Effects of photosynthetic bacteria on nonspecific immunity and water quality of Cherax quadricarinatus

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Abstract: In order to clarify the effects of photosynthetic bacteria on water quality improvement and non-specific immunity in Australian freshwater crayfish culture environment. Seedlings with an average body weight of 0.265 g were placed in a 50 L sink and various concentrations of Rhodop seudanonas palustris were applied. Ammonia nitrogen, nitrite nitrogen and pH were measured once every 5 days, and lysozyme and acid phosphatase were determined at 25 days. The results showed that the concentration of photosynthetic bacteria was 4×104cfu/ml, which could effectively control the increase of ammonia nitrogen and nitrite nitrogen, stabilize the pH value, increase the activity of lysozyme and acid phosphatase, and increase the survival rate and increase of seed. The concentration of photosynthetic bacteria was 4×104cfu/ml. The ammonia nitrogen and nitrite increased to different degrees, the pH decreased, the activity of lysozyme and acid phosphatase increased significantly, and the survival rate and weight gain rate of seedlings decreased significantly. The results showed that the photosynthetic bacteria concentration of 102-103 cfu / ml can effectively improve the water quality of breeding, improve the immunity and survival rate of shrimp, and the concentration may be inhibited if the concentration is too high.

1. Introduction

Australian freshwater lobster, belonging to the arthropod, crustacea, decapod, crayfish, serrata, and the species Cherax quadricarinatus. It is one of the most valuable freshwater economic shrimp species in the world. Individuals generally weigh 100 to 250 grams. They are native to the tropical regions of northern Australia. They are called Australian freshwater lobsters because they are as strong as lobsters in the sea [1].Australian freshwater lobster is rich in protein, low in fat, low in cholesterol, and contains some minerals and vitamins and essential calcium and various amino acids [2].Long-term consumption of Australian freshwater lobster can improve human immunity and is a good source of protein. The global consumption of crayfish is very large. China only consumes 1.2 million tons in 2018, with an output value of 350 billion yuan. Australian freshwater lobsters are much larger than individual crayfish. In 2018, the output reached 3,000 tons, which is gradually favored by the market.

Water quality is the key to the success of farming. Environmentally-friendly microorganisms such as photosynthetic bacteria have good removal effects on harmful substances such as ammonia and nitrogen in water [3], and are widely used in aquaculture [4].After the photosynthetic bacteria are added to the aquaculture water, the water quality index changes, and the change of the immune-related enzymes of the Australian freshwater lobster will not be reported. Therefore, this study provides a basic theoretical reference for the improvement of aquaculture technology by studying the addition of photosynthetic bacteria in aquaculture water to determine the change of water quality index and the activity of immune-related enzymes in Australian freshwater lobster seedlings.

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2. Materials and Methods

2.1 Experimental Materials

The Australian freshwater lobster was provided for the Zhejiang Freshwater Fisheries Research Institute. It was transported back to the laboratory on August 15, 2018, and stocked in an aquarium of 100 cm × 60 cm × 50 cm. It was kept for 10 days, and the young shrimp feed of Macrobrachium rosenbergii was fed every day. Select healthy and energetic shrimps for experimentation.

The photosynthetic bacterial strain was purchased from Rhodes seudanonas palustris in Nanjing Haishusi Biotechnology Co., Ltd. After the purchase, the cells were inoculated with photosynthetic bacteria optimized medium [5], placed in a constant temperature light box, and cultured at 30 ° C for 7 days, and then used for the experiment, and the concentration of the bacteria was 1.0×109 cfu/ml.

The experimental water was tap water aerated for 7 days, pH 7.6, NH4+<0.05 mg/L, NO2-<0.05 mg/L, and the hardness was 132 mg/L in terms of CaCO3.

