Pakistan J. Zool., vol. 51(3), pp 919-923, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.919.923

Effect of Two Microecological Preparations on the Breeding of White Leg Shrimp, *Litopenaeus vannamei*

Song Jiang, Fa-Lin Zhou, Jian-Hua Huang, Qi-Bin Yang* and Li-Shi Yang

South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences/Key Laboratory of South China Sea Fishery Resource Exploitation and Utilization, Ministry of Agriculture, Guangzhou 510300, China

Article Information
Received 09 February 2018
Revised 21 July 2018
Accepted 04 September 2018
Available online 21 March 2019

Authors' Contribution
SJ and FLZ designed the study. QBY and JHH took part in the execution of study. LSY, QBY, and SJ implemented the study and involved in sampling as well as testing. FLZ and SJ drafted the manuscript.

Key words
***Litopenaeus vannamei*, Microecological preparation, Breeding, Body weight, Survival rate, stress resistance.**

ABSTRACT

To explore the effect of microecological preparations on the growth and stress resistance of *Litopenaeus vannamei* larvae, different amounts of *Bacillus* and *Lactobacillus* bacteria were added to the *L. vannamei* aquaculture water during seeding. Seven groups were established and the microecological preparations were added every 5 d. *Bacillus* concentrations in the Y1, Y2 and Y3 groups were 10 ppm, 15 ppm and 20 ppm, respectively, and the *Lactobacillus* concentrations in the R1, R2 and R3 groups were 20 ppm, 25 ppm and 30 ppm. The control (C) group had no microecological preparation added. Results showed that the Y2 larvae achieved the heaviest body weights, which were significantly higher than those of all other groups ($P < 0.05$), except for Y3 and R3 ($P > 0.05$). The body weights of the Y2 group were 27.18%, 11.97%, 27.18% and 23.58% higher than the weights of the C, Y1, R1 and R2 groups, respectively. Furthermore, except for that of the R2 group ($P > 0.05$), the survival rate of the R3 group was significantly higher than that of the C (34.86%), Y1 (21.49%), Y2 (17.60%), Y3 (15.75%) and R1 (15.75%) groups ($P < 0.05$). Microecological preparation addition also improved the stress resistance of *L. vannamei* larvae. Results showed that under stress conditions (60 min in fresh water and a sudden temperature change of 5°C), the survival rate in the two treatment groups was markedly higher than that in the C group (14.46% and 9.65%, respectively; $P < 0.05$). These results demonstrated that microecological preparations significantly enhanced the growth, survival and stress resistance of *L. vannamei* larvae.

INTRODUCTION

Litopenaeus vannamei, also known as *Penaeus vannamei*, has experienced rapid development in the Chinese aquaculture industry since its introduction in the 1990s (Yao *et al*., 2007). To control the massive environmental pressure of high density cultures, many farmers have injected huge amounts of chemical additives to the aquaculture water during the breeding process, resulting in irreversible effects on the ecological environment (Wu *et al*., 2006). In recent years, outbreaks of white spot syndrome and acute hepatopancreas necrosis syndrome have caused the near extinction of *L. vannamei* aquaculture (Argue *et al*., 2002). To save the industry, considerable attention has been paid to ecological breeding (Moss *et al*., 2001, 2007, 2011). Of note, research has shown that microecological preparations used during the breeding of Chinese shrimp (*Penaeus chinensis*) can significantly improve the seed emergence rate (Li *et al*., 2007).

Microecological preparations are a general term for probiotics that can be used in aquaculture (Jiang *et al*., 2014). *Bacillus* and *Lactobacillus* bacteria are two kinds of probiotics commonly used in aquaculture and breeding (Zeng *et al*., 2007). Studies have shown that *Bacillus* can produce a variety of digestive enzymes to help animals digest and absorb nutrients, thereby contributing to their growth and survival (Hu *et al*., 2012; Samocha *et al*., 2004; Gong *et al*., 2012). *Lactobacillus* are gram-positive bacteria that produce lactic acid (Qiu *et al*., 2005), and in aquaculture water can reduce the ammonia nitrogen content, decompose undesirable material and effectively control water quality (Shen *et al*., 2004; Yang *et al*., 2001). In the present study, we added *Bacillus* and *Lactobacillus* regularly during the entire breeding process to study their effects on *L. vannamei* seed growth and survival and impact resistance of young shrimps. This research will provide reference data for the improvement of *L. vannamei* aquaculture breeding.

