BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, October 2014. Vol. **11**(Spl. Edn. 1), p. 297-305

Effectiveness of *Lactobacillus* sp (AMET1506) as Probiotic against Vibriosis in *Penaeus monodon* and *Litopenaeus vannamei* Shrimp Aquaculture

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doi: http://dx.doi.org/10.13005/bbra/1423

(Received: 15 August 2014; accepted: 10 October 2014)

Vibriosis is the one of the major pathogenic bacterial disease in shrimp aquaculture. Improving the health status of culture organisms using beneficial microbes as probiotic is the better method to control the pathogens. In this present study the *Lactobacillus* sp AMET1506 (Which shows strongest antagonistic activity against pathogenic bacteria such as, *E.coli, V. cholerae, V.parahaemolyticus, Salmonella* sp. and S*higella* sp) was previously isolated from curd sample. While checking the antibacterial activity of *Lactobacillus* sp (AMET1506) against *V.harveyi* maximum inhibition activity was observed. So, the strain was potentially chosen and it was incorporated in shrimp feed by standard method. A total of 400 *Penaeus monodon* and *Litopenaeus vannamei* (each 200) shrimps larvae were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. After acclimation of seven days, the average weights of the shrimps were divided into twelve 50 L plastic tanks each containing 25 juvenile shrimps. The experimental tanks were treated with feed supplemented with 10^6 CFU g-1 of *Lactobacillus* sp (AMET1506), and the control tanks were fed with a control diet. Shrimp in all the groups were fed twice daily at 5.0% of biomass and the water temperature was maintained at 28 ± 1°C. After 30 days of culture, shrimp in all the control and experimental tanks were exposed to *V. harveyi* (10^5 CFU ml-1) for 10 days. During the experiment, the accumulated mortality of the shrimp and the microbial load in the shrimp and culture water was recorded. Among that, the shrimp *P.monodon* treated with *Lactobacillus* sp AMET1506 resulted in 6% final mortality as compared to 80% in the control group and in *L.vannamei* treated with *Lactobacillus* sp AMET1506 resulted in 12% final mortality as compared to 100% in the control group. Based on these results, the work has suggested to use this potential strain *Lactobacillus* sp AMET1506 as a probiotic in shrimp aquaculture feeds to improve the shrimp microbiota (GIT) and also to control the vibriosis in shrimp aquaculture.

Key words: Shrimp Aquaculture, Vibriosis, *Lactobacillus* sp, Probiotic.

Shrimp farming is one of the most important aquaculture in worldwide especially in Asia due to their economic value. Recently, it is estimated that approximately more than 5 million metric tons of shrimp are annually produced but the current global demand for both the wild and farmed shrimp is approximately more than 6.5 million metric tons per annum. So, in recent times there are many shrimp farms are being created throughout the world to solve this increasing food demands (FAO 2012). However, fast development of these shrimp industry has produced various ecological, economical and social issues. In general, intensive

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shrimp farming is the main aquaculture activity which has been frequently affected by bacterial pathogens especially in Asian countries. Among that, vibriosis is the common bacterial disease responsible for mortality of cultured shrimp (Sivakumar *et al*., 2014). Using antibiotics and chemotherapeutic agents to be an important disease controlling measures has developed drug resistance microorganisms (Verschuere *et al*., 2000). In recent times, an alternative that has been widely engaged in the aquaculture industry is the dietary supplementation with probiotic bacteria, because probiotic bacteria are a "live microbial cells administered to cultured organisms to colonize the digestive tract and improve their immune response" (Vine *et al*., 2006).

