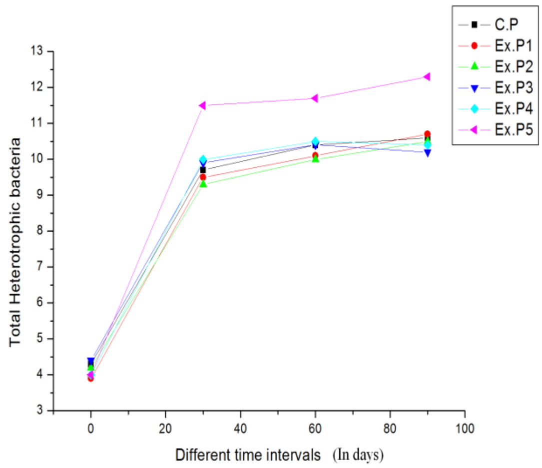

Figure 12

The higher count in the probiotic treated ponds can be explained as the probiotic bacteria dominated the pathogenic bacterial groups by competitive exclusion. Figure 12 demonstrates the results of effect of probiotics on total heterotrophic bacteria of control and experimental ponds.

Estimation of total vibrio count

Vibrios are the most widely distributed and well known as most economic loss creating bacterial agents in shrimp aquaculture. The higher vibrio count indicates the reduced water quality in ponds. The probiotic bacteria in the experimental ponds might have inhibited the vibrios by competitive inhibition mechanism and also their capability to produce bacteriocin [25]. Commericial probiotics like Pro-w, Super biotic, zymatin, Environ-AC, and Super PS had showed the appreciably low count of vibrios and promoted good water quality variables. Total vibrio count was found to be higher in control ponds and significantly lower in probiotic treated ponds. Figure 13 and 14 demonstrate the results of effect of probiotics on Total vibrio count of *Vibrio alginolyticus* and *Vibrio parahaemolyticus* in control and experimental ponds.

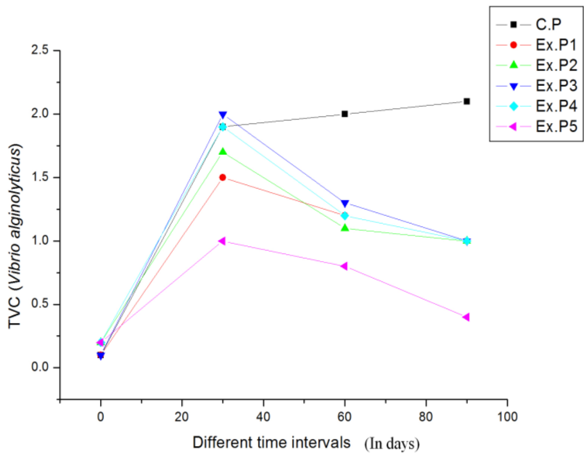

Figure 13

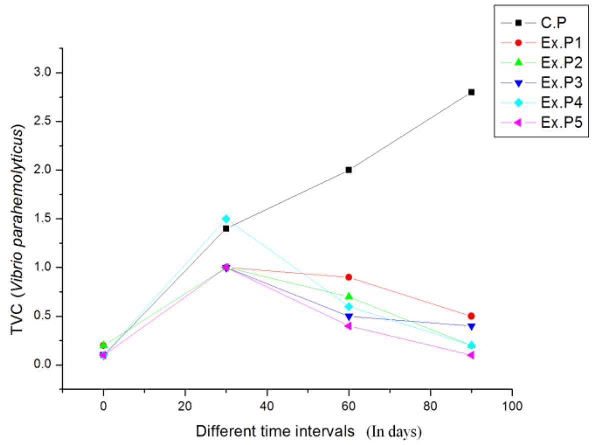

Figure 14

Effect of isolated probiotic bacteria on the growth and survival rate of the shrimp

Promising reports were available for the beneficial effects of probiotics on the growth and survival rate of the shrimp. Though the means by which the probiotics improve the growth and survival were not understandable, it is expected that the probiotics made the shrimp to consume more feed with reduced wastage and increase feed conversion ratio and enhance the function of digestive enzymes in the shrimp thereby play a significant job in feed digestion and consumption.