2.2 Experimental Methods

A total of 720 shrimps (body weight 0.265 g ± 0.043 g) with similar body lengths were randomly selected and randomly placed in a square non-toxic plastic water tank containing 50 L of aerated 7-day tap water. There are 40 shrimps per sink, a total of 6 groups, 3 for each repetition.200 g of coral stone is placed in each sink, and 20 grams of Elodea is planted in coral stone so that juveniles can hide. Five photosynthetic bacteria concentration groups were set in the experiment, and the non-injection group was used as the control group B. The concentration of the bacteria in the water was 4.0×102, 4.0×103, 4.0×104, 4.0×105, and 4.0×106 cfu/ml, respectively.(numbered as group A1-5), 10% water is discharged every 5 days, and the same concentration of fresh water is added. Feeding 0.1 g of peanut swarm powder daily (peanut meal 7.4%, soybean meal 20%, soybean phospholipid 4%, yeast powder 4%, fish meal 23%, shrimp powder 12.5%, flour 6%, sub-powder 15%, vegetable oil) 1.5%, calcium lactate 0.5%, calcium dihydrogen phosphate 2.6%, premix 3.5%), fed at 8:00 (20%) and 18:00 (80%).The experimental time was 25 days, and pH, ammonia nitrogen, and nitrite nitrogen were measured every 5 days, and the death was recorded every day. At the end of the experiment, the body weight and body length of each group of shrimps were measured, and the enzyme activities in the muscle tissues were measured.

2.3 Water Quality Measurement Method

The pH is determined by the glass electrode method, the ammonia nitrogen is the Nash reagent method, and the nitrite nitrogen is the diazo-azo method [6].

2.4 Determination of Enzyme Activity in Tissues

At the end of the experiment on day 25, the quality of the shrimps was weighed using an analytical balance (sartoriusf, 0.0001 g), the body length was measured with a vernier caliper, the shrimp was dissected on an ice pack, and all muscles were picked. The shrimp seedlings were placed in a homogenizer after sterilization, and 9 times by weight of physiological saline was added, and the mixture was ground in an ice bath at 2,500 rpm, centrifuged at 2 ° C for 10 minutes, and the supernatant was taken for testing. The activity of acid phosphatase (ACP) and lysozyme (LZM) in muscle tissue was determined by enzyme kit of Nanjing Jiancheng Bioengineering Research Institute.

2.5 Determination of Growth Indicators

The weight was weighed with an analytical balance at the beginning and at the end of the experiment, and the vernier caliper was used to measure the body length.

Weight gain rate (%) = (mm0) / m0 × 100%

Growth rate (%) = (ll0) / l0 × 100%

Survival rate (%) = (xx0) / x0 × 100%

m is the mass at the end of the test, m0 is the initial body mass; L is the body length at the end of the test, L0 is the initial body length; x is the number at the end of the test, and x0 is the initial amount.

2.6 Data Statistics and Analysis

All data were sorted by Excel2016 and averaged. The data were analyzed by SPSS19.0 statistical software. ANOVA and Duncan multiple comparisons were used to compare the significance of the mean between the groups. $p<0.05$ was a significant difference, and $p>0.05$ was no significant difference.

3. Results and Analysis

2.1 Water Quality Changes

The water quality changes of each group are as follows:

Tab 1. Changes of water quality indexes in each group with using photosynthetic bacteria

Group	pH		Ammonia Nitrogen(mg/l)		Nitrite Nitrogen(mg/l)	
	Original	Terminal	Original	Terminal	Original	Terminal
B	8.35	8.59±0.06d	0.040	0.608±0.026d	0.006	0.063±0.021d
A1	8.35	8.47±0.05cd	0.040	0.466±0.073c	0.006	0.044±0.016cd
A2	8.35	8.36±0.04bc	0.040	0.246±0.034a	0.006	0.025±0.009ab
A3	8.35	8.32±0.05ab	0.040	0.308±0.049ab	0.006	0.013±0.004a
A4	8.35	8.31±0.03ab	0.040	0.348±0.081bc	0.006	0.034±0.007bc
A5	8.35	8.19±0.07a	0.040	1.278±0.137e	0.006	0.061±0.019d

2.1.1 PH Value

After adding photosynthetic bacteria to the culture water, the pH value of the control group and the A1, A2, and A3 groups increased on the 20th day compared with the initial value. The pH and initial values of the A4 and A5 groups were not significantly different before the 20th day, and were lower than the initial value on the 25th day, which was significantly lower than the control group ($p<0.05$).It indicates that the use of photosynthetic bacteria does not have a large impact on the environmental pH, and the high concentration uses the effect of stabilizing the pH of the water.

