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Effects of Ecological Base on Microbial Community Structure in Aquaculture Ponds

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Abstract

At present, the most modes of aquaculture in our country are high-density intensive culture. With the development of this model, pollution of the ecological environment of aquaculture is becoming more obvious gradually. As a result, more and more Chinese and foreign scholars have studied the pollution of aquaculture environment, and the means to deal with the pollution problem have become more and more abundant. The purpose of this study is to explore the impact of ecological base technology on the culture environment of aquaculture ponds. The main research object is ecological base, and the changes of microbial community structure in the water and ecological base in the culture environment are analyzed, so as to explore the mechanism of ecological base in the culture environment. The water quality index showed that ammonia nitrogen, nitrate nitrogen, nitrite nitrogen and phosphate were all in a stable state of decline during the whole experiment, and the contents were all within the safe concentration range. The microbial diversity in the experimental group was lower than that in the control group, indicating that the microorganisms in the experimental group would transfer and attach to the ecological base, and the unique structure of the ecological base provided an ideal habitat for microorganisms, thus increasing the diversity of microbial community structure. The results showed that the influence of ecological base on water quality index was not obvious, but on bacterial community.

Keywords

Breeding environment, Ecological restoration, Ecological base, Diversity

1. Introduction

At present, the global Marine ecological environment pollution intensifies, not only affects the global economic development, but also affects the ecological environment of the earth on which we rely for survival. It also brings bad news to the world's fisheries industry. According to the Ministry of Agriculture, Chinese aquaculture output accounted for 70% of the world [1]. The development of aquaculture industry cannot be separated from good water ecosystem resources, and the health of water quality is the core issue for aquaculture industry. The core concept of healthy and sustainable development of aquaculture industry is restricted whether the aquaculture environment can be protected and restored at the same time with the rapid development of aquaculture [2]. Therefore, the problem of water quality is very important. It is necessary and obligation for us to make due contribution to our living environment.

Microbe exists widely in nature and is the basic biological group of nature ecological balance. In aquaculture environment, microorganisms play a catalytic role in the degradation of pollutants by their own functions of absorption, metabolism and degradation of environmental pollutants. The principle of microbial (beneficial microbial) restoration is that beneficial microorganisms in water use nutrients and decompose and consume organic matter such as humus, and transform into harmless substances after a series of micro-ecological reactions [3]. These beneficial microorganisms include photosynthetic bacteria, bacillus and other microorganisms, which can decompose the sediment nutrient elements in the pond, and can affect the growth of algae in the pond, thus affecting the water quality and micro-ecological environment of the aquaculture pond. Relevant studies have shown that the use of microorganisms and algae as food to feed aquaculture has achieved good results [4]. Microorganisms directly or indirectly affect the objects and environment of aquaculture. They can decompose organic matter such as excrement, bait residues and phytoplankton residues of aquaculture organisms. In the process of purifying the water quality of aquaculture, they can transform organic matter into simple compounds through the effects of peroxidation, reduction, photosynthesis, assimilation and dissimilation, which plays a stabilizing role in the normal water quality of aquaculture. Thus, the balance among aquaculture organisms, pathogens and water quality in the aquaculture ecosystem can be better maintained [5].

2. Experiments and methods

2.1 Experimental Base

In this study, the microbial community structure of four breeding ponds was analyzed. The four ponds were divided into two groups: experiment 1 and control 1, and experiment 2 and control 2. All the breeding objects in the pond were white prawn.

2.2 Sample collection and index measurement

Water samples were taken from 250 mL glass bottles directly in the middle of the pond, with sampling intervals of 20 days. After the initial collection of water samples from the experimental group and the blank control group, the ecological base samples with a wet weight of 2 g were clipped from the central position of the pond and brought back to the laboratory with a sampling period of 20 days. The samples were placed in 250 mL sterilized glass bottles, and then sterilized deionized water was added to the 250 mL scale, and then the glass bottles were fixed in the shaking incubator. After being shaken at 270 r/min for 2.5 h, 100 mL water samples were taken out and vacuumized with 0.2 μm filter membrane. After each filtration, the filter membrane was cut with sterilized scissors and stored in a 50 mL sterilized centrifuge tube for DNA extraction. The rest of the treatment steps are the same as the water treatment method. The indicators of water are detected according to the national water quality standard (GB11607-89), including ammonia nitrogen, nitrate, nitrite and phosphate.

The extraction method of genomic DNA of water and ecologic-based microorganisms was extracted according to the specific procedure of OMEGA Water Bacterial DNA Extraction Kit. Finally, 100 mL sterilized deionized water was used to elute the DNA. The 1% DNA agarose gel electrophoresis (110 V, 230 mA, 25 min) was used to detect the fragment size and concentration. Store at –20℃ for later use.

