Dynamic Change of Microbiota in Culture Pond Based on High-throughput Sequencing Technique

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Abstract

In recent years, aquaculture is one of the highly developed major industries in China. The annual supply of fish food through aquaculture occupies the main consumption of Chinese people’s fish food. The traditional aquaculture technology is subject to technical level and other subjective or objective factors, resulting in low-quality and expensive fish product, which greatly restricts the development of aquaculture industry. The culture pond technology, an emerging aquaculture technology in recent years, can effectively filter out microbiota that are harmful to fish in the water and further validly improve the quality of aquaculture, which is of great significance to promote the development of this industry. For this purpose, based on the high-throughput sequencing technology, this paper studies the dynamic changes of microbiota in the culture pond. The conclusion is as follows: the richness of microbiota in No.3 recirculating aquaculture system is significantly higher than that of the other two recirculating aquaculture systems, and there is a high degree of similarity between No.1 recirculating aquaculture system and No.2 recirculating aquaculture system. As far as bacteria are concerned, the main nitrifying bacteria are nitrosomonas and nitrospira. This study has provided a reference for the application of the recirculating aquaculture system in the aquaculture industry.

Keywords: High-throughput sequencing, Culture pond, Microbiota.

1. RESEARCH OVERVIEW

1.1 Research background

1.1.1 High-throughput sequencing technology

High-throughput sequencing technology, also known as a new generation of sequencing technology, is a new technology capable of determining the sequence and properties of massive DNA molecules at a time. Its birth is a milestone event in the field of biology and provides a new approach for genomics research. The traditional first-generation sequencing technology is expensive and defective compared to high-throughput sequencing technology, so it has gradually been replaced. In the context of this technology, the genome of more species can be studied in depth, so that genomics is moving towards a new era. In terms of the same species whose study has been completed, it is possible to make an in-depth study on the other varieties in the same species.

1.1.2 Microbiota in culture pond

Culture pond technology is the basic technology of the aquaculture industry in China. The traditional culture pond is featured with an uncontrollable environment and a relatively low efficiency. Some natural ecological problems are prone to occur, and its quality is difficult to be guaranteed. The recirculating aquaculture technology can effectively solve the shortcomings of the traditional aquaculture technology, which is not only the main form of aquaculture technology at present, but also the main development direction of future aquaculture technology. The core technology of recirculating aquaculture is to affect the structure and changes within the microbiota through biological filtration, so that water quality can be better at culture and aquatic products with a better quality can be achieved. Therefore, the study on the dynamic changes of microbial communities in the environment of culture pond is of great significance to the improvement of the prospect of the aquaculture industry.
1.2 Literature review

The water quality and environment of the recirculating aquaculture system (RAS) are controllable. Besides, the RAS is more efficient and is able to effectively protect the water quality and the ecological environment in the surrounding areas, and the quality of its products has been greatly improved. The RAS is the main development direction of the aquaculture field and also the most advanced aquaculture technology model at present, which is of great significance to promote the development of aquaculture industry. Its core technology is biological filtration, and toxic ammonia and nitrogen in the water are converted mainly through nitrifying bacteria and then filtered out by the biofilter. Filtration and maintenance have became the key technology of the RAS and also the focus and difficulty of the whole system (Xia and Jia, 2014). In the RAS, the dynamic changes of the microbiota in the biofilter have been the focus of the researchers in China. A number of methods were adopted, but limited by the scientific and technical level, most of them employed the traditional methods to analyze only part of the microorganisms. The study on microbiota diversity is not comprehensive. The application of high-throughput sequencing technology can effectively solve the drawbacks of traditional methods. With an enormous reduction in sequencing costs, a more large-scale analysis can be conducted to make an accurate and comprehensive description of the dynamic change characteristics of the microbiota in the RAS environment (Li et al., 2014). High-throughput sequencing technique is applied to analyze the microbiota in three filter chambers of the garrupa recirculating culture system. The following conclusions can be drawn: first, the microbiota is mainly composed of proteobacteria and bacteroides, and other species is in a relatively small amount. Secondly, a quantity of bacteria of proteobacteria can adopt the organic carbon source in the recirculating aquaculture pond to conduct exclusion reactions. Thirdly, the three recirculating aquaculture ponds were nitrified, and only the bacteria reaction in two ponds was recorded and the third pond did not work. Fourthly, there was a high degree of similarity in the dynamic changes and structure of the microbiota in two recirculating aquaculture ponds, while the other pond did not show this feature (Wang et al., 2015).