Researchers also have demonstrated about the use of probiotic bacteria in aquaculture to improve the water quality and immune system by balancing bacterial flora in water and reducing pathogenic bacterial load (Kesarcodi-Watson *et al*. 2008). Among the probiotic bacteria used in aquaculture, the lactic acid bacteria are found to be great due to their easy multiplication, production of antimicrobial compounds (bacteriocins, hydrogen peroxide, organic and lactic acids) and the stimulation of the non-specific immune response of the host (Gatesoupe, 2008). Some, studies also have demonstrated about the beneficial effect of lactic acid bacteria bacteria in several aquatic species culture by their nutritional benefits and strong antimicrobial activity against pathogenic microorganisms (Gilliland et al., 1985; Rossland et al., 2003; Ajitha et al.,2004; Gatesoupe, 2008; Qi et al., 2009; Ismail and Soliman, 2010 and Sivakumar *et al*., 2012) but no probiotic bacteria has been employed especially against the shrimp pathogen *Vibrio harveyi.* Thus the present study was carried out to evaluate the probiotic potential of *Lactobacillus* sp (AMET1506) to control the pathogenic *Vibrio harveyi* in juvenile shrimp (*Penaeus monodon and Litopenaeus vannamei*) culture at laboratory scale experiments.

MATERIALS AND METHODS

Bacterial Strains

The *Lactobacillus* sp (AMET1506) strain used in this study was previously isolated from curd sample by dilution plating on de Man, Rogosa and Sharpe (MRS) media (Himedia, India) and it was identified by biochemical examination using Bergey's Manual of Determinative Bacteriology (1989). The strain has shown strongest antagonistic activity against different seafood bacterial pathogens such as, *E.coli, V.cholerae, V.parahaemolyticus, Salmonella* sp and S*higella* sp (Karthik *et al*., 2013).

Antibacterial activity of *Lactobacillus* sp (AMET1506) against *V.harveyi*

The potential culture of *Lactobacillus* sp (AMET1506) was grown in 100 mL MRS broth for 24 h at 30°C. After the incubation period it was centrifuged at 10,000 rpm for 10 min and the obtained supernatant was passage through a 0.25 µM syringe driven filter and neutralized (pH 7.0) with 2 N NaOH. The pathogenic bacteria *V.harveyi* was obtained from AMET Microbial Culture Collection Centre. Mueller-Hinton agar plates were prepared and swabbed with 100 µL of *V.harveyi*. The sterile disk (6 mm), impregnated with 20 µL of filtered supernatant (Obtained from *Lactobacillus* sp (AMET1506)) were positioned on the plate and kept for 24 hours incubation at 30°C. After the incubation period the diameter of the clear zone around the disk was measured (Sivakumar *et al*., 2012).

Preparation of *Lactobacillus* sp (AMET1506) incorporated feed

The strain *Lactobacillus* sp (AMET1506) was grown in MRS broth in a shaking incubator at 30°C for 24 hours. After the incubation period, the cells were harvested by centrifuging at 2000 rpm and the obtained pellet was washed twice with phosphate-buffered saline (pH 7.2) and re-suspended in the same buffer. Then, the absorbance at 600 nm was adjusted to 0.25 ± 0.05 in order to standardize the number of bacteria (10^6 CFU mL-1) by dilution plating method. The commercial shrimp feed was obtained for the supplementation of *Lactobacillus* sp (AMET1506). In order to reach a final concentration (10^6 CFU g-1) the bacterial suspension was slowly sprayed onto the feed for mixing. The amount of *Lactobacillus* sp (AMET1506) in the feed was determined by standard plate count method on MRS agar (Ajitha *et al*., 2004).

Probiotic treatment and *Vibrio* challenging study of shrimp

A total of 400 *Penaeus monodon* and

Litopenaeus vannamei (each 200) shrimps larvae were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram District, Tamil Nadu, India. After acclimation of seven days, the average weight of the shrimps were divided into twelve 50 L plastic tanks (Six tanks for *Penaeus monodon* and another six tanks for *Litopenaeus vannamei*) each containing 25 juvenile shrimps. In both the culture experiments, six tanks (Three tanks for *Penaeus monodon* and another three tanks for *Litopenaeus vannamei*) were treated with feed supplemented with 10^6 CFU g-1 of *Lactobacillus* sp (AMET1506) for 30 days, and the another six tanks (Three tanks for *Penaeus monodon* and another three tanks for *Penaeus vannamei*) were served as control and they were fed with a control diet during the entire trial period. Shrimp in all the groups were fed twice daily at 5.0% of biomass and the water temperature was maintained at 28 ± 1°C. After 30 days of culture the weight and the survival of the shrimp were recorded and three shrimps were removed from all the control and experimental tanks for microbiological examination. After 30 days of probiotic supplementation, the experimental infection was carried out by the immersion method. *V. harveyi* was grown for 24 h at 30°C in TCBS broth (Himedia, India). Shrimp in all the control and experimental tanks were exposed to *V. harveyi* (10^5 CFU ml-1) for a period of 10 days and the accumulated mortality of the shrimp was recorded (Sivakumar *et al*., 2012).