MATERIALS AND METHODS

We purchased *L. vannamei* nauplii from the Shenzhen Global Group (China). The *Bacillus* preparation, containing 1.2×10^9 CFU g^{-1} of viable bacteria, was obtained from

* Corresponding author: 515641795@qq.com
0030-9923/2019/0003-0919 $ 9.00/0

Table I.- Feeding and management during the breeding of *Litopenaeus vannamei*.

Growth stage	Diets	Feeding time	Temperature(°C)	Inflation	Light intensity
Nauplii	Do not feed, using their own yolk nutrients	-	30	Microwave Shape	Avoid hard light
Zoea	Opening bait (unicellular algae, shrimp slices)	Every 3h	30	Slightly boiling	Avoid hard light
Early Mysis	Rotifers, prawn crackers	Every 4h	30	Boiling	Enhancing light
Mysis medium, larval shrimp	Increase investment of larva of *Artemia*	Every 6h	30	High boiling	Increase brightness

the Chinese Academy of Fishery Sciences South China Sea Fisheries Research Institute (Guangzhou, China). The *Lactobacillus* preparation (1 × 108 CFU·mL^{-1}) was purchased from Xinhailisheng Biotechnology Co., Ltd., (Guangzhou, China) in liquid form.

The nauplii of *L. vannamei* were cultured in indoor glass fiber buckets (300 L), each containing 200,000 larvae. The nauplii were divided into seven groups, including a control group (C), three *Bacillus* culture groups (Y1, Y2, Y3), and three *Lactobacillus* culture groups (R1, R2, R3). The experiments were carried out for 21 d (from 28 August to 18 September 2016). Table I shows the breeding management methods during the seeding process. *Bacillus* were added to Y1 (10 ppm), Y2 (15 ppm) and Y3 (20 ppm) on days 0, 1, 6, 11 and 16. *Lactobacillus* bacteria were added to R1 (20 ppm), R2 (25 ppm) and R3 (30 ppm) on the same days. No probiotics were added to the C group. Seawater (temperature from 27.9°C to 31.1°C, salinity from 30.0 PSU to 32.0 PSU, pH from 7.5 to 8.0) was unchanged during the experiment, with continuous aeration for 24 h and dissolved oxygen maintained above 5.0 mg·L^{-1} (Table I).

After the nauplii metamorphized to mysis stage, water samples (1 L) were collected from the upper, center and bottom of the barrels. After further metamorphosis, those mysis that turned into larval shrimp were collect. At the end of the 21-d experiment, all larvae in each barrel were weighed (W_1, corrected to 0.001g), after which 100 shrimp seeds were randomly collected from each barrel and again weighed (W_2). The average weight of the individual shrimp (W) and the survival rate of the larvae (SR) were then determined using the following formulae:

$$W = W_2 / 100$$

$$SR = [(W_1 / W) / 20000] \times 100\%$$

Resistance test of shrimp seeds

Fresh water survival measurement: 100 shrimp seeds were used for each group and transferred to fresh water, with the number of live shrimp seeds after 30 min and 60 min then counted. Survival rate after temperature change: 100 shrimp seeds were used for each group and were transferred to water with a temperature of 5°C and the same salinity; after 2 min the shrimp were removed and transferred into the original culture barrels. The number of shrimp were then counted after 15 min.

Statistical analyses

The data were analyzed using SPSS 17.0 for Windows software. One-way ANOVA was used to compare the differences between periods in the same group and *t*-tests were used to determine the differences between groups in the same period. Percentage data were analyzed by square root sine transformation, with the results shown as per the original data. We considered $P < 0.05$ to represent significant differences among the data.