2. MATERIALS AND METHODS

2.1 RAS

The RAS takes culture pond as an overall frame and adopts arc sieves, protein separators, tertiary submerged biofilter, trickling filter, UV disinfection equipment, ozone generators, liquid oxygen tanks and other equipment. Its operation is stable and automated. The most significantly applied technology is culture pond shunt drainage technology, which mainly includes the approaches of bottom outlet and surface overflow. Specifically, bottom outlet mainly excludes some precipitated particles with a relatively high density, and the corresponding overflow pipe is set on the surface so as to discharge the oil and foam on the water surface and to ensure the qualified water quality of the recirculating aquaculture ponds. Meanwhile, the RAS can also be applied to maintain the water level and to make a wide range of effects (Yan et al., 2015).

2.2 Water quality testing methods

The microbiota in the RAS is in dynamic changes. It is necessary to detect the water temperature, dissolved oxygen and PH value of the inlet and outlet, and these values should be measured twice in each day of a week. The values of ammonia nitrogen, nitrite nitrogen, nitrate nitrogen and chemical oxygen demand should be measured once every two days. The method of measurement should be consistent with the standard water quality measurement method (Yan et al., 2015).

2.3 Sample extraction method

The entire recirculating aquaculture systems were arranged in the direction of water flow, which was recorded as No.1 RAS, No.2 RAS and No.3 RAS, and samples were separately extracted. Six samples were taken at different locations of each RAS, respectively. In addition, polyculture should be conducted in different RASs. A certain amount of sample was selected and placed in a centrifuge tube and precipitated according to standard procedures. This part of the precipitate will be used for DNA extraction (Li et al., 2014).

2.4 High-throughput sequencing

To perform high-throughput sequencing on the above samples, first, it is necessary to amplify the target DNA through the main primers - 338F and 506R in accord with the standard implementation method. At the end of
the amplification, the obtained product was subjected to gel electrophoresis to verify the DNA amplification effect. When the testing ended, high-throughput sequencing was carried out.

2.5 Data analysis

The data analysis level directly affects the accuracy and comprehensiveness of biological information. Therefore, it is necessary to strictly control data information, to examine the obtained information in the database, and to remove invalid and erroneous data and chimeric sequences. In addition, Bayesian algorithm should be employed to conduct the taxonomy analysis, and the relevant computational software should be adopted for modeling and calculation. Also, Chaol index and Simpson index need to be calculated. Chaol index indicates the richness of microbiota in the RAS. The higher the index is, the richer the microbiota in the RAS is. Simpson index reflects the diversity of microbiota in the RAS. The higher the index is, the lower the microbiota diversity in the RAS is (Chen et al., 2015). The formula is as follows:

\[ S_{\text{Chao1}} = S_{\text{obs}} + \frac{n_1(n_1-1)}{2(n_2+1)} \]  

where \( S_{\text{obs}} \) represents the OTU index, namely the operational taxonomy unit; \( n \) stands for the number of OUT; \( n_1 \) is one sequence; \( n_2 \) represents two sequences.

\[ D_{\text{Simpson}} = \frac{n_{\text{obs}} n_1(n_1-1)}{N(N-1)} \]  

According to the above formula, the tree structure was constructed by means of software computation and the similarity of the three recirculating aquaculture ponds was calculated (Huang et al., 2016).

3. EXPERIMENTAL RESULTS

3.1 Water quality situation

After a period of experiment and operation, the three recirculating aquaculture ponds were able to effectively purify the water quality and and to operate stably. The parameters data of the recirculating aquaculture ponds is illustrated in Table 1:

<table>
<thead>
<tr>
<th>Table 1 Data of Each Parameter in a Recirculating Aquaculture Pond</th>
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<tbody>
<tr>
<td>Water quality index</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Water temperature (°C)</td>
</tr>
<tr>
<td>Dissolved oxygen (mg·L⁻¹)</td>
</tr>
<tr>
<td>PH value</td>
</tr>
<tr>
<td>Total ammonia nitrogen (mg·L⁻¹)</td>
</tr>
<tr>
<td>Nitrates (mg·L⁻¹)</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg·L⁻¹)</td>
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</tbody>
</table>

As indicated by the table, the differences of water temperature, PH value and nitrate in the inlet and outlet of the RAS were insignificant, but dissolved oxygen, total ammonia nitrogen and chemical oxygen consumption of the RAS demonstrated a major distinction. The treated water quality has fully satisfied the required standards of the recirculating aquaculture ponds (Wu et al., 2016).
3.2 Microbiota diversity in the RAS

![Biological filter No.1, Biological filter No. two, Biological filter No. three](image)

**Figure 1. Dilution Curve of Biological Filter**

According to the chart, the curves of No.1 RAS and No.2 RAS were roughly equal, while the curve of No.3 RAS was obviously higher than that of No.1 RAS and No.2 RAS, which proves that the diversity of the microbiota in No.3 RAS was significantly higher than that in No.1 RAS and No.2 RAS. In addition, as far as the three RASs were concerned, its growth rate showed a clear trend in general. When the number of sample sequences was between 0 and 20,000, they grew rapidly. When the number exceeded 30,000, the grow rate was slowed down and tended to be stable (Cao et al., 2016).