Microbiological analysis

Shrimps and the culture water samples were taken on 30th day (before Vibrio challenging study) and 40th day (after Vibrio challenging study) from all the control and experimental tanks. Total heterotrophic bacteria (THB), *Lactobacillus* sp and *Vibrio* sp load in the shrimp intestine and culture water was enumerated by growth on Zobell Marine agar, MRS agar and TCBS agar (Himedia, India) respectively. For isolation of other pathogenic bacteria such as, *E.coli*, *Salmonella* sp, *Shigella* sp and *Listeria* sp MPN technique was followed using EMB agar, SS agar and PALCAM agar (Himedia, India) respectively (Sivakumar *et al*., 2012; Karthik *et al*., 2013).

Statistical analysis

All the experiments were repeated at least 3 times, and the data were expressed as the mean standard deviation (±SD).

RESULTS AND DISCUSSION

In normal, diseases in aquaculture practices are mostly caused by luminous bacteria *Vibrio harveyi,* and it has been referred as the largest economic loss in the shrimp aquaculture due to mass mortalities (Natesan *et al*., 2014). To control the pathogens, the use of probiotics in aquaculture is increasing demand for its more environment friendly aquaculture practices (Petlu Nitya *et al*., 2013). The *Lactobacillus* sp (AMET1506) strain used in this study was potentially selected due to its strongest antagonistic activity against different seafood bacterial pathogens such as, *E.coli, V. cholerae, V.parahaemolyticus, Salmonella* sp. and S*higella* sp (Karthik *et al*., 2013). While checking its antibacterial activity against *Vibrio harveyi* the maximum inhibition zone (18mm) was observed around the well. Natesan *et al*., 2012 also observed the maximum zone of inhibition (16mm) against *V. alginolyticus* using their strain *L. acidophilus* 04. The previous authors also described that, the antibacterial activity of *Lactobacillus* sp against the pathogenic microbes may be due to the production of its metabolites such as, organic acids (lactic and acetic acid), hydrogen peroxide, diacetyl and bacteriocins (Valenzuela *et al*., 2010).

Nowadays, the use of probiotics in aquaculture might represent a valuable mechanism to increase shrimp growth and survival rate. In general, the gastro intestinal tract (GIT) of the aquatic animal is mainly composed of gram negative bacteria (Vine *et al*., 2006). So, the incorporation of beneficial gram positive (probiotic) bacteria in feed can modify its gastro intestinal tract (Vieira *et al*., 2007). In our study, the potential strain *Lactobacillus* sp (AMET1506) was incorporated in the range of 10^6 CFU g-1 in shrimp feed using standard protocols. The *Lactobacillus* sp (AMET1506) incorporated feeds were fed to the shrimps in the experimental tanks and the control diet was fed to the shrimps in control tanks. The experiment was carried out for 30 days with zero water exchange. During the culture period the water temperature was maintained at 28 ± 1°C. After 30 days of culture, no shrimp mortality was observed in both *P.monodon* and *L.vannamei* culture in all the control and experimental tanks. The higher survival of shrimp fed with probiotic supplemented

Table 1: Microbial load on *P.monodon* and *L.vannamei* shrimp intestine and culture water on 30th day (before *Vibrio harveyi* challenging study) in both control and experimental groups