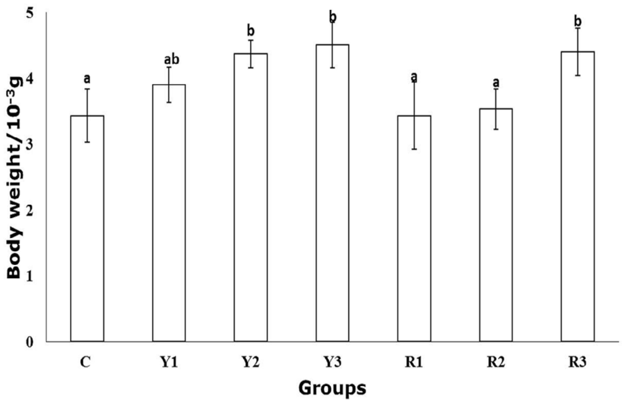

Fig. 1. Body weight of *L. vannamei* post larvae in different groups. The different letters are significantly different from each other (P<0.05), the same letters are not significantly different from each other (P>0.05); the same case in the following figures.

RESULTS

Effect of different microecological preparations on seed quality

After 21 d of experimentation, the *L. vannamei* seed weights (Fig. 1) of the control group and experimental groups with different microecological preparations demonstrated obvious differences. From a numerical point of view, group C exhibited the lowest body weights and group Y2 exhibited the highest. There were no significant differences between group C and groups Y1, R1 and R2 ($P > 0.05$) and no significant differences between Y2, Y3 and R3 ($P > 0.05$). The body weights of the Y2 larvae were 27.18%, 11.97%, 27.18% and 23.58% higher than those in groups C, Y1, R1 and R2 ($P < 0.05$), respectively. The body weights of the shrimp in Y1 showed no significant differences with the other groups ($P > 0.05$).

Effect of different probiotics on L. vannamei *survival at all metamorphic stages*

After the *L. vannamei* shrimp seeds metamorphosed to the mysis stage, the survival rate of all groups ranged from 70%–80% and exhibited no significant differences. However, after the shrimp seeds transformed to the larval stage, the survival rates of the experimental groups were markedly higher than that of the control group ($P > 0.05$). The survival rate of the shrimp larvae in R3 was 10.45% and 5.21% higher than that of the C and Y1 groups, respectively ($P < 0.05$), although the survival rates of larvae in groups Y1, Y2, Y3, R1 and R2 were not significantly different ($P > 0.05$). At the twenty-first day of the experiment, R3 demonstrated the highest survival rate and, except for R2, was significantly higher than that in groups C, Y1, Y2, Y3 and R1 (34.86%, 21.49%, 17.60%, 15.75% and 15.75%, respectively; $P < 0.05$). However, the survival rate was not significantly different among groups C, Y1, Y2, Y3 and R1 ($P > 0.05$).

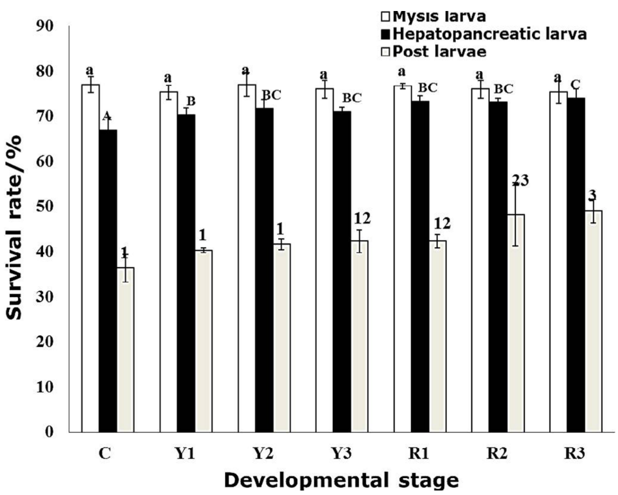

Fig. 2. The survival rate of *L. vannamei* in each stage of metamorphosis in different groups. The numbers are compared among the same series.

