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Research article

Contrasting microbial community composition and function perspective of the biofilms in shrimps (*Macrobrachium nipponense*) cultured systems

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ABSTRACT

Biofilm microbial communities and the water quality environment were studied in either carrier biofilm in isolation, or in combination with shrimp (*Macrobrachium nipponense*) from Baiyangdian Lake. Use of biofilm treatment effectively improved the water quality environment, however, the content of NH_4-N, TN, and TP increased by 2.49, 1.93, and 0.94 folds, with the addition of high shrimp populations into the aquatic environment over a long-term period (75 d). The relative abundance of dominant phyla in carrier biofilms was *Proteobacteria* (50.20%), *Cyanobacteria* (23.31%) and *Planctomycetes* (8.14%) in control group (no shrimps). The relative abundance of *Cyanobacteria* decreased by 60.6%, whereas *Planctomycetes* increased by 1 folds with the high shrimp population addition. In addition, the inclusion of low density shrimp populations decreased by 21.1%, and 31.6% in NH_4-N and TP concentrations compared to controls over a short-term period (15 d), respectively. The abundance of *Planctomycetes* increased by 92.9% with the addition of low shrimp populations on 15 d. Our results showed that the addition of low shrimp population (286 animals/m^2) could improve the water quality environment containing carrier biofilm according to the regulation of the bacterial diversity in the biofilm system in short-term period (15 d).

1. Introduction

With aquaculture development, nutrient pollution of the environment has reached serious levels, with the effect particularly apparent in water quality when the aquaculture wastewater are discharged directly to the water. Developing a value way to remove the nutrients and make valuable biomass has been more and more important for aquaculture development.

The technology of composite biofilms can effectively convert different forms of nutrient to proteins for food sources according to the microbial communities from a simulated aquaculture waste water (Abreu et al., 2007; Viau et al., 2012). Biofilms were formed of microbial communities, which accumulated on an organic matrix surface and submersed in a waterbody (Rittmann, 2018). Biofilms are widely used to treat wastewater, as well as having wider beneficial effects on aquatic ecosystems, such as provide a valuable food source to improve the growth of crustacean species (Ramesh et al., 1999; Tanner et al., 2018).

Baiyangdian Lake is an important aquaculture base for *Macrobrachium nipponensis* feeding and previous reports have shown that biofilms could be a valuable alternative food source and/or