Microbial Load	*P.monodon*				*L.vannamei*			
	Control		Experiment		Control		Experiment	
	SI	CW	SI	CW	SI	CW	SI	CW
THB CFU/g/ml	$2.5 \pm 0.2 \times 10^7$	$3.8 \pm 0.2 \times 10^7$	$1.5 \pm 0.2 \times 10^8$	$1.7 \pm 0.2 \times 10^8$	$2.8 \pm 0.2 \times 10^7$	$4.2 \pm 0.2 \times 10^7$	$1.8 \pm 0.2 \times 10^8$	$1.7 \pm 0.2 \times 10^8$
Vibrio sp CFU/g/ml	$0.8 \pm 0.2 \times 10^8$	$1.0 \pm 0.2 \times 10^8$	$0.1 \pm 0.2 \times 10^8$	$0.1 \pm 0.2 \times 10^8$	$1.1 \pm 0.2 \times 10^8$	$1.5 \pm 0.2 \times 10^8$	$0.2 \pm 0.2 \times 10^8$	$0.2 \pm 0.2 \times 10^8$
Lactobacillus sp CFU/g/ml	$0.3 \pm 0.2 \times 10^8$	0±0	$8.8 \pm 0.33 \times 10^6$	$5.1 \pm 0.33 \times 10^6$	$0.1 \pm 0.2 \times 10^8$	0±0	$8.5 \pm 0.33 \times 10^6$	$5.1 \pm 0.33 \times 10^6$
E.coli MPN/100mL	60±0	50±0	26±0	21±0	70±0	60±0	33±0	27±0
Salmonella sp MPN/100mL	12±0	9±0	-	-	14±0	12±0	-	-
Shigella sp MPN/100mL	9±0	7±0	-	-	12±0	9±0	-	-
Listeria sp MPN/100mL	7±0	6±0	-	-	9±0	7±0	-	-

SI : Shrimp Intestine CW : Culture Water

Table 2. Microbial load on *P.monodon* and *L.vannamei* shrimp intestine and culture water on 40th day (After exposed to *Vibrio harveyi* for 10 days) in both control and experimental groups

Microbial Load	*P.monodon*				*L.vannamei*			
	Control		Experiment		Control		Experiment	
	SI	CW	SI	CW	SI	CW	SI	CW
THB CFU/g/mL	$4.2 \pm 0.2 \times 10^6$	$4.8 \pm 0.4 \times 10^6$	$0.9 \pm 0.2 \times 10^8$	$1.1 \pm 0.02 \times 10^8$	$4.3 \pm 0.2 \times 10^6$	$5.0 \pm 0.4 \times 10^6$	$1.0 \pm 0.2 \times 10^8$	$1.3 \pm 0.2 \times 10^8$
Vibrio sp CFU/g/mL	$4.4 \pm 0.2 \times 10^8$	$4.6 \pm 0.4 \times 10^8$	$5.1 \pm 0.2 \times 10^8$	$6.1 \pm 0.02 \times 10^8$	$4.4 \pm 0.2 \times 10^8$	$4.5 \pm 0.4 \times 10^8$	$6.3 \pm 0.2 \times 10^8$	$7.1 \pm 0.2 \times 10^8$
Lactobacillus sp CFU/g/mL	0±0	0±0	$0.5 \pm 0.33 \times 10^6$	$2.1 \pm 0.2 \times 10^6$	0±0	0±0	$0.3 \pm 0.33 \times 10^6$	$1.1 \pm 0.33 \times 10^6$
E.coli MPN/100g/mL	110±0	90±0	40±0	50±0	140±0	110±0	60±0	70±0
Salmonella sp MPN/100g/mL	27±0	21±0	-	-	34±0	30±0	-	-
Shigella sp MPN/100g/mL	30±0	34±0	-	-	34±0	40±0	-	-
Listeria sp MPN/100g/mL	34±0	30±0	-	-	40±0	50±0	-	-

SI : Shrimp Intestine CW : Culture Water

feed might be related to an immune reactive effect of probiotics on the host immune system, and the lactic acid bacteria are the main microbes which produce extracellular compounds to stimulate the non specific immune response in vertebrates (Marteau *et al*., 2002; Gill, 2003).

