Research in
ISSN : P-2409-0603, E-2409-9325
AGRICULTURE, LIVESTOCK and FISHERIES
An Open Access Peer Reviewed Journal

Open Access
Research Article

Res. Agric. Livest. Fish.
Vol. 5, No. 1, April 2018 : 127-135.

USE OF INDIGENOUS BENEFICIAL BACTERIA (*Lactobacillus* spp.) AS PROBIOTICS IN SHRIMP (*Penaeus monodon*) AQUACULTURE

Fatema Ahmmed[1,*], Mirja Kaizer Ahmmed[1,2], Md. Saifuddin Shah[1] and Ghausiatur Reza Banu[1]

[1]Fisheries and Marine Resources Technology, School of life science, Khulna University, Bangladesh; [2]Feculty of Fisheries, Chittagong Veterinary and Animal Sciences University, Bangladesh.

***Corresponding author:** Fatema Ahmmed; E-mail: fatemafmrt@gmail.com

ARTICLE INFO

Received
05 April, 2018

Accepted
26 April, 2018

Online
30 April, 2018

Key words
Probiotic
Shrimp
Lactobacillus
Antagonistic effect
Aquaculture

ABSTRACT

The present study was conducted to study *in-vitro* the antagonistic effect of *Lactobacillus* spp. against the pathogenic bacterial *vibrio harveyi* on shrimp. For this purpose, shrimp samples were collected from three different Ghers at Batiaghata upazilla, Khulna. Gills and intestines were taken out from the samples to identify the load of *Lactobacillus* spp. and *Vibrio* spp. The results revealed that the load of *Lactobacillus* spp. was found more than *Vibrio* spp. both in gills and intestines; the gills also contained higher load of *Vibrio* spp. than in the intestines. *V. harveyi* was separated from the isolated *Vibrio* spp. with different types of biochemical tests: Gram stain, Motility test, Indole test, VP test, MR test, Arginine dihydrolase, Salt tolerance test, growth at different temperature ranges and colony color on TCBS agar media. The isolated *V. harveyi* was subjected for *in-vitro* test. In *in-vitro* challenge test, the potential antagonistic effect of *Lactobacillus* spp. against *V. harveyi* was gradually obtained at 0, 4th, 8th, 12th hour of treatments. Interesting finding was that, with the time, the load of *V. harveyi was* reduced gradually and the lowest load was obtained after 12 hours of probiotic inoculation. The present study revealed an excellent *in-vitro* antagonistic probiotic effect of *Lactobacillus* spp. on *V. harveyi.* Therefore the result suggested that probiotic treatment might be an effective alternative to the use of antibiotics in treatments of bacterial diseases in shrimp aquaculture.

To cite this article: Ahmmed F, MK Ahmmed, MS Shah and GR Banu, 2018. Use of indigenous beneficial bacteria (*Lactobacillus* spp.) as probiotics in shrimp (*Penaeus monodon*) aquaculture. Res. Agric. Livest. Fish. 5 (1): 127-135.

INTRODUCTION

Shrimp aquaculture has been recognized as a profitable business in Bangladesh. In spite of having great potentiality, this sector is affected with a wide range of microbial disease. It is one of the limiting factors to shrimp production. A dozen of *Vibrio* species are responsible for bacterial disease. Specially *V. harveyi, V. logei,, V. alginolyticus, V. pelagicus, V. splendidus, V. alginolyticus, V. vulnificus, V. parahaemolyticus and V. damsella,* are commonly found as causative agent of vibriosis (Lightner, 1983). Gram-negative bacterium *V. harveyi* as the causative agent of luminous bacterial disease and it is considered a serious pathogen of larval shrimp in hatcheries (Lavilla-Pitogo et al., 1990; Karunasagar et al., 1994). Both juvenile and adult shrimp can be attacked by this bacterium causing mass mortalities. From over the years, a verity of antibiotics has been used to control various pathogenic bacteria (Baticados et al*.,* 1990). Disinfectants and antimicrobial drugs (antibiotics) are not easy to buy and most of time it is applied in semi-intensive and intensive aquaculture run by the rich farmers. But the situation is different in our country where most of the people farmer live below the poverty line during the starting of the culture and practice traditional or improved traditional culture systems. It is really difficult for them to afford such extra expenses. Furthermore, antibiotics is not encouraged to use nowadays as there is a growing concern about the abuse of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture (Alberman, 1988). Frequent use of chemotherapeutic agents, especially antibiotics, leads to the emergence of resistant strains bacteria (Akoki, 1975) pathogenic to the animals. If antibiotics or disinfectants are used to kill bacteria, some bacteria will survive, because they carry genes for resistance. These will then grow rapidly because their competitors are removed (Moriarty, 1999). Vaccinations to prevent infections have been successful in laboratory scale but yet to be proved under field conditions.