3.3 Microbiota composition of the RAS

In different circulating aquaculture systems, the microbiota composition has some differences, and its distribution is illustrated in Figure 2.

![Microbiota Structure Distribution in Three Biological Filters](image)

**Figure 2. Microbiota Structure Distribution in Three Biological Filters**

As indicated by the analysis on the above figure, there were a total of 14 diversified microbial communities in three types of RASs. Specifically, the number of proteobacteria was the largest, accounting for about 50%; bacteroidetes constituted about 30%; chloroflexi took up about 5%; nitrospira occupied about 4%; the remaining proportion was other types of bacteria. The composition of the microbial communities in the three RASs was broadly similar. Among them, pacificibacter, ruegeria and rhodobacteraceae-unclassified are a type of purple non-sulfur bacteria, which can form denitrification with a variety of organic carbon source in the RAS and play a significant role in optimizing water quality. Besides, muricauda, lutibacter, maribacter and flavobacteriaceae-unclassified are a type of facultative anaerobic bacteria that can react through nitrates to achieve anaerobic respiration, which proves that the RAS has received denitrification for the normal use. Haloferula is a facultative anaerobe that can carry out photosynthesis. However, this photosynthesis has a certain degree of specificity, and oxygen can not be produced through photosynthesis and facultative nitrogen fixation is not possible. Ammonia oxidizing bacteria (nitrite bacteria) and nitrite oxidizing bacteria (nitrifying
bacteria) have a nitrification reaction in the RAS, and there is a certain reaction with nitrospira and nitrosomonas, so the RAS is optimized (Yang et al., 2017).

3.4 Microbiota similarity in the RAS

A total of 712 OTUs were included in No.1 RAS; 635 OTUs were contained in No.2 RAS in total; a total of 825 OTUs were involved in No.3 RAS. Specifically, only 41 OTUs were overlapped between No.1 RAS and No.2 RAS; 133 OTUs were crossed between No.1 RAS and No.3 RAS; 488 OTUs were overlapped between No.2 RAS and No.3 RAS plus No.2 RAS; 82 OTUs were overlapped between No.2 RAS and No.3 RAS. A conclusion could be drawn by the clustering analysis on the three RASs. No.1 RAS is highly similar with No.2 RAS, and the similarity between No.3 RAS and the other two systems is relatively low. But at the beginning of the experiment, the original prediction result was that No.3 RAS had a high similarity, which is vastly different with the results. Based on the analysis, the primary reason is non-unified parameters among various RASs.

4. DISCUSSION

4.1 Nitrifying bacteria

The main purpose of constructing a RAS was to convert ammonia nitrogen that is harmful to aquaculture in the culture pond into harmless nitrate nitrogen by filtration or chemical reaction. This process was divided into two steps. The first step was to oxidize the ammonia in water to form nitrite, and the second step was to continue the oxidation of nitrite generated in the last step into nitrates. Specifically, the reacting bacteria in the first step was ammonia oxidizing bacteria and the one in the second step was nitrite-oxidizing bacteria. These two bacteria are the focus of this study. Therefore, it is indicated that the RAS can effectively solve the shortcomings in the traditional culture system. However, the role assumed by the RAS largely varies due to different types of fish as well as the major distinction of the environment, water quality and other aspects (Lu et al., 2017).

4.2 Anammox bacteria

In an oxygen-deficient environment, anammox bacteria can convert ammonium ions into nitrogen through chemical reactions, which not only exerts the desired effect for the aquaculture industry, but also presents a new approach for the global nitrogen cycling. In the traditional concept, the RAS is often considered in an oxygen-rich state. In essence, because of the presence of anammox bacteria, the RAS is regarded to be deficient in oxygen. Two main reasons might be involved. The first reason is the lack of oxygen supply in the RAS. As a result of the uneven distribution of oxygen supply equipment, some part of water is rich in oxygen and the other part is in anoxic state. Secondly, the overly thick biofilm on the surface of the filtration equipment hinders the flow of oxygen to certain degree, resulting in long-term oxygen depletion in the filtering membrane. This also brings a new idea to the setting of the RAS, which means to change the location of the traditional oxygen supply equipment to make it evenly distributed, thereby ensuring that the water in each position is rich in oxygen. In addition, the filter chambers of the RAS should be regularly discharged to improve the circulation rate of oxygen and to reduce the problem of oxygen deficit in water, which is of great significance to guarantee the normal operation of the RAS (Zheng and Jia, 2013).

ACKNOWLEDGMENTS

1. Study on the NIH shift of Bacillus sp. B1 involved in 4HBA degradation pathway, National Natural Science Foundation of China (31400099); 2. Study on the degradation of 4HBA by Bacillus sp. B1 and the NIH shift, Jiangsu Science and Technology Agency Project (SBK20140780); 3. The Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD)

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