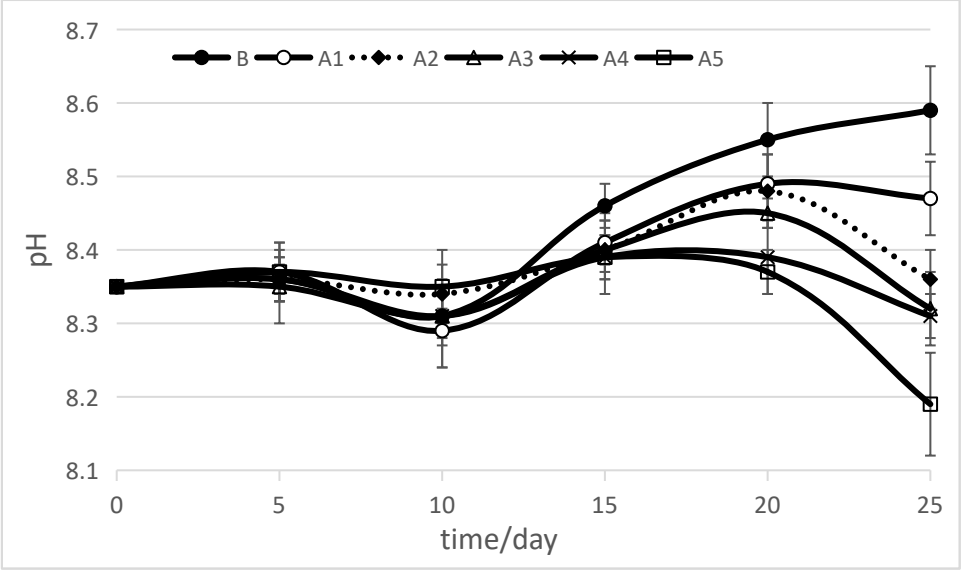

Fig .1 Changes of pH in each group with using photosynthetic bacteria

2.1.2 Ammonia Nitrogen

The change of ammonia nitrogen in the cultured water body after photosynthetic bacteria is

shown in Figure 2.In the 5th day of the experiment, the ammonia nitrogen concentration of each group gradually increased, which was significantly higher than the initial value ($p<0.05$).In the control group, ammonia nitrogen gradually increased before the 10th day, and gradually decreased on the 15th-25th day, with an average concentration of 0.83 mg/l.The ammonia nitrogen concentrations in the A1 and A2 groups did not change much from day 10 to 25, with mean values of 0.41 mg/l and 0.24 mg/l, respectively, which were significantly lower than the control group ($p<0.05$).Ammonia nitrogen was significantly higher in the A3 and A4 groups than in the first 10 days ($p<0.05$), and the mean values at 15-25 days were 0.23 mg/l and 0.30 mg/l, respectively, which were significantly lower than the control group ($p<0.05$).The ammonia nitrogen in the whole culture process of A5 group was significantly higher than other groups ($p<0.05$).The results show that the use of photosynthetic bacteria can effectively control the increase of ammonia nitrogen. However, when the concentration is higher than 4.0×104 cfu/ml, a short-term increase occurs, and when the concentration exceeds 4.0×105 cfu/ml, the opposite effect is obtained.

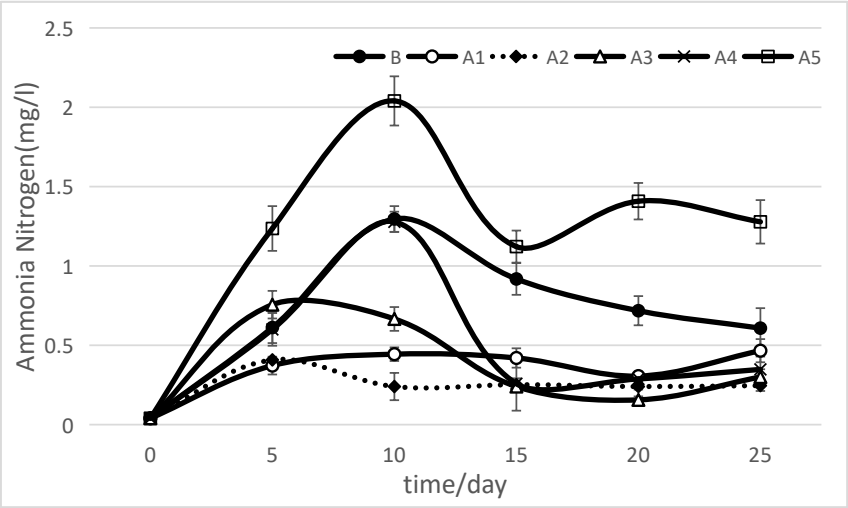

Fig .2 Changes of ammonia nitrogen in each group with using photosynthetic bacteria