Moreover, while measuring the final weight of shrimps in all the groups, a significant difference was observed. The maximum mean final weight of *P.monodon* (Control-1.1 ± 0.1 gm in Experiment- 1.6 ± 0.3) and *L.vannamei* (Control-0.96 ± 0.1 gm in Experiment- 1.5 ± 0.3) was observed in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed compared to control groups fed with unsupplemented control diet (Fig 1). Similar, results were observed by previous authors while checking other probiotics for the same purpose (Li *et al*., 2006; Far *et al*., 2009). Rengpipat *et al*., 2000, also observed the better growth in shrimps when fed with *Bacillus* S11 (probiotic) supplemented feed in *Penaeus monodon*. But, our results were comparatively better than Dennis et al. (2000). Because, in their studies, they used commercial bacteria as a supplement for the culture of *L. vannamei* and they reported that it did not show increase mean final weight and FCR of the shrimps. So, the potential strain *Lactobacillus* sp (AMET1506) as proven its probiotic effectiveness in both *P.monodon* and *L.vannamei* shrimp culture at laboratory scale experiments. Venkat *et al*., 2004, also reported that the dietary supplementation of *Lactobacillus acidophillus* and *L. sporogenes* for *Macrobrachium rosenbergii* increased shrimp growth rate.

In *P.monodon* culture, whereas checking the microbial load in the culture water and shrimp intestine from both the control and experimental groups on 30th day, the higher total heterotrophic bacterial count was observed in shrimp intestine ($2.5 \pm 0.2 \times 10^7$) and culture water ($3.8 \pm 0.2 \times 10^7$) in control groups fed with unsupplemented control diet, and it was slightly decreased in shrimp intestine ($1.5 \pm 0.2 \times 10^8$) and culture water ($1.7 \pm 0.2 \times 10^8$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Moreover, the higher vibrio load also observed in shrimp intestine ($0.8 \pm 0.2 \times$

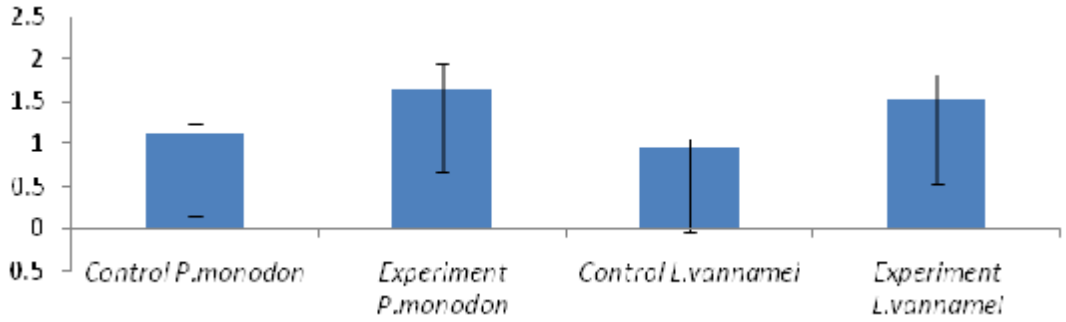

Fig. 1. Mean final weight gain of shrimp (on 30th day) fed with probiotic of *Lactobacillus* sp (AMET1506)

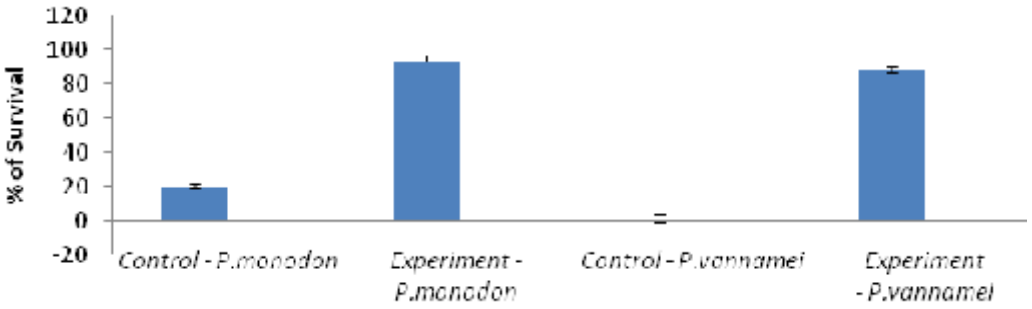

Fig. 2. Survival (%) of shrimps on 40th day (after challenging study) after feeding with control and probiotic *Lactobacillus* sp (AMET1506) supplemented feeds