Effect of different probiotics on stress resistance of L. vannamei *seeds*

After the shrimp seeds were placed in fresh water for 30 min, the survival rate for all groups was 100% (Fig. 3) and there were no significant differences ($P > 0.05$). After 60 min, however, the survival rates of the experimental groups were significantly higher than that of the control group ($P < 0.05$), and were 14.05%, 16.53%, 14.05% 13.22%, 14.05% and 14.88% higher in Y1, Y2, Y3, R1, R2 and R3, respectively. There were no significant differences among the experimental groups ($P > 0.05$). When the temperature changed abruptly (Fig. 4), the survival rates of groups Y1, Y2, Y3, R1, R2 and R3 were 9.29%, 9.84%, 10.38%, 8.74%, 9.29% and 10.38% higher, respectively, than that of the control group ($P < 0.05$), though there were no significant differences among the experimental groups ($P > 0.05$).

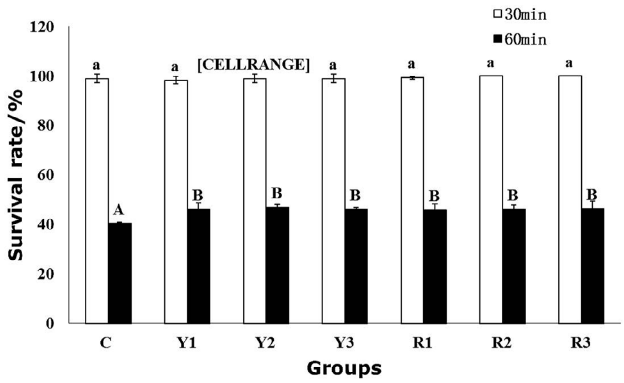

Fig. 3. The survival rate of post larvae in fresh water experiment.

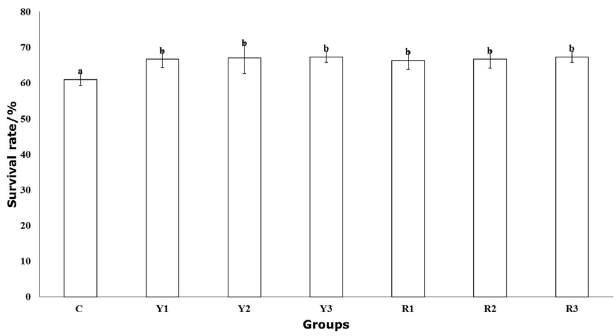

Fig. 4. The survival rate of post larvae in temperature mutations.

DISCUSSION

Effect of microecological preparations on growth performance of shrimp seeds

Microecological preparations have been applied within the Chinese aquaculture industry since the 1990s (Jiang *et al*., 2014). Microecological preparations are mainly comprised of either species that directly act on the breeding animals to promote growth and improve digestion functions and the utilization of feed, or species that act on the water medium to improve the environment to promote survival, ecological breeding and healthy farming (Zeng *et al*., 2007). Studies have indicated that *Bacillus* can improve the activity of digestive enzymes in aquatic animals, increase the digestibility of animal feed and improve the conversion rate of nutrients (Amaya *et al*., 2007). *Lactobacillus* bacteria can reduce the content of ammonia nitrogen in water, decompose detritus and organic matter in bottom water and effectively control water quality (Li *et al*., 2001; Jiang *et al*., 2014). Our experimental results showed that by regularly adding *Bacillus* and *Lactobacillus* to the aquaculture water, the weights of the *L. vannamei* seeds improved, albeit to different degrees, in accordance with previous research on Chinese white shrimp (*Fenneropenaeus chinensis*; Wu *et al*., 2006). The body weights of groups Y2, Y3 and R3 were significantly higher than the weights of groups C, R1 and R2. *Bacillus* can break down organic material and improve rearing water (Yang *et al*., 2001). In this experiment, floc-like material was observed in the *Bacillus* treatment groups. As larvae fed on these *Bacillus*, adding the correct amount significantly improved the body mass of emerging seeds. *Lactobacillus* bacteria regulate water quality, whereby they reproduce to occupy niches and inhibit the growth of harmful bacteria (Samocha *et al*., 2004). The R1 and R2 experimental groups, with relatively small amounts of *Lactobacillus*, did not display significant catalytic roles in improving the body mass of the emerging shrimp seeds. Therefore, we next isolated and identified the bacterial species in the water of the *Lactobacillus* experiment group to determine the mechanism of *Lactobacillus* bacteria for improving the aquaculture environment; in addition, the microecological preparations were mixed and used in the nursery process to analyze and evaluate their effects.