However, it is an urgent issue to find an alternative of antibiotics that would be easy to get and will no harm the nature in anyway. The promising alternative approach for using antibiotics for controlling fish diseases is the use of probiotics or beneficial bacteria. Probiotics bacteria could prevent the establishment of pathogenic bacteria by out-competing them for adhesion and colonization sites in the intestines and other tissues of the animal (Vine et al., 2004a). They could also produce inhibitory substances actively preventing pathogen establishment (Verschuere et al., 2000). When added to rearing water, they may act as bioremediation agents improving water and sediment quality, augment nutrient cycling in the system and initiate colonization of other beneficial micro-flora affecting an overall positive impact on growth rates and productivity (Prabhu et al., 1999). Based on above background the study was undertaken *in-vitro* to find out the beneficial bacteria (*Lactobacillus* spp.) from shrimp for possible use as probiotics on *V. harveyi* infected shrimp.

MATERIALS AND METHODS

Sampling and sample size

The present study was conducted on three ghers of Batiaghata upazilla, Khulna. The ghers were randomly selected for collecting shrimp samples. Nine (9) specimens of shrimps, *Paeneus monodon* (8.10cm-12.4cm in length and 13.12g-22.6g in weight) were collected from three ghers of the study area.

Preparation of stock solution of the target organs

The target organs (gill and intestine) of the samples were separated aseptically and weighed in electric balance. Organs (gills weight: 0.22g-0.34g and weights of intestinal tracts: 0.13g-0.25g.) were taken into eppendorf tubes with peptone water (James and Hirsch, 1960) for isolating *Lactobacillus* spp. and alkaline saline peptone water was used for isolating *Vibrio* spp. Then they were homogenized using tissue homogenizer. Then homogenized solutions were centrifuged at 3000 rpm for 3 minutes (James and Hirsch, 1960). After centrifugation the supernatant liquid portion was collected with a micropipette and taken into eppendorf tube and preserved in deep freeze.

Experimental design

Isolation of probiotic bacteria (*Lactobacillus* spp.)

Lactobacillus spp. was isolated from gills and intestinal tracts of the collected shrimp samples. The stock solution was diluted (tenfold dilution) with peptone water (James and Hirsch, 1960). 0.1 ml suitable dilution of each stock solution was inoculated in MRS *Lactobacillus* agar media and incubated at 37^0C temperature for 24-48 hours. After incubation at 37^0C temperature for 24–48 h, cream-colored colonies with yellow halos were collected and preserved for further experiment.

Isolation of *Vibrio* spp.

Vibrio spp. was isolated from the gills and intestinal tracts of the collected shrimp samples from study area. ISO method was followed for isolating *Vibrio* spp. 0.1ml stock solution of each gill and intestinal tract was taken and mixed with 0.9ml alkaline saline peptone water (ASPW) in sterilized test tubes. Then the mixture was incubated at 37 °C for 6 ±1 hr. After that, whole culture of each test tube obtained in first selective enrichment was taken and transferred into other test tubes each containing 10 ml ASPW. Then the solution was incubated at 41.5 °C for 18 ± 1 hr. This was the second selective enrichment. Then serial dilution (tenfold) was done and 0.1ml suitable dilution of each culture was inoculated in thiosulfate citrate bile and sucrose (TCBS) agar plates. Then the inoculated TCBS agar plates were incubated at 37 °C. After 24 h ± 3 h of incubation, the plates were examined for the presence of typical colonies of presumptive *Vibrio* spp. (ISO/TS 21872-1, 2007).