10^8) and culture water ($1.0 \pm 0.2 \times 10^8$) in control groups fed with unsupplemented control diet, however it was mostly decreased in shrimp intestine ($0.1 \pm 0.2 \times 10^8$) and culture water ($0.1 \pm 0.2 \times 10^8$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Similarly, the *Lactobacillus* sp count was decreased in the shrimps intestine ($0.3 \pm 0.2 \times 10^8$) and not even a single colony was isolated from the culture water samples in control groups fed with unsupplemented control diet, but it was increased in shrimp intestine ($8.8 \pm 0.33 \times 10^6$) and culture water ($5.1 \pm 0.33 \times 10^6$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Moreover, when assessing other pathogenic microbial load in the shrimp and culture water using MPN technique, the maximum pathogenic bacterial load was observed in the control groups and minimum in the experimental groups respectively (Table 1). Sivakumar *et al*., 2012 also observed the similar results, when incorporating *L. acidophilus* 04 has potential probiotic to control pathogenic *V. alginolyticus* in *P.monodon* shrimp culture.

Comparable results were observed in *L.vannamei* culture, the maximum total heterotrophic bacterial count was observed in shrimp intestine ($2.8 \pm 0.2 \times 10^7$) and culture water ($4.2 \pm 0.2 \times 10^7$) in control groups fed with unsupplemented control diet, and it was slightly decreased in shrimp intestine ($1.8 \pm 0.2 \times 10^8$) and culture water ($1.7 \pm 0.2 \times 10^8$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. In addition, the higher vibrio load also observed in shrimp intestine ($1.1 \pm 0.2 \times 10^8$) and culture water ($1.5 \pm 0.2 \times 10^8$) in control groups fed with unsupplemented control diet, however it was mostly decreased in shrimp