2.1.3 Nitrite Nitrogen

The change of nitrite nitrogen after the release of photosynthetic bacteria in aquaculture water is shown in Fig. 3.The nitrite nitrogen of all groups increased in the 5th day, which was significantly higher than the initial value ($p<0.05$).On the 10th day, the concentration of the A1-A3 group decreased significantly, which was significantly lower than that of A4, A5 and the control group ($p<0.05$).On the 10th to 25th day, the average concentration of nitrite in the A1-A3 group was 0.034 mg/l, 0.023 mg/l, and 0.013 mg/l. results showed that the use of photosynthetic bacteria began to exert a water quality improvement effect after 5 days, but when the concentration exceeds 4.0×104 cfu/ml, the nitrite nitrogen concentration is increased.

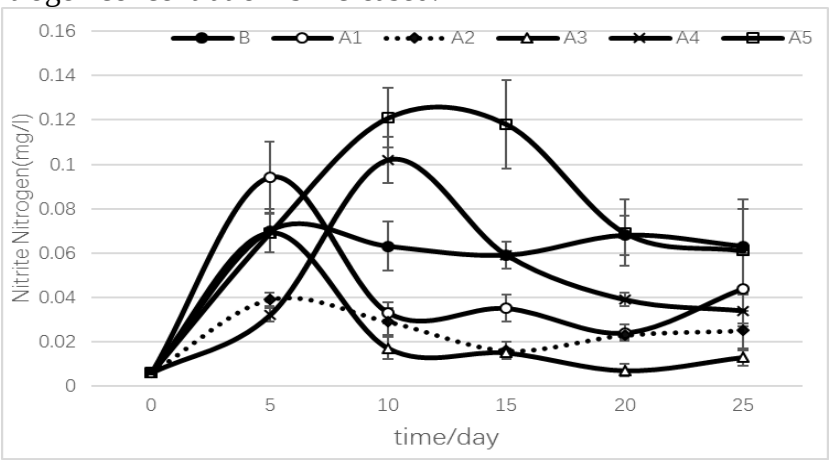

Fig .3 Changes of nitrite nitrogen in each group with using photosynthetic bacteria

2.2 ACP and Lysozyme Activity Changes

2.2.1 Changes in lysozyme activity

The changes in lysozyme activity of freshwater crayfish in Australia after 25 days of feeding of photosynthetic bacteria in aquaculture water are shown in Figure 4.The activity of lysozyme in A3-A5 group with photosynthetic bacteria concentration higher than 4.0×104 cfu/ml was significantly higher than that in control group and A1 and A2 groups ($p<0.05$).The activity of lysozyme in group A2 was significantly higher than that in control group. The results showed that the addition of photosynthetic bacteria caused an increase in the activity of Australian crayfish lysozyme.