The dominant degree can be calculated as follows: $d=N_{max}/N$, where N_{max} is the number of individuals of the largest species in the community, and N is the number of individuals. The formula for density calculation is: $N=C_s/(F_s \cdot F_n) \cdot V/v \cdot P_n$, where C_s is the area of the counting frame, F_s is the area of a field of vision, F_n is the number of counted fields, V is the volume of 1 L water sample after precipitation and concentration, v is the volume of the counting frame, P_n is the number of counted phytoplankton individuals in F_n fields.

2.3 Data Analysis

Excel data processing software was used for data processing, and single factor analysis of variance (ANOVA) in SPSS17.0 software was used to analyze the significant difference of the data. The experimental data were expressed as mean±SD, $P<0.05$ was considered as significant difference. BIO-RAD Quantity One 4.6.2 software was used to process DGGE (denaturing gradient gel electrophoresis) fingerprint information, similarity comparison and cluster analysis of bacterial community structure (UPGMA). The bacterial phylogenetic Tree was constructed using the Test Neighbor-Joining tree method in MEGA 5.0.

3. Results

3.1 Hydration index measurement and analysis

At the end of this experiment, through the water quality test results, there was no significant difference in the parameters in the whole experiment process ($P<0.05$), which was similar to the results of water quality parameters in the study on the influence of ecological base on water microorganisms [6], as shown in Table 1. But on the whole, the variation of parameters showed a steady trend during the experiment, indicating that the ecological base played a role in the stability of water environment.

The phosphate group was significantly lower than the control group at 40 days ($P<0.05$); There was no significant difference in the ammonia nitrogen groups during the whole experiment ($P<0.05$), but the concentration is high, which may be caused by the increase of nitrogen concentration caused by denitrification caused by the action of nitrate in microorganisms; Compared with the control group, nitrate was not significant at all time periods ($P<0.05$); Nitrite in the experimental group was significantly lower than that in the control group from 40 days to the end of the experiment ($P<0.05$).

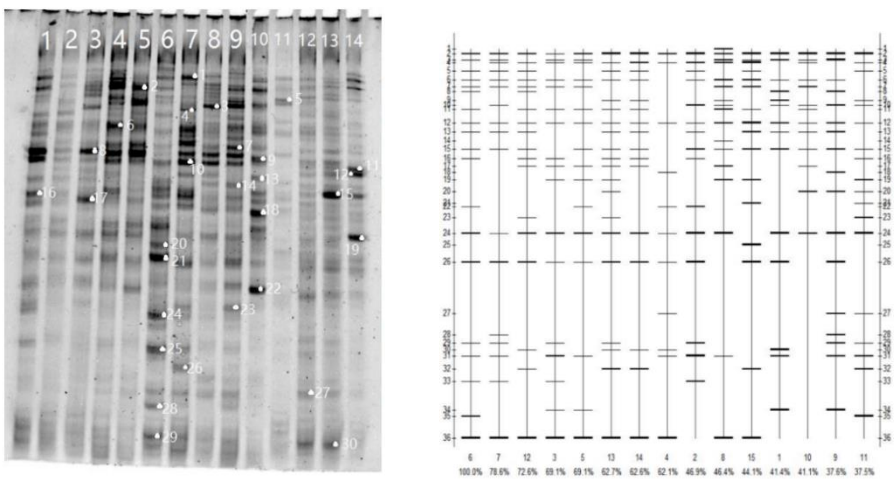
Table 1. Pond water quality index

Index	Control group	Experience group
TP	0.49±0.07a (0.41,0.59)	0.34±0.13a (0.22,0.54)
NH_3-N	0.41±0.06a (0.32,0.47)	0.37±0.05a (0.31,0.44)
NO_3-	0.08±0.02a (0.05,0.08)	0.06±0.11a (0.05,0.07)
NO_2-	0.06±0.01a (0.07,0.09)	0.05±0.01a (0.06,0.07)

Note: the letters in the same column in the table are marked as significant differences ($P < 0.05$). The data in brackets below are the minimum and maximum values of the data obtained in the experiment.

3.2 Analysis of 16SrDNA DGGE results of bacteria in water

The result bands of DGGE of bacterial 16SrDNA in cultured water are shown in Figure 1. There are 36 bands obtained this time: Band 2, 6, 8, 16 and 17 were the bands belonging to the experimental group. Band 1, 3, 4, 7, 9, 10, 13, 14, 18, 20, 21, 22, 23, 24, 25, 26, 28, 29 were 18 bands of the control group. Band 5, 11, 12, 15, 19, 27, and 30 are the bands of the ecological base. Among them, the stripe of all lanes is 1, 2, 6, 7, 8, 19, 21, 25 and 27. As can be seen from Figure 1b, the similarity of DGGE maps in the experimental group changed little over time, while that in the control group changed significantly, which reflected that the ecological base played a role in the stability of microbial community structure in water. After 40 days, bacterial abundance in each group showed a downward trend, which may be related to the changes of physical and chemical indexes in water. The overall bacterial diversity showed a fluctuating state diagram 1b. Although the bacterial abundance on the ecological base also decreased, it was significantly higher than that in the experimental group and the control group. Because the ecological base can develop the microbial community in the aquatic ecosystem, and can absorb the survival elements needed by planktic algae, the microbial community with higher abundance than the water body is formed.