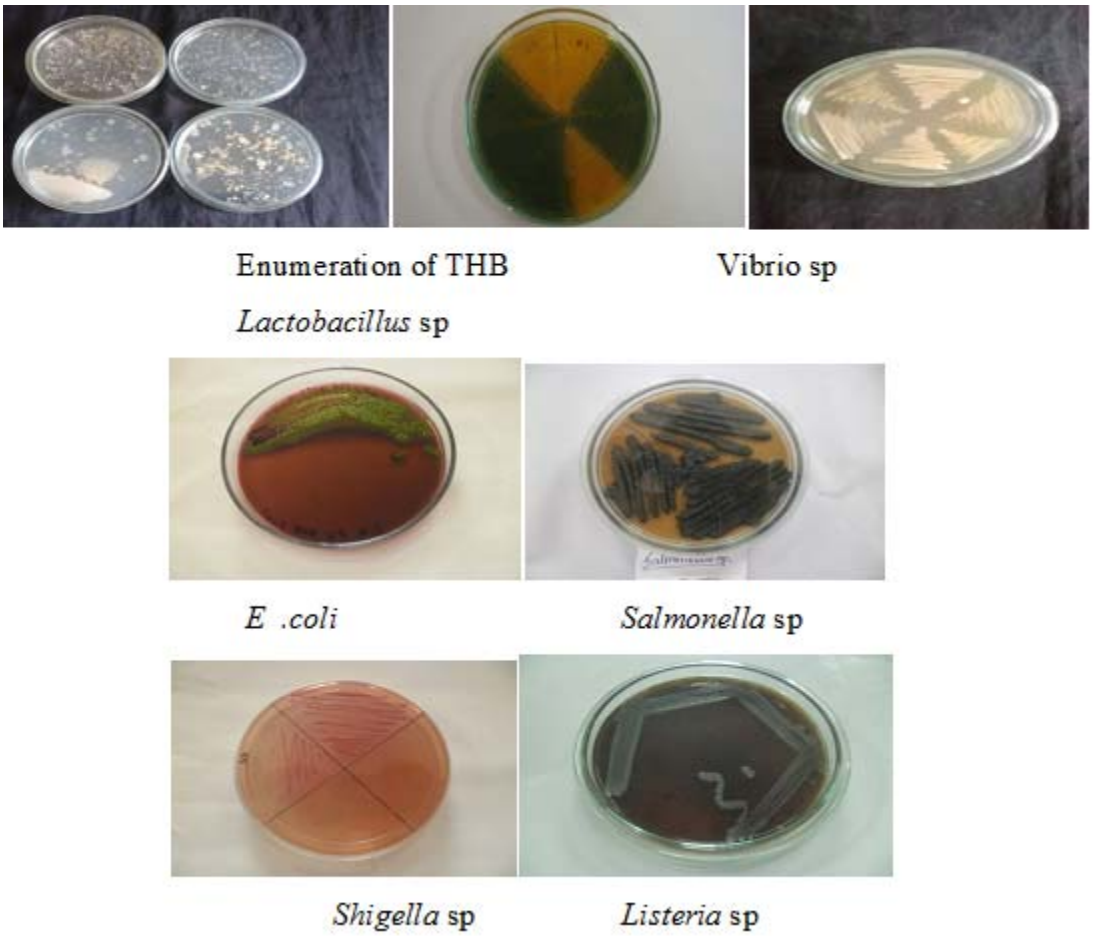

Fig. 3. Isolation of bacterial strains from shrimp intestine and culture water

intestine ($0.2 \pm 0.2 \times 10^8$) and culture water ($0.4 \pm 0.2 \times 10^8$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. In the same way, the *Lactobacillus* sp count also decreased in the shrimps intestine ($00.1 \pm 0.2 \times 10^8$) and not even a single colony was isolated from the culture water samples in control groups fed with unsupplemented control diet, but it was increased in shrimp intestine ($8.5 \pm 0.33 \times 10^6$) and culture water ($6.1 \pm 0.33 \times 10^6$) in the experimental groups fed with probiotic *Lactobacillus* sp (AMET1506) supplemented feed. Furthermore, when assessing other pathogenic microbial load in the shrimp and culture water using MPN technique, the maximum pathogenic bacterial load was observed in the control groups and minimum in the experimental groups respectively (Table 1). Jeevan Kumar *et al*., 2013 also reported that, they observed increase growth pattern of *Penaeus vannamei* when fed with *B.subtilis* incorporated diet and *L.rhamnosus* incorporated diet compared to control groups. Therefore, the reduction of pathogenic microbial load in the shrimp intestine and culture water may be due to the production of acid end products and antimicrobial peptides produced by the lactic acid bacteria (Vinothkumar *et al*., 2013).

In general, among the aquatic pathogens vibrio species are highly dangerous and it will detached with shrimp epithelium and affect highly by eliminating the two layers which protects the shrimp from infections and finally end with high mortality (Martin *et al*. 2004). Normally, probiotics may prevent the pathogens from the shrimp gut by production of antimicrobial compounds (Balcazar *et al*., 2006a). Whereas, to check the probiotic potential of *Lactobacillus* sp (AMET1506) to control the pathogenic microbes and to increase the shrimp growth as well as survival rate, the shrimps (*P.monodon* and *L.vannamei*) in the both control and experimental tanks were exposed to *V.harveyi* (10^5 CFU ml-1) on 31st day (Only once) and the experiment was carried out for 10 days with zero water exchange by maintaining the water temperature at 28 ± 1°C. After 10 days of culture, the final mortality of the shrimps was observed. In *P.monodon* treated with *Lactobacillus* sp AMET1506 resulted in 6% final mortality as compared to 80% in the control group and in *L.vannamei* treated with *Lactobacillus* sp AMET1506 resulted in 12% final mortality as compared to 100% in the control group (Fig 2). The results, were comparatively better than, Ajitha *et al*., (2004) who observed the survival of shrimp *P.indicus* (56 to 72%) when treated with probiotic supplemented feed groups challenged with *V.alginolyticus*.