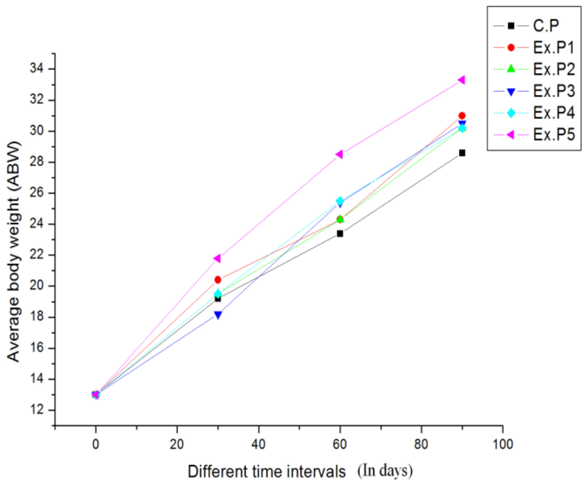

Figure 15

The results of average body weight showed increased body weight of the shrimps in all ponds but considerably higher in probiotic treated ponds, among the probiotic treated ponds the experimental pond which was applied with mixture of all probiotic bacteria showed higher average body weight readings when compared to rest of experimental ponds.

The survival rate was recorded to be higher in experimental ponds whereas the control pond showed decreased survival rates which decreased as the culture period prolongs. Reduced survival rate was also recorded in experimental ponds but not significantly [26, 27, 28].

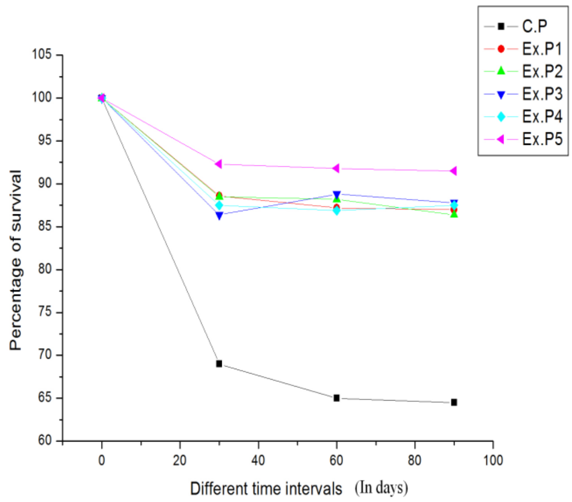

Figure 16

Figure 15 and 16 demonstrates the results of effect of probiotics on Average body weight (ABW) and Percentage of Survival in Control in Control (untreated) and Experimental ponds.

IV. CONCLUSION

Usage of antibiotics in shrimp aquaculture has led to emergence of drug resistant bacteria. Drug resistance in shrimp pathogen may be transferred to environmental and human pathogenic bacteria. Moreover, the residues of drugs in fish can be potentially risky to consumers and the environment. Therefore the demand for safer alternative approaches to control the infections has been increasing. One of the most promising methods is to strengthen the defense mechanisms of fish using probiotic bacteria. In the present research laboratory studies proved that the isolated bacteria VVRANU-1, VVRANU-2, VVRANU-3 and VVRANU-4 were having considerable probiotic ability and the molecular and phylogenetic analysis proved that the identified bacteria do not belong to any prawn pathogenic group. From the field studies it was proved that the isolated probiotic bacteria were capable of surviving in the pond ecosystem and having positive influence on the *Litopenaeus vannamei.* With all the obtained results we can bring to a close that in the present study we have successfully isolated probiotic isolates of host origin, non-pathogenic, suitable for industrial processes, acid- and bile-fast, adhere to the gut epithelial tissue, produce antimicrobial substances, modulate immune responses and influence the metabolic activities of the gut. Although several probiotics are already on the market, new strains with beneficial properties are continually being sought.

V. REFERENCES

[1]. M.D., Ibrahem, (2015). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. J Adv Res., 6: 765-791.

[2]. S.A., Mansour, M.D. Ibrahem, H.I. Abass and S.E. Ali, (2012). Effects of dietary supplementation of Diamond V on growth performance, liver and kidney functions and digestive enzymes of catfish Clarias gariepinus. Global J Fish Aquacult Res., 5: ISSN 18191.