(a) (b)

Figure 1. Experimental group (1-5), control group (6-10) and 11-14 (denaturing gradient gel electrophoresis (DGGE)) (a) and schematic diagram (b).

4. Discussion

4.1 Changes of hydration index

Ecological base can enrich, transfer and remove nutrient elements such as heavy metal ions, nitrogen and phosphorus in water, so as to effectively reduce the outbreak of water and bloom and increase the transparency of water. It does not release harmful substances and can be used for a long time [7]. In the whole process of this experiment, the overall change of hydration index in the experimental group was relatively stable, and the contents of nitrate, nitrite, ammonia nitrogen and phosphate in the water were reduced to a certain extent, indicating that the ecological base had a stabilizing and purifying effect on the water quality of aquaculture ponds. The surface of the ecological substrate has micropore structure, which provides a favorable environment for algae and microorganisms to adhere and survive, and forms a unique biofilm structure on the ecological substrate. The design shape of the ecological base can smooth the water flow in the pond, provide a good water environment for microorganisms (bacteria, beneficial algae, etc.) to attach to the ecological base, and increase the effective contact area between the ecological base and the water body. Biofilms are attached to and grow nitrifying bacteria and nitrifying bacteria, and the growth of bacteria requires nitrogen and phosphorus to provide nutrients for them. Therefore, these bacteria can effectively utilize various forms of nitrogen-containing substances in water to degrade nitrogen content in water. The kinetic energy of the suspended solids in water decreases rapidly in the process of collision with the biofilm, which promotes its full settlement. At the same time, due to the bioflocculation on the surface of the suspended biofilm, substances in the water can condenses on the surface of the ecological base, and after a period of microbial action, they fall off and degrade and are deposited at the bottom of the pond.

4.2 Analysis of bacterial community diversity in water

The PCR products of the samples were isolated by DGGE with different bands in number, strength and migration location (Figure 1), and these bands at different locations corresponded to different bacterial species. The more bands in electrophoresis are, the higher the diversity of bacterial species in the samples is. The higher the concentration of the strip is, the higher the number of bacteria represented by the strip is, which belongs to the dominant population under the current conditions.

In this experiment, there are 30 bands could be identified, among which Proteobacteria accounted for the main category, Bacteroidetes followed, Fibrinbacteria, green flexbacteria and actinomyces were the third category, and the last category was unknown. Proteobacteria have strong adaptability in nature, so they often appear. In the whole process of this experiment, Proteobacteria were the dominant bacteria. Proteobacteria are important bacteria participating in the nitrogen cycle in the water environment. Their presence and abundance are affected by nutrient salts such as ammonia nitrogen in the water environment. Nitrosomonas and Nitrosospira from B-Proteobacteria are the main promoters of ammooxidation and nitrite oxidation in the aquatic environment of shrimp ponds. In the important link of denitrification, Azoarcus and other bacteria groups play a driving role. Actinomyces can degrade organic compounds and participate in other cycles in the water environment like Proteobacteria, thus producing a variety of chemicals that can benefit the water environment and effectively purify water quality. Actinomyces are famous antibiotic producing bacteria. The enzymes produced by actinomyces have a wide range of functions in sewage treatment. It also plays a role in the nitrogen cycle in nature. Compared with common bacteria and fungi, actinomycetes grow slowly, usually in the late stage of decomposition of organic matter can accelerate growth and reproduction, thus speeding up the formation of humus and promoting the decomposition of organic matter. Bacteroides are dominant bacteria in water purification or water treatment and play a very important role in the microtreatment of substances in water environment. Whether they appear in the process of water treatment and whether they can be an advantage has an important impact on the final effect.

5. Conclusion

Ecologic-based technology has been involved in many fields at home and abroad, such as industrial wastewater treatment, domestic sewage treatment, lake water environment restoration, aquaculture wastewater treatment, aquaculture environment restoration, and has achieved good results. In our country, the ecological base technology is mostly used in the south, this paper applied the ecological base technology in the north of the breeding pond environment, and studied its effect, and obtained some results. In this experiment, the PCR-DGGE technology was used to study the impact of ecological base technology on the culture environment in the aquaculture pond. The results showed that the ecological base could enrich microorganisms and beneficial algae in the water, form biofilms to develop microbial communities, and microorganisms could absorb and degrade nutrients and pollutants in the water through their own functions. It can stabilize the physical and chemical indexes of aquaculture water, reduce the content of nitrogen and phosphorus in water, indirectly affect the change of microbial community structure in water, affect the distribution of bacteria and make them transfer and attach to the ecological base, and play a role in purifying water quality.

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