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Effect of enriched rotifer (*Brachionus plicstilis*) with probiotic lactobacilli on growth, survival and resistance indicators of western white shrimp (*Litopenaeus vannamei)* larvae

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Abstract

This study examined the effects of a commercial Lactobacillus probiotic on growth, survival and resistance of western white shrimp (*Litopenaeus vannamei*) larvae against salinity and formalin stresses in the Persian Gulf and Oman Sea Ecology Research Center. In this experiment, larvae were fed 6 times a day from mysis I (M_1) step to post larvae 5 over 3 treatments including a control treatment C (without probiotic) and 2 experimental treatments namely A (having probiotic enriched rotifer) and B (having probiotic enriched rotifer and adding probiotic powder directly to the water). Larvae were stocked in 9 plastic tanks (20-liter) containing 10 L of seawater at a density of 50 larvae per liter. Three replicates were used for each treatment. At the end of the experimental period biometric larvae were studied under salinity and formalin stresses. Results showed that using probiotic bacteria had significant and positive effects on shrimp resistance, survival and growth (p<0.05) and that growth and survival rate of larvae fed with A and B treatments were significantly higher than that of the control group (p<0.05). The results of the stress tests also indicated that probiotic containing treatments (A and B) had significantly higher survival rates in salinity and formalin stresses than the control treatment (p<0.05). The highest survival rate and resistance were observed in treatment B which was not statistically different from that of treatment A (p>0.05). According to the results we may conclude that the use of probiotic powder is effective in increasing growth, survival and resistance rate of western white shrimp in the larval and post larval stages.

Keywords: *Brachionus plicatilis*, Probiotic, Resistance, *Litopenaeus vannamei*

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Introduction

Nowadays, aquaculture is the most important economic activity in the world (Balcazar *et al*., 2006). At present, many types of shrimp are being reproduced one of which is the western white shrimp. Many countries are reproducing this shrimp and have achieved significant success (Shakouri, 1997). However larval culture, especially in the initial stages of feeding, is the main obstacle in the development of the aquaculture industry (Sorgeloos *et al.*, 1998).

Today, the biotechnology industry presents various microbial products to modify the quality of water in aquaculture ponds (Aguirr-Guzma *et al*., 2002) and many individuals and producers are tending to use these live bacteria (probiotics) to improve production conditions in this area (Boyd and Gross, 1998). Elli Mechinkov was the first who used probiotics scientifically (Mosaffa, 2008). The term "probiotic" is a Greek word meaning "for life" (Gismondo *et al*., 1999). In a more comprehensive definition Fuller emphasizes the live essence of probiotics and says: "probiotics are useful bacteria which positively influence a host's health and growth by improving microbial equilibrium in the intestine" (Fuller, 1992). Accordingly, it is proposed to use probiotics to enhance growth and health of aquatic animals.

Various studies have been done on the use of probiotics in aquaculture in the world. Jafarian *et al.* (2010) used 4 species of bio-assisted lactobacilli as probiotics to increase larval growth and survival in rainbow trout. Other researchers found that probiotics prevent diseases in salmon (Austin *et al.*, 1992, 1995), larvae of scallop (Riquelme *et al*., 1997) and black tiger shrimp (Rengpipat *et al*., 1998).

The present paper tries to evaluate the effect of commercial lactobacilli (as probiotics) by rotifer enrichment and adding its powder to a watery environment to investigate its role in improving resistance and growth indicators in western white shrimp. The results of this study can be helpful in applying commercial probiotic bacteria to improve growth and survival rate in western white shrimp larvae and reduce the use of chemicals and medicines in aquaculture, especially in the shrimp reproduction industry.

Materials and methods

The present study was conducted at the aquaculture laboratory, Department of Persian Gulf and Oman Sea Ecology Research Center, Bandar Abbas, Iran.

Preparation of probiotics

Probiotic bacteria used in this study were in the form of a powder product with the commercial name, Protexin containing 7 species of bio-assisted bacteria, including: *Lactobacillus plantarum, Lactobacillus delbrueckii, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus salivarius, Enterococcus faecium*. This bacterial product contains 2×10^9 bacteria cells per gram.