The identification of *Vibrio harveyi*

The identification of the *Vibrio harveyi* colonies was done by performing various biochemical tests viz, motility test, methyl red test, VP test, arginine dihydrolase, indole ring test, Salt tolerance test (0%, 1%, 3%, 5%, 7%, and 10%) and growth at (4°C, 28°C, 37°C, 55°C).

Biochemical tests

The isolates were identified at the species level with the use of biochemical key.

Gram Staining

Gram stain was done to identify the gram positive and gram negative bacteria (Cowan and Steel's, 1993).

Indole test

The Indole test was performed in a 48h culture in peptone water adding about 1ml ether; shake; run 0.5 ml Ehrlich's reagent down the side of the tube. A red color in the solvent indicates positive reaction (Cowan and Steel's, 1993).

Voges-Proskauer (VP) test

The VP test was performed after completion of the methyl red test adding 0.6ml 5% α-naphthol solution and 0.2 ml 40% KOH aqueous solution; shake well, slope the tube, and examine after 15 min & 1 h. A positive reaction is indicated by a strong red color (Cowan and Steel's, 1993).

Arginine hydrolysis

Arginine hydrolysis inoculated 5ml Arginine broth & after incubation for 24h adding 0.25ml Nessler's reagent. Arginine hydrolysis is indicated by the development of a brown color (Cowan and Steel's, 1993).

MR test

The MR test inoculated the isolated bacteria with buffered glucose broth & incubated at 37°C for 48h. After incubation adding a few drops of methyl red solution to the culture, read immediately. A red color represents a positive test (Cowan and Steel's, 1993).

Motility test

The motility test stabbed inoculates tubes of motility medium to a depth of about 5mm. Incubate at or below the optimum growth temperature. Motile organisms migrate throughout the medium, which becomes turbid; growth of non-motile organisms is confined to the stab inoculum (Cowan and Steel's, 1993).

Salt Tolerance Test

This test was done on nutrient agar media supplemented with varying amounts of NaCl (0%, 1%, 3%, 6%, 8%, and 10%). This was performed to study the salt tolerance range of the isolated species and the optimum concentration, once determined was supplemented in various media required to test their biochemical properties (Cowan and Steel's, 1993).

Growth at different temperature range

The isolated *Vibrio* spp. was kept in incubator after inoculation at subsequent interval at temperature 4^0C, 28^0C, 37^0C, 55^0C and checked for their survivability and colony formation obtained at 28^0C and 37^0C (Cowan and Steel's, 1993).

In-vitro challenge test and determination of antagonistic activity of the probiotics

After identification of *Vibrio harveyi* by biochemical test one colony of *V. harveyi* was taken into eppendorf tube by isolating loop into 0.9ml peptone water. Prepared stock solution of *V. harveyi* (ISO/TS 21872-1, 2007). The stock solution was diluted (tenfold dilution) with peptone water (James and Hirsch, 1960) and prepared test solution of *V. harveyi*. Then 0.5 ml isolated probiotic solution was separately mixed with 0.5ml of test solution (*V. harveyi)*. 0.1ml suitable dilution of the mixer solution was inoculated in TCBS agar media after at subsequent intervals of 4 hour up to 12hour. This procedure was done for 2 times. Test solution of *V. harveyi* ; without probiotic was also inoculated in TCBS agar media at 0 hour, 4^{th} hour, 8^{th} hour and 12^{th} hour subsequently. All the inoculated TCBS agar plates were incubated at 37^0 C for 24± 3 hours. Standard plate count was done after incubation.