Whereas analyzing the microbial load in *P.monodon* culture groups on 40th day, the maximum total heterotrophic bacterial count was observed in the shrimp intestine ($4.2 \pm 0.2 \times 10^6$) and culture water ($4.8 \pm 0.4 \times 10^6$) and it was decreased in the shrimp intestine ($0.9 \pm 0.2 \times 10^8$) and culture water ($1.1 \pm 0.02 \times 10^8$) in the experimental groups. Besides, the higher vibrio load also observed in shrimp intestine ($4.4 \pm 0.2 \times 10^8$) and culture water ($4.6 \pm 0.4 \times 10^8$) in the control tanks, however it was mostly decreased in shrimp intestine ($5.1 \pm 0.2 \times 10^8$) and culture water ($6.1 \pm 0.02 \times 10^8$) in the experimental tanks. Moreover checking *Lactobacillus* sp load, not even a single colony was isolated from the culture water samples collected from control tanks, but it was increased in shrimp intestine ($7.8 \pm 0.33 \times 10^6$) and culture water ($4.1 \pm 0.33 \times 10^6$) in the experimental tanks fed with *Lactobacillus* sp (AMET1506) supplemented feed. Moreover, when assessing other pathogenic microbial load in the shrimp and culture water using MPN technique, the maximum pathogenic bacterial load was observed in the control groups and minimum in the experimental groups respectively (Table 2).

Parallel results were observed in *L.vannamei* culture on 40th day, the maximum total heterotrophic bacterial count was observed in the shrimp intestine ($4.3 \pm 0.2 \times 10^6$) and culture water ($5.0 \pm 0.4 \times 10^6$) and it was decreased in the shrimp intestine ($1.0 \pm 0.2 \times 10^8$) and culture water ($1.3 \pm 0.2 \times 10^8$) in the experimental tanks. Moreover, the higher vibrio load also observed in shrimp intestine ($4.4 \pm 0.2 \times 10^8$) and culture water ($4.5 \pm 0.4 \times 10^8$) in the control tanks, however it was mostly decreased in shrimp intestine ($6.3 \pm 0.2 \times 10^8$) and culture water ($7.1 \pm 0.2 \times 10^8$) in the experimental tanks fed with *Lactobacillus* sp (AMET1506) supplemented feed. In addition, checking *Lactobacillus* sp load, 100% mortality was observed and not even a single colony was isolated from the culture water in the control tanks, but it was increased in shrimp intestine ($7.5 \pm 0.33 \times 10^6$) and culture water ($5.1 \pm$

0.33×10^6) in the experimental tanks where the shrimps were fed with *Lactobacillus* sp (AMET1506) supplemented feed. While assessing other pathogenic microbial load in the shrimp and culture water using MPN technique, the maximum pathogenic bacterial load was observed in the control groups and minimum in the experimental groups respectively (Table 2). The effect of commercial probiotic in aquaculture has been investigated by previous researchers but some of their research results has not shown any positive effects on the growth parameters or survival rate (Jeevan Kumar *et al*., 2013). Based on the shrimp survival rate, pathogenic microbial load and water quality in the experimental groups in both the *P.monon* and *L.vannamei* culture, our results were comparatively better than previous authors, who reported about the effect of lactic acid bacteria on the inhibition of *V. harveyi* in *invitro* (Vaseeharan and Ramasamy, 2003; Vieira *et al*., 2007). From the results, the study concluded that the *Lactobacillus* sp (AMET1506) strain will be helpful to manage the pathogenic luminous bacteria *V. harveyi* and other pathogenic bacteria. The study also suggested that, incorporating this kind of potential beneficial bacterial strain in feed will enhance the shrimp production in ecofriendly aquaculture practices.

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