Different probiotics affected the survival rate of all metamorphic stages of *L. vannamei*. After the shrimp underwent metamorphosis from zoea stage to mysis stage larvae, there were no significant differences between the experimental and control groups in the metamorphosis rate, consistent with previous research (Yang *et al*., 2011). This was likely due to the relatively good water quality and suitable bait during the early period of the experiment, which allowed healthy shrimp seeds to metamorphose to the mysis stage. After the mysis stage larvae metamorphosed into juvenile shrimp, there was a certain degree of deterioration in water quality due to the accumulation and decomposition of high-protein residual bait and feces. As probiotics can adjust water quality to a certain extent, the metamorphosis rate of juvenile shrimp in the experimental group was significantly higher than that of the control group, with R3 demonstrating the highest rate and Y1 (with the smallest amount of *Bacillus*) demonstrating an obviously lower rate. This was possibly due to the *Lactobacillus* bacteria possessing better regulative ability compared with the *Bacillus* bacteria, or that the propagation speed of *Lactobacillus* bacteria was faster than that of *Bacillus* in the nursery waters. Thus, we next measured seeding water quality indicators and total number of different bacteria.

Effect of microecological preparations on the resistance of L. vannamei *seeds*

Stress resistance, also referred to anti-stress, is an index of the ability of an animal to adapt to sudden changes in environment indicators. When faced with a rapid increase or decrease in temperature or salinity, unexpected bacterial infection outbreaks, or sudden changes in food availability, animals will experience stress. Stress resistance is not only related to genetic factors but also to an animal's health status (Yang *et al*., 2001; Peng *et al*., 2004). Water temperature and salinity changes are common stress factors in shrimp breeding (Yang *et al*., 2001). Therefore, we studied stress resistance in the shrimp seeds based on these factors. Results showed that almost no shrimp died under low stress conditions (salinity of 0 in fresh water for 30 min). These findings indicate that *L. vannamei* shrimp seeds have a broad ecological amplitude, and can withstand and survive salinity changes (from normal salinity to a salinity of 0) for at least 30 min. However, with the extension of time in fresh water, considerable differences were observed among the control and experimental groups. Specifically, the survival rate of larvae in the control group was significantly lower than that in the experimental groups ($P < 0.05$), though there were no significant differences among the various treatment groups ($P > 0.05$). The temperature experiments also obtained the same results. Our study further showed that in the *L. vannamei* breeding process, only a small amount of probiotics was required to significantly improve the resistance of shrimp. Here, the *Bacillus* promoted the digestive function of shrimp as well as the protease activity of the seeds, whereas the *Lactobacillus* improved the water environment of the nursery. Thus, the two probiotics improved and facilitated the physical fitness of the shrimp seeds in different ways. Based on our research results, probiotics should be added during the breeding process of *L. vannamei* to enhance the resilience of shrimp seeds and reduce their mortality rate during transportation or after seeding into aquaculture ponds.

Considering our experimental findings in regard to actual production, the addition of microecological preparations during the breeding stage can increase the survival rate and individual weight of *L. vannamei* seeds, and also enhance the constitution and stress resistance of shrimp seeds. Furthermore, the amount of *Bacillus* should be greater than that of *Lactobacillus* (calculated by living bacteria). The spraying cycle and amount of microecological preparations are closely related to factors such as seeding pool load and water quality. Therefore, application of microecological preparations should be flexibly adjusted according to the effective microbial content, physiological activity and ecological characteristics of the various preparations to achieve the best results.

ACKNOWLEDGEMENTS

This research was supported by the Special Scientific Research Pilot Project of Sanya (2014KS03).