Data collection and analysis

Collected data were stored, explored and analyzed using Microsoft Excel (Microsoft office, 2007) and Statistical Package for the Social Sciences (SPSS version 16.0; SPSS, Inc., Chicago, IL). Independent sample t-test was applied to address the differences between the treatment and control at 1% significance level using SPSS (version 16.0).

RESULTS

For the enumeration of bacterial loads, gills and intestines were aseptically taken out from the samples. The organs were taken into eppendorf tubes with peptone water **(**James and Hirsch, 1960) for isolating *Lactobacillus* spp. and alkaline saline peptone water was used for isolating *Vibrio* spp**. (**ISO/TS 21872-1, 2007). Then the colonies of *Lactobacillus* spp. and *Vibrio* spp**.** were counted**.** The result revealed that the load of *Lactobacillus* spp. were more (1.65×10^5, 1.65×10^5, 1.18×10^5 CFU/g and 4.14×10^4, 2.74×10^4, 3.10×10^4 CFU/g) than *Vibrio* spp. (1.29×10^4, 1.90×10^4, 1.40×10^4 CFU/g and 5.44×10^3, 4.00×10^3, 8.49×10^3 CFU/g) both in gills and intestines; the gills also contained higher load of *Vibrio* spp. than the intestines (Figure 1).

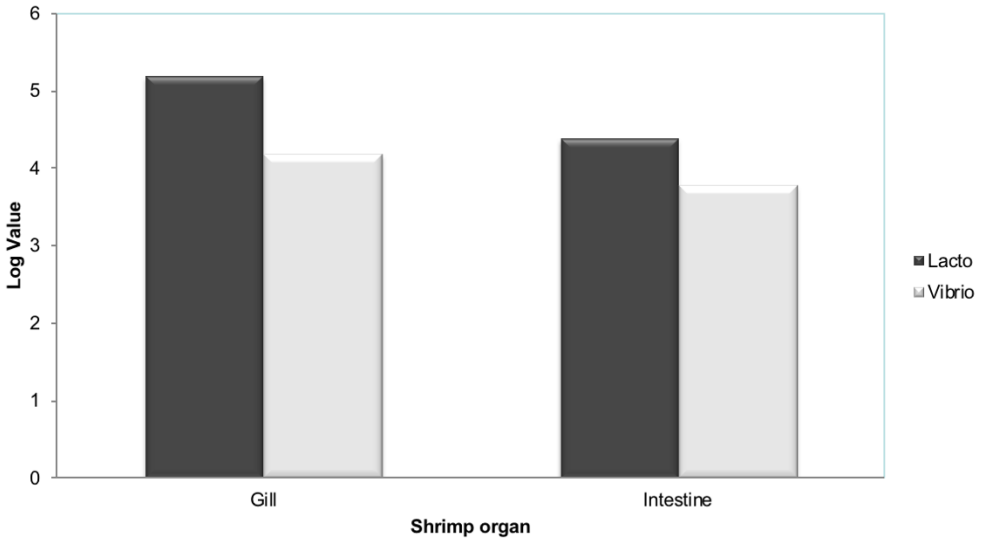

Figure 1. Average Load of *Vibrio* spp. and Lactobacillus spp. in Gill and intestine