[3]. B. Lakshmi, , B. Viswanath and D.V.R. Sai Gopal, (2013). Probiotics as Antiviral Agents in Shrimp Aquaculture. Journal of Pathogens., 13: ID 424123.

[4]. N. Uddin, , and H. Ahmed, (2012). Bacterial flora of polycultured common carp (Cyprinus carpio) and African catfish (Clarias gariepinus). International Aquatic Research., 4: 10-15.

[5]. P. Priyadarshini, D. Balaraman, R. Thirunavukkarasu, E. G. J George, K. Subaramaniyan and S. M. B. Sadayappan, (2013). Probiotics in aquaculture. Drug Invention today., 5: 55-59.

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

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

[8]. J. R. Tagg, and A. R. Mc Given, (1971). Assay system for Bacteriocins. Applied Microbiology., 21: 943-946.

[9]. P., Beth. (2008). Biochemical Tests. Microbiology 202. Penn State Erie, The Behrend College. 30 Oct. 2008 - 11 Nov.

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

[11]. R.P., Harris,. J.F. Samain, J. Moal, V. Martin-Jezequel and S.A. Poulet, (1986). Effects of algal diet on digestive activity in Calanus helgolandicus. Marine Biology., 90: 353-361.

[12]. G., Dalmin, K. Kathiresan and A. Purushothaman, (2001). Effect of probiotics on bacterial population and health status of shrimp in culture pond ecosystem. Indian Journal of Experimental Biology., 39: 939-942.

[13]. S. Mustafa, and A. R. Ridzwan, (2000). Sustainable marine aquaculture recent developments with special reference to South East Asia. Kotakinabula, Malaysia.

[14]. S., Sambasivam, R. Chandran and S. Ajmal Khan (2003). Role of probiotics on the environment of shrimp pond. Journal of Environmental Biology., 24: 103-106.

[15]. T. Hagi, and T. Hoshino, (2009). Screening and Characterization of Potential probiotic Lactic acid Bacteria form cultured common carp intestine., Bioscience, Biotechnology and Biochemistry., 73: 1479- 1483.

[16]. M., Begley, C.G.M. Gahan, and C. Hill, (2005). The interaction between bacteria and bile. FEMS Microbiology Reviews., 29: 625-651.

[17]. S.E., Gilliland, T.E. Staley and L.J. Bush, (1984). Importance in bile tolerance of Lactobacillus acidophilus used as a diatery adjunct. Journal of Dairy Science., 67: 3045-3051.

[18]. H.M. Begley, Colin, and C. G. M. Gahan, (2006). Bile Salt Hydrolase Activity in Probiotics. Applied and Environmental Microbiology., 72 : 1729-1738.

[19]. G.N., Moll, W.N. Konings and A.J. Driessen, (1999). Bacteriocins: mechanism of membrane insertion and pore formation. Antonie Leeuwenhoek., 76: 185-198.

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

[21]. M.I., Hossain, M.M. Kamal, M.A. Mannan, M.A.B. Bhuyain and M.I. Hossain, (2013). Effects of Probiotics on Growth and Survival of Shrimp (Penaeus monodon) in Coastal Pond at Khulna, Bangladesh. Journal of Scientific Research., 5: 363-370.

[22]. T., Rajinikanth, P. Ramasamy and V. (2010). Ravi Efficacy of Probiotics, Growth Promotors and Disinfectants in Shrimp Grow out Farms. American-Eurasian Journal of Agriculture & Environment Science., 7: 347-354.

[23]. L.M., Gilles, (2001). Environmental factors affect immune response and resistance in Crustaceans, The Advocate., 18.

[24]. Y.B., Wang, Z.R. Xu and M. S. Xia, (2005). The effectiveness of commercial probiotics in northern white shrimp Penaeus vannamein ponds. Fisheries Science., 71: 1036-1041.

[25]. R., Fuller, (1992). Probiotic the scientific basis. 1st edition Chapman & Hall London, UK.

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

[27]. R., Palaniappan, (1982). 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.

[28]. P.S., Begum, G. Madhavi, S. Rajagopal, B. Viswanath, M.A. Razak and V. Venkataratnamma, (2017). Probiotics as Functional Foods: Potential Effects on Human Health and its Impact on Neurological Diseases. International journal of nutrition, pharmacology, neurological diseases., 7: 22-33.