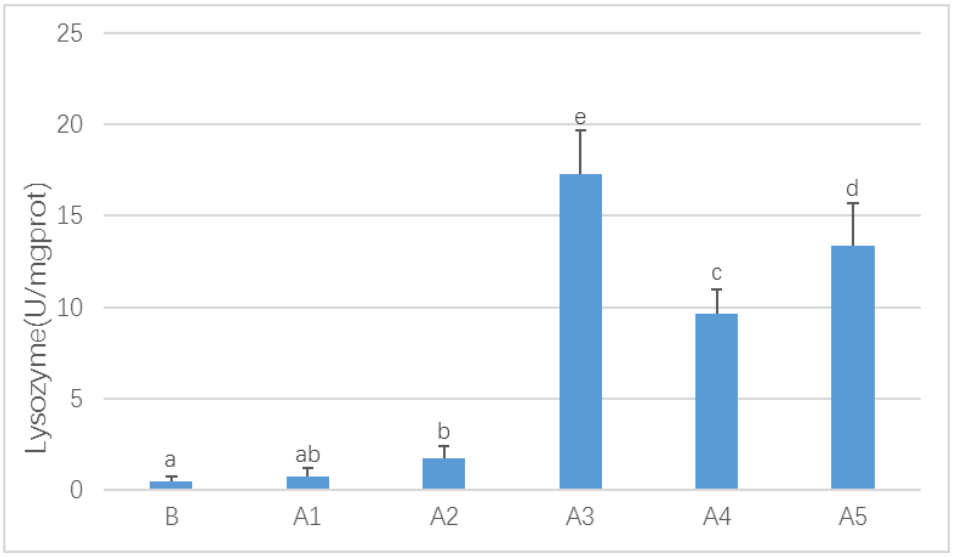

Fig .4 Activity of lysozyme in each group with using photosynthetic bacteria

2.2.2 Acid Phosphatase (ACP) Activity Changes

The change of acid phosphatase activity of freshwater crayfish in Australia after 25 days of feeding of photosynthetic bacteria in aquaculture water is shown in Fig. 5.The activity of lysozyme in the A3-A5 group with photosynthetic bacteria concentration higher than 4.0×104 cfu/ml was significantly higher than that in the control group and A1 and A2 groups ($p<0.05$).There was no significant difference between the A1 and A2 groups and the control group.

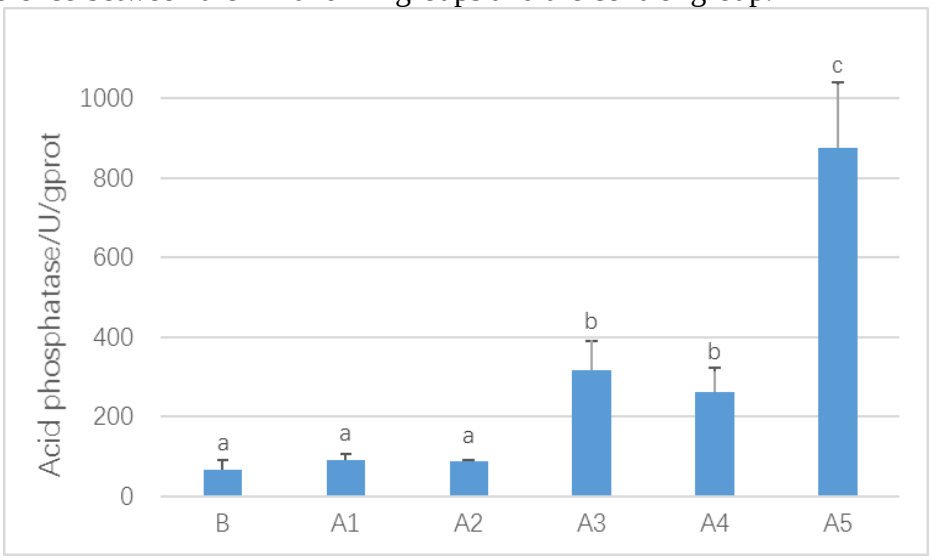

Fig .5 Activity of acid phosphatase in each group with using photosynthetic bacteria

2.3 Shrimp Growth

Within 25 days of the experiment, the quality of the Australian freshwater crayfish in the control

group was doubled .In the experimental group with photosynthetic bacteria, the survival rate decreased gradually with the increase of photosynthetic bacteria concentration, among which A1 group had the highest weight gain rate, and A1 and A2 groups were significantly higher than A3-A5 group ($p<0.05$).The composition of A2 had the highest survival rate, and gradually decreased after the concentration increased, which was significantly higher than that of A3-A5 group and control group ($p<0.05$).