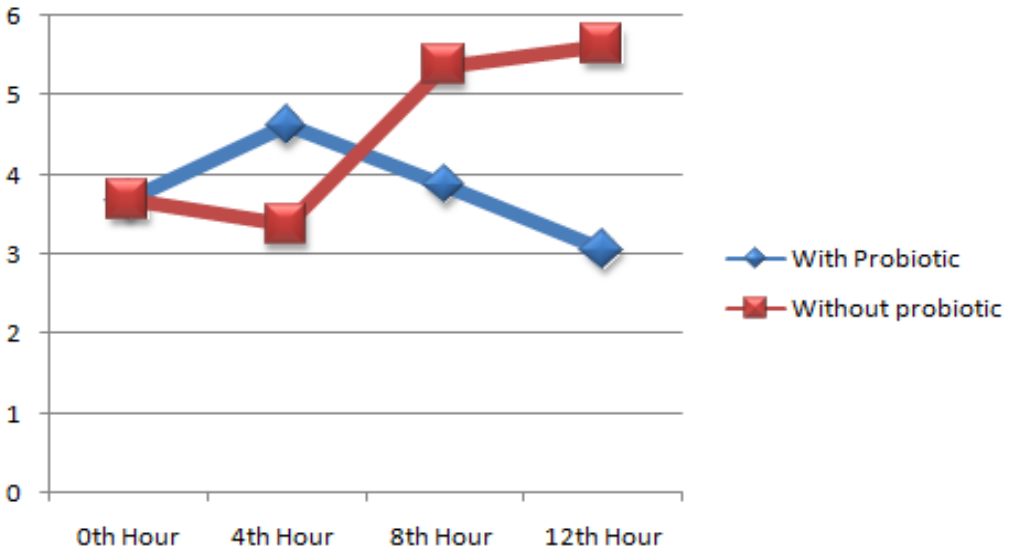

Figure 2. *In-vitro* challenge test with isolated probiotics on *vibrio harveyi*

The average load of *Lactobacillus* spp. in the gills and intestinal tracts of the shrimp samples collected from 3 experimental ghers were 1.65×10^5, 1.65×10^5, 1.18×10^5 CFU/g and 4.14×10^4, 2.74×10^4, 3.10×10^4 CFU/g respectively (Table-1). The average load of *Vibrio* spp. in the gills and intestinal tracts of the shrimp samples collected from 3 experimental ghers were 1.29×10^4, 1.90×10^4, 1.40×10^4 CFU/g and 5.44×10^3, 4.00×10^3, 8.49×10^3 CFU/g respectively (Table 1).

Table 1. Load of *Lactobacillus* spp. in gills and intestinal tracts of collected shrimp

Gher No.	*Load of Lactobacillus spp. in gills (CFU/g)*	*Load of Vibrio spp. in gills (CFU/g)*	*Load of Lactobacillus spp. in intestinal tracts (CFU/g)*	*Load of Vibrio spp. intestinal tracts (CFU/g)*
01.	1.65×10^5	1.29×10^4	4.14×10^4	5.44×10^3
02.	1.65×10^5	1.90×10^4	2.74×10^4	4.00×10^3
03.	1.18×10^5	1.40×10^4	3.10×10^4	8.49×10^3

Table 2. Biochemical test to identify *Vibrio harveyi*

Biochemical test	***C1***	***C2***	***C3***	***C4***	***C5***	***C6***	***C7***	***C8***	***C9***	***C10***
Gram stain	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	N/D	+	+	+	N/D	+
Indole formation	+	+	+	+	-	+	+	+	-	+
VP test	-	-	-	-	+	-	-	-	+	-
Arginine dihydrolase	-	-	-	-	N/D	-	-	-	N/D	-
MR test	+	+	+	+	-	+	+	+	-	+
Growth in NaCl at										
0%	+	+	+	+	+	+	+	+		+
1%	+	+	+	+	+	+	+	+		+
3%	+	+	+	+	+	+	+	+		+
5%	+	+	+	+	+	+	+	+	N/D	+
7%	-	-	-	-	+	-	-	-		-
10%	-	-	-	-	N/D	-	-	-		-
Growth at										
4°C	-	-	-	-		-	-	-		-
28°C	+	+	+	+	N/D	+	+	+	N/D	+
37°C	+	+	+	+		+	+	+		+
55°C	-	-	-	-		-	-	-		-
Colony color on TCBS	G	G	G	Y	G	G	G	G	G	G
Identification	Vh	Vh	Vh	Vh	vs	Vh	Vh	Vh	vs	Vh