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Rotifer enrichment

The strain of rotifer used was *B. plicatilis*. The rotifers were obtained from intensive production tanks. After filtration, and washing they were transferred to 20-liter tanks containing sterile seawater, and enriched based on their density. In order to enrich rotifers, considering their density (200–300 indv mL^{-1}) 0.43 mg mL^{-1} of probiotic powder was used (M.Planas *et al*., 2004). The measured probiotic powder was dissolved in sterile distilled water and was mixed using a shaker to create a usable emulsion and was added to enrichment tanks (Jafarian *et al.*, 2010). After 6 hours of enrichment, rotifers were harvested and washed and then used to feed larvae.

Experimental design

This experiment was planned and implemented in 3 treatments with 3 repetitions in the form of a fully random plan with a control treatment C and experiment treatments of A and B. The shrimp larvae were produced from a single parent. Based on treatments, larvae from step Mysis I (M_1) were stored in 20-liter plastic tanks containing 10 liters of filtered seawater at 30ppt salinity. The concentration of larvae in these tanks was 50 larvae per liter (500 larvae in each tank). Based on larval steps, from M_1 up to PL_5, larvae were fed six times daily at 4:00, 8:00, 12:00, 16:00, 20:00 and 24:00 h with Chaetoceros (3×10^4 -8×10^4 cell mL^{-1}), rotifer (4.5-20 per mL) and concentrated food produced by the INVE company in Thailand (1-2 mg/L) (Lavens and Sorgeloos, 1996; Stottrup and Mc Evoy, 2003). Treatment conditions differed based on the probiotics received (Table 1) so that larvae in the control group did not receive any probiotics.

Table 1: Conditions of treatments.

Treatment	Feeding and experiment plan (M_1-PL_5)
C	Chaetoceros + concentrated food + normal rotifer (control, no probiotics received)
A	Chaetoceros + concentrated food + probiotic enriched rotifer
B	Chaetoceros + concentrated food + probiotic enriched rotifer + probiotic powder solved in water

Larvae in treatment A received probiotics through feeding on enriched rotifer and those in treatment B received probiotics both through feeding on enriched rotifer and by adding probiotic powder to the water they live in (dissolving 1 gram of powder in 1 liter of water) (Pazir *et al.*, 2008). To maintain similar environmental conditions in all treatments, water quality parameters including temperature, pH, salinity, and dissolved oxygen were also controlled every day. At the end of the experimental period shrimp (PL_5) were harvested. Their weight and length was

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measured and survival rate was evaluated through enumerating the number of larvae. Besides, stress tests were used to study the resistance of larvae influenced by bio-assisted lactobacilli since using environmental stresses is one of the most appropriate methods to evaluate quality and resistance of shrimp larvae in Penaeoidea family (Dhert *et al*., 1992; Racotta *et al*., 2004). Hence, 30 randomly selected shrimp from each repetition of every treatment were exposed to salinity stresses of 10 and 45 ppt and formalin stress of 100 ppm in small plastic pots over a 60-minute period (Konatra, 1997; Palacios *et al*., 2004). At the end of these tests, mortality was enumerated and survival rate was recorded. The data obtained were analyzed by SPSS and Microsoft Excel software and through One-way ANOVA method based on Tukey test at level of 0.05.

Results

The effect of the probiotic lactobacilli on growth and survival parameters of western white shrimp are presented in Table 2.

Table 2: Growth average and survival percentage of shrimp in various treatments during experiment period (M_1-PL_0)

Treatments / Parameters	A Mean ± SD	B Mean ± SD	C Mean ± SD
Primary length (mm)	2.84±0.11 [a]	2.84±0.11 [a]	2.84±0.11 [a]
Total length (mm)	7.39±0.14 [a]	7.4±0.58 [a]	4.87±0.13 [b]
Enlargement percentage	160±5.3 [a]	164±19.4 [a]	71±3.8 [b]
Weight average (mg)	3.38±0.01 [a]	3.86±0.58 [a]	2.31±0.06 [b]
Highest weight (mg)	4.46	12.92	3.31
Lowest weight (mg)	2.71	2.37	1.6
Survival percentage average	79.07±0.98 [a]	80.53±1.67 [a]	62.8±3.89 [b]

Non-similar letters in each row show the significant difference in the level of 5% ($p<0.05$).