Tab 2.Growth and survival of Cherax quadricarinatus larvae with using photosynthetic bacteria in 25 days

Group	Initial mass(g)	Final mass(g)	Weight gain rate(%)	Survival rate(%)
B	0.265 g±0.043	0.531±0.177b	100.34±36.94b	65.75±13.45b
A1	0.265 g±0.043	0.734±0.254c	176.95±51.86c	68.35±16.83bc
A2	0.265 g±0.043	0.627±0.303bc	136.55±43.33bc	88.24±21.18c
A3	0.265 g±0.043	0.305±0.179a	35.13±18.65a	54.38±15.31b
A4	0.265 g±0.043	0.376±0.095a	41.93±26.02a	32.16±11.65a
A5	0.265 g±0.043	0.325±0.082a	22.53±12.06a	18.69±10.95a

4. Discussion

Photosynthetic bacteria are one of the earliest types of photoautotrophic prokaryotes on the earth. It can survive in anaerobic and aerobic conditions and is widely distributed in all corners of the earth. Photosynthetic bacteria contain bacterial chlorophyll and carotenoids, and have a wide absorption spectrum. Under anaerobic conditions, water-based organic matter is used for non-oxygen photosynthesis [7].Therefore, the application of photosynthetic bacteria in fishery production can degrade harmful substances such as aquatic animal feces, residual baits, corpses, etc., and use ammonia nitrogen, nitrite nitrogen, hydrogen sulfide, etc. in the water as growth nutrients to purify water quality [3].Shi Junyan [8] and other photosynthetic bacteria were applied in the culture pond of Penaeus vannamei for 15 days. The total organic matter content at the bottom of the pond decreased by 37.5% on average, and the degradation amount reached 2 times of the control group; the sulfide content decreased by 27%, while the control pool increased by 25%. .Wang Yiya [9] reported that a photosynthetic bacterium isolated from it, the cumulative removal rate of ammonia nitrogen was as high as 94.42%, and the removal rate of nitrite nitrogen reached 76.44%, which effectively reduced the content of ammonia nitrogen and nitrite nitrogen and improved the water quality of culture. Xie Quansen et al reported that the pH of the water increased after application of photosynthetic bacteria in the pond of Taiwan Paramisgurnus dabryanus [4].The results of this experiment showed that the pH value of the photosynthetic bacteria was increased when it was below 4.0×104 cfu/ml, and it was gradually decreased when the concentration was higher than this concentration. Ammonia nitrogen and nitrite nitrogen in the low concentration group below 4.0×104 cfu/ml showed good removal effect, but the high concentration group was reversed. This may be because after the photosynthetic bacteria are put into the breeding environment, there is not enough nutrients to affect the growth and reproduction of the photosynthetic bacteria, and even the decomposition of death, but the ammonia nitrogen, nitrite and other indicators are increased. In particular, the Australian freshwater crayfish breeding environment, the water is relatively clear [10].Therefore, the amount of photosynthetic bacteria used should not be too high.

Zhang Ke et al. added a concentration of 104 cfu/mL of photosynthetic bacteria in tilapia culture water to detect a significant increase in serum ACP activity and lysozyme activity [11].Lao Huiyan et al. added photosynthetic bacteria to Ctenopharyngodon idellus diet for 30 days. Ctenopharyngodon idellus percentage of leukocyte phagocytosis, serum total superoxide dismutase (SOD) activity, serum and liver glutathione peroxidase (GSH-Px) The vitality is significantly higher than that of Ctenopharyngodon idellus [12], which indicates that the photosynthetic bacteria can improve the non-specific immune function of fish by oral administration and external application. The injection of Rhodobacter sphaeroides into the culture water of Caridina serrata by Liu Tao [13]

found that the immunity of Caridina serrata was significantly improved. In this experiment, photosynthetic bacteria were added to the culture water, and the activities of lysozyme and acid phosphatase increased with the increase of photosynthetic bacteria concentration. When the concentration is greater than 4.0×104 cfu/ml, the enzyme activity is particularly high, showing pathological state, and the survival rate and growth rate are not good at high concentration, and the removal effect of ammonia nitrogen and nitrite nitrogen is also poor. The experiment showed that the photosynthetic bacteria concentration of 102-103 cfu/ml is the most suitable for Australian freshwater crayfish. At this time, the activity of non-specific immune-related enzymes is increased, which improves the immunity of shrimp. The specific performance is the highest survival rate and growth rate, and the removal effect of ammonia nitrogen nitrite nitrogen is the best.

The results of this study indicate that the use of microecological preparations such as photosynthetic bacteria in Australian freshwater crayfish farming can improve the non-specific immunity of shrimps and improve the water quality of the water, but not as much as possible, preferably at 104 cfu/ Within the ml concentration.

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