Vh = *Vibrio harveyi*, + = positive, - = negative, G = Green, Y = Yellow, Vs = *Vibrio* Sp

Nine biochemical tests were performed (Table 2) to identify *V. harveyi* from the isolated colonies of *Vibrio* spp. The isolated colonies were inoculated in different biochemical media, supplemented with 1% NaCl to provide the optimum condition for growth, and incubated overnight at 30°C. The Gram stain result was observed under total magnification by using a light microscope and showed all *V. harveyi* isolates to be Gram negative short rods. All *V. harveyi* isolates showed positive results to the Motility test, Indole ring, MR test. Majority of *V. harveyi* isolates (96.7%) were able to grow in salt tolerance test at 0% to 5%. These bacterial isolates showed inhibition at temperatures of 4°C and 55°C but were able to grow well at temperatures of 28°C and 37°C. The green and yellow colonies were observed on TCBS agar plates.

The biochemical test was done with 10 colonies of *Vibrio* spp. From the conformation test it was found that 80% was *V. harveyi* among 10 *Vibrio* spp. After that with the isolated *V*. *harveyi* colonies were further *in-vitro* tested to determine the antagonistic effect of *Lactobacillus* spp. on *V. harveyi.*

The result of the *in-vitro* challenge test with and without probiotics had been presented in Figure 2. *In-vitro* challenge tests were performed to investigate the antibacterial effect of the isolated probiotics (*Lactobacillus* spp.) on the *Vibrio harveyi* of infected shrimp. When *in-vitro* challenge tests were run it was found that the average load of *V. harveyi*. in zero (0) hour was 4.69×10^3 CFU/g and it was 2.30×10^4, 2.36×10^5 and 4.24×10^5 at 4th, 8th and 12th hour respectively. However, after treatment with the isolated probiotics, the average load of *V. harveyi* at 4th, 8th and 12th hour were reduced to 1.16×10^4, 7.41×10^3 and 1.13×10^3 respectively. The present study showed a slight reduction of *V. harveyi* load at the 4th hour of probiotic application where as a drastic and significant reduction in the load of *V. harveyi* had been obtained at the 8th and 12th hour of probiotic application.

DISCUSSION

The present study showed that the load of *Lactobacillus* spp. and *Vibrio* spp. was more in gills than in the intestinal tracts of shrimp samples. Gill is an essential organ for the respiration of fish and always remains in contact with the aquatic environment. Water is the major source of various types of microorganisms. So, gill possesses the greater possibility of association with different types of bacteria. The present study was also similar with some other research work. Such as, potential pathogens were able to maintain themselves in the external environment (water) of the animal and proliferate independently of the host animal (Hansen and Olafsen, 1999; Verschuere et al., 2000).

Various potential pathogens are taken up constantly by the animal through the processes of respiration, osmoregulation and feeding. This might be the main reason of such better occurrence of *Lactobacillus* spp. and *Vibrio* spp. in gills than intestinal tracts of the experimental samples. This result also agreed well with the findings of Ringo and Gatesoupe (1998). They reported that, *Lactobacillus* spp. is less abundant in intestinal tract. It is also well known that the population level of lactic acid bacteria associated with the digestive tract is affected by nutritional and environmental factors like dietary polyunsaturated fatty acids, chromic oxide, stress etc. These affecting factors might be another reason why there was less abundance of *Lactobacillus* spp. in intestinal tracts than in the gills.

Moriarty (1990) reported that, aquatic farmed animals are surrounded by an environmental milieu that supports opportunistic pathogens independently of the host animal, and so the pathogens can reach high abundance on the external organs of the animal. In aquaculture ponds, where animal and algal population densities are very high, *Vibrio* spp. numbers can also become high compared to the open sea. This might be another reason of such better occurrence of *Vibrio* spp. in gills than the intestinal tracts of the experimental samples, which was found in the present study. Verschuere et al., (2000) stated that although lactic acid bacteria are not dominant in the normal intestinal microbiota of larval or growing fish, several trials have been undertaken to induce an artificial dominance of lactic acid bacteria in aquatic animals. This statement supports the findings of the present study.