Statement of conflict of interest

The authors declare that there is no conflict of interests.

REFERENCES

Amaya, E., Davis, D. and Rouse, D., 2007. Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **262**: 419-425. https://doi.org/10.1016/j.aquaculture.2006.11.001

Argue, J., Arce, M. and Lotz, M., 2002. Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome Virus. *Aquaculture*, **204**: 447-460. https://doi.org/10.1016/S0044-8486(01)00830-4

Gong, H., Jiang, D.H. and Frank, A., 2012. Effects of dietary protein level and source on the growth and survival of two genetic lines of specific-pathogen-free Pacific white shrimp, *Penaeus vannamei*. *Aquaculture*, **338-341**: 118-123. https://doi.org/10.1016/j.aquaculture.2012.01.030

Hu, X.J., Li, Z.J. and Cao, Y.C., 2012. Dynamic characteristic of bacterial community in *Litopenaeus vannamei* culture ponds under the condition of strong weather disturbances in the Western Guangdong Province. *South. Aquat. Sci*., **8**: 52-59.

Jiang, S., Fan, S.G., Wen, W.G. and Yu, D.H., 2014 mpacts of microecological agent on growth, activity of digestive enzymes and water quality of *Holothurian scabra*. *Mar. Fish*., **36**: 335-341.

Jiang, S., Wang, J.H. and Fan, S.G., 2014. Effects research of adding photosynthetic bacteria on the *holothuria* seedling stages. *South. Aquat. Sci*., **11**: 78-82.

Li, X.Y., Dong, Z.G. and Yan, B.L., 2007. Effects of Compound probiotics on pond water quality and growth performance of Chinese shrimp. *China Feed*, **19**: 27-29.

Moss, M., Arce, M. and Argue, J., 2001. Greening of the blue revolution: Efforts toward environmentally responsible shrimp culture. *World Aquacul. Soc*., **25**: 1-19.

Moss, R., Arce, A. and Otoshi, A., 2007. Effects of inbreeding on survival and growth of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, **27**: 30-37. https://doi.org/10.1016/j.aquaculture.2007.08.014

Moss, R., Arce, A. and Otoshi, A., 2011. Shrimp breeding for resistance to Taura syndrome virus. *Glob. Aquacul. Advocate*, **36**: 40-41.

Peng, Z.R., Zang, W.L. and Gao, Y., 2004. Toxic effects of ammonia nitrogen and nitrite on *Penaeus vannamei* juvenile. *J. Shanghai Fish. Univ*., **13**: 274-278.

Qiu, D.Q. and Yang, S.P., 2005. Changes in content of organic matter in shrimp high-density culture water. *Aquat. Sci*., **10**: 12-14.

Samocha, T., Davis, A. and Saoud, P., 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, **231**: 197-203.

Shen, W.Y., Hu, H.G. and Pan, Y.J., 2004. Effects of temperature and pH value on digestive enzyme activity of white leg shrimp *Penaeus vannamei*. *Oceans and Lakes*, **35**: 543-548.

Wu, G., Zhang, C.F. and Lu, J., 2006. Application of photosynthetic bacteria in prawn. *China Fish*., **10**: 74-77.

Yang, Z.W. and Li, S.W., 2001. Tolerance test of low salinity environment for *Penaeus vannamei* lavae. *Ocean Sci*., **25**: 12-13.

Yang, Z.W., Lu, X.N., Zheng, Y.Y. and Le, Z.L., 2011. Effects of temperature on the growth, metamorphosis and survival rate of larvae of *Penaeus vannamei*. *Taiwan Strait*, **30**: 81-85.

Yao, X.M., Huang, B. and Zhang, J.T., 2007. Comparative study on the growth and survival of SPF shrimp *Litopenaeus vannamei* F1, F2 and hybrid generation. *China Fish. Sci*., **14**: 326-330.

Zeng, D.G., Lei, A.Y. and Ma, N., 2007. Test of immobilization *Bacillus subtilis* in purifying water culture. *Agricul. Sci*., **36**: 685-687.