At the end of the experiment, treatments A and B (containing probiotics) showed an increase in body total weight and length and significantly differed from those of treatment C ($p<0.05$). Shrimps in treatment B (receiving probiotics as powder dissolved in water and rotifer enrichment) had the highest weight (3.86±0.58 mg) and length (7.4±0.58 mm) increase among the experimental treatments but they did not differ significantly ($p>0.05$) from those in treatment A (receiving probiotics only by enriched rotifer) with length and weight increase of 7.39±0.14 mm and 3.38±0.01, respectively. In our experiment the lowest growth was observed in treatment C (without probiotics) (Fig. 1). Besides, the enlargement rate in A and B was significantly higher than that in C ($p<0.05$). Changes related to post-larvae and their survival rate also showed better results in treatments A and B and were significantly different between the control group (C) and the treated group (A and B) (Table 2 and Fig. 2).

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Figure 1: The difference in larval growth rates in treatment C (shrimps in the top row) and treatments A and B (those in bottom row) at the end of experiment period.

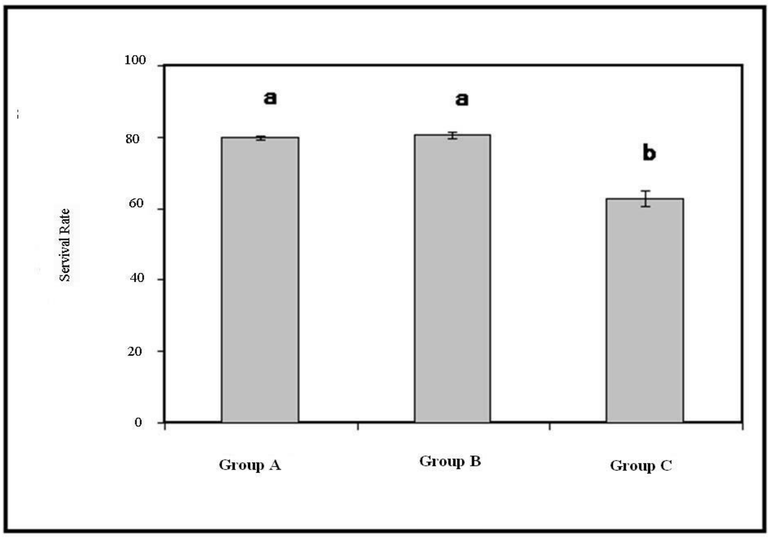

Figure 2: Comparison of effects of probiotic on average survival rate (percent) of western white shrimp in various treatments. Non-similar letters in each row show the significant difference at the level of 5% (*p*<0.05).

Results of Tukey test evaluation indicated that there were no significant differences between treatments A and B (with survival rate of 79.07±0.98 and 80.53±1.67, respectively) at the level of 5% ($p>0.05$).

The results of effect of the lactobacillus bacteria on resistance and survival of shrimp against stress tests are shown in Table 3. Data showed that larvae have different resistance levels against stress tests. The shrimp treated with probiotics (A and B) significantly showed higher survival when compared to the control group ($p<0.05$) (Fig. 3). As for salinity tensions of 10 ppt and 45 ppt, the observed survival rates of shrimps in treatment B were 66.67±3.33 and 92.22±2.94 percent, respectively which were not statistically different from that of treatment A ($p>0.05$).

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Table 3: Average (percent) of shrimp survival during various treatments at the end of experiment period (PL_5) against stress tests.

Parameters \ Treatments	A	B	C
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Salinity test (10 ppt)	62.22±2.22[a]	66.67±3.33[a]	46.67±1.92[b]
Salinity test (45 ppt)	90.00±1.92[a]	92.22±2.94[a]	71.11±2.94[b]
Formalin test (100 ppm)	7.33±8.82[a]	76.67±8.82[a]	60.00±3.85[b]

Non-similar letters in each row show the significant ($p<0.05$).

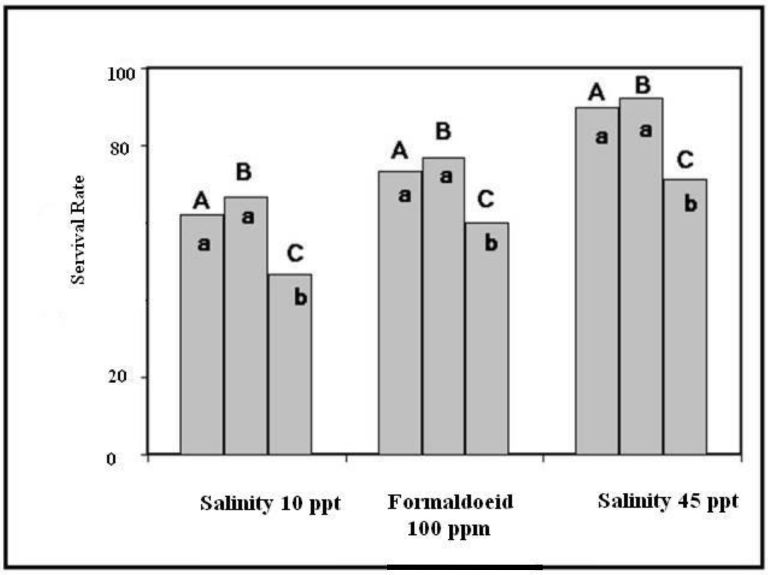

Figure 3: Comparison of probiotic effect on survival average (percent) of western white shrimp against tests performed in various treatments. Non-similar letters in each row show the significant difference in the level of 5% ($p<0.05$).

However, the lowest survival rate against this tension was significantly observed in shrimp of the control treatment (C) ($p<0.05$). Results of Formalin (100 ppm) tension indicated that the survival rate of shrimp in treatment A and B was significantly higher than that in the control treatment and they have more resistance ($p<0.05$).

Discussion

The use of probiotics and the beneficial effects of them in aquatic animals has been reported by several workers (Irianto and Austin, 2003; Carnevali *et al.*, 2004; Jafaryan *et al*., 2009; Ramazani-fard *et al*., 2014). In the present study rotifer (*B. plicatilis)* was used as a carrier transferring lactobacilli probiotics to western white shrimp larvae to increase their growth and survival rates. Results obtained from biometry of larvae at the end of the experiment period showed that the treated groups were influenced by probiotic lactobacilli and growth parameters are significantly different from that in the control group. Therefore, this investigation supports previous works and also shows that probiotics could be passed through rotifer which is routinely used to feed

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shrimp larvae and clearly shows that rotifer was an effective probiotic carrier. Pazir *et al.* (2008) achieved similar results in evaluating the effects of bacillus probiotics on western white shrimp growth so that the growth parameters of the shrimp treated by probiotics (at 30 ppt salinity) significantly changed compared to the control treatment. In fact, as Ziaeinejad *et al*. (2005) demonstrated, an increase in food uptake, digestion and absorption leads to increase of growth factors in probiotic treatments.

Based on survival rates in the present study, the used probiotic was significantly effective for shrimp survival during larval culture. Findings of Balcazar *et al*. (2007) confirm our results. They demonstrated that bio-assisted lactobacilli lead to an increase in fish immunity responses and are associated with higher survival rates. In some cases, it has been reported that the use of probiotic bacteria modifies the microflora of shrimp larvae (Rengpipat *et al*., 2000), improves larval survival (Rengpipat *et al.*, 2003; Mirbakhsh *et al*., 2012) and shrimp growth (Rengpipat *et al.*, 1998). Another study reported that adding probiotic powder to water of shrimp ponds has significantly increased dissolved oxygen in water, useful bacterial flora in shrimp intestines and shrimp production (p<0.05) (BO Wang *et al.*, 2006).

In experiments of resistance against stresses, probiotic lactobacilli enhanced larval resistance against stresses and increased their lifespan. In formalin shock and salinity stresses probiotic containing treatments showed a significant difference from the control group in terms of resistance and survival rates. There is evidence that bio-assisted bacteria such as lactobacillus stimulate the host's immunity system and lead to an increase in its resistance against environmental stresses and enhance the survival rate (Nikoskelainen *et al*., 2003; Panigrahi *et al*., 2004). The results of applying probiotic lactobacilli in the present research showed that probiotics play a positive role in the shrimp culture process, so our method may prove beneficial as an enhancement for hatchery postlarvae or for the improvement of young shrimp survival at the initial stages of earthen pond culture.

Mechanisms of probiotic action in the host are not fully understood but the purpose of probiotic use in aquaculture is to reduce the dependence on antibiotics and chemicals, thus improving environmental safety. In fact, today probiotics are being used instead of antibiotics (Zeheng and Yangl, 2004). Nowadays, probiotics are widely used in shrimp farms as a bacterial treatment. However further research is needed in this field.

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