The present study revealed that *Lactobacillus* spp. loads were higher than *Vibrio* spp. both in gill and intestine. *Lactobacillus* spp. is a beneficial bacteria and the presence of higher amount of *Lactobacillus* spp. in gill and intestine is a good sign. Because this *Lactobacillus* spp.; act as natural probiotics in infected shrimp. These results also agreed with the findings of two other separate experiments (Lee et al*.,* 2000) and (Vine et al., 2004) who stated that successful probiotics bacteria are usually able to colonize the intestine, at least temporarily, by adhering to the intestinal mucosa. The adhesive probiotics bacteria could prevent the attachment of pathogens, such as coliform bacteria and clostridia, and stimulate their removal from the infected intestinal tract. Both of these experiments revealed the temporary colonization and adherence of probiotic bacteria. The gills may appear susceptible to bacterial penetration because they are covered by a thin exoskeleton (Taylor and Taylor, 1992), but their surfaces are cleaned by the setobranchs.

From the biochemical test it was found that most of the *Vibrio* spp. were *Vibrio harveyi* . From the present it has been proved that there is a significant antagonistic effect of *Lactobacillus* spp. against *V. harveyi.* In *in-vitro* challenge test, the potential antagonistic effects of *Lactobacillus* spp. against *V. harveyi* was gradually obtained at 4^{th}, 8^{th} and 12^{th} hour of probiotics treatment. This finding is very much similar to Balca'zar, (2003) also works on effect of probiotics on shrimp, his findings also relate with the findings of the present study. He found that the administration of a mixture of bacterial strains (*Bacillus* spp. and *Vibrio* spp.) positively influenced the growth and survival of juveniles of white shrimp and presented a protective effect against the pathogens *Vibrio harveyi* and white spot syndrome virus.

Sumon et al., (2018) also found the antagonistic effect of probiotic bacteria on *Vibrio harveyi* that also supports the present study. Austin and Brunt, (2008) also mentioned that the probiotics actively interfere with the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients, oxygen and/or space, by alteration of microbial metabolism, and/or by the stimulation of the innate immune response, including enhancement of phagocytic and respiratory burst activities and lysozyme reduction. Jiravanichpaisal and Chuaychuwong et al., (1997) used successfully *Lactobacillus spp.* as the probiotic bacteria in the tiger shrimp (*P. monodon* Fabricius). They designed to investigate an effective treatment of *Lactobacillus spp.* against vibriosis and white spot diseases in *P. monodon*. Results of the present study indicated that probiotics treatment offers a promising alternative to the use of antibiotics in shrimp aquaculture. These works strongly suggest the effective control of microflora in fish and shellfish in culture environments by antibiotic-producing bacteria.

CONCLUSION

From this *in-vitro* challenge test with *Lactobacillus* spp. it was found that it significantly reduced the *V. harveyi* load of the selected shrimp samples. From this *in-vitro* test it was proved that *Lactobacillus* spp., had the inhibitory property of a biocontrol agent for use in control of shrimp pathogens and might be useful for replacing the commercial antibiotics. The present investigation also clearly demonstrated that, putative probionts isolated from infected shrimp possesses an excellent antibacterial effect and could be applied in aquaculture operation as an effective tool of treatment to prevent and cure shrimp diseases caused by *V. harveyi*. On the basis of the results obtained in this work, it could be concluded that Probiotics, as 'bio-friendly agents' such as *Lactobacillus* spp., could be introduced into the culture environment to control and compete with pathogenic bacteria as well as to promote the growth of the cultured organisms.

ACKNOWLEDGEMENT

The authors would like to thank all the people who helped in the collection of prawn juveniles and pond preparation. The authors gratefully acknowledge the financial support of Bangladesh Fisheries Research Institute (BFRI).

REFERENCES

1. Alberman DJ, 1988. Fisheries chemotherapy: a review. In: Recent Advances in Aquaculture, 3: 1-61.
2. Akoki T, 1975. Effects of chemotherapeutics on bacterial ecology in the ponds and the intestinal tracts of cultured fish. Journal of Microbiology, 19: 7-12.
3. Austin B and J Brunt, 2008. Probiotics and disease control in aquaculture. Journal of the World Aquaculture Society, 6: 43-48
4. Bacazar E, 2003, Anti-infectious immune effectors in marine invertebrates: potential tools for disease control in larviculture. Journal of Aquaculture, 227: 427–438.
5. Baticados MCL, CR Lavilla-Pitogo, ER Cruz-Lacierda, LD de la Pena LD and MA Sunaz, 1990. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeus monodon* larvae and rearing water. Disease of Aquatic Organisms, 9: 133-139.
6. Cowan and Steel's, 1993. Manual for the Identification of Medical Bacteria. pp. 201-233.
7. Garriques D and G Arevalo, 1995. An evaluation of the production and use of alive bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vennamei*. Journal of Microbiology, 3: 43-51.
8. Hansen GH and JA Olafsen, 1999. Bacterial interactions in early life stages of marine cold water fish. Journal of Microbial Ecology, 38: 1–26.
9. ISO/TS 21872-1, 2007. Microbiology of food and animal feeding stuffs-Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. - Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.
10. James and Hirsch, 1960. Comparative bacterial activity of blood serum and plasma serum. Journal of Biology, 66: 391-398.
11. Jiravanichpaisal P, P Chuaychuwong and P Menasveta, 1997. The use of *Lactobacillus* sp. as the probiotic bacteria in the giant tiger shrimp, *Penaeus monodon*. Proceedings of the poster session of the 2nd Asia-Pacific marine biotechnology conference and 3rd Asia-pacific conference on algal biotechnology, 2: 13-16.
12. Karunasagar L, P Pai, GR Malathi and L Karunasagar, 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Journal of Aquaculture, 128: 203-209.
13. Lavilla-Pitogo CR, MCL Baticados, ER Cruz-Lacierda and LD Pena, 1990. Occurence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Journal of Aquaculture, 91: 1-13.
14. Lee YK, WL Lim, AC Teng, EM Ouwehand, EM Tuomola and S Salminen, 2000. Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. Journal of Applied Environmental Microbiology, 66: 3692–3697.
15. Lightner DV, 1983. Diseases of cultured Penaeid shrimps. *In:* McVey, J.P. (Ed.). CRC Handbook of Mariculture, CRC Press, Boca Raton, FL. pp. 289–320.
16. Moriarty DJW, 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Journal of Aquaculture, 164: 351-358.
17. Prabhu NM, AR Nazar, S Rajagopal and SA Khan, 1999. Use of probiotics in water quality management during shrimp culture. Journal of Aquaculture in the Tropics, 14: 227–236.
18. Ringo E and F Gatesoupe, 1998. Lactic acid bacteria in fish: a review. Journal of Aquaculture, 4: 177-203.
19. Sumon MS, F Ahmmed, SS Khushi, MK Ahmmed, MA Rouf, MAH Chisty and MG sarower, 2018. Growth performance, digestive enzyme activity and immune response of Ma*crobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets, Journal of King Saud University, 30:21-28.
20. Taylor HH and EW Taylor, 1992. Gills and lungs: the exchange of gases and ions. *In:* Harrison, F.W. and Humes, A.G (Eds.). Microscopic Anatomy of Invertebrates, Wiley-Liss, New York, 10: 203–293.
21. Verschuere L, G Rombaut G, P Sorgeloos and W Verstraete, 2000. Probiotic bacteria as biological control agents in aquaculture. Journal of Molicular Microbiology, 64: 655–671.
22. Vine NG, WD Leukes, H Kaiser, J Baxter and T Hecht, 2004. Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. Journal of Fish Disease, 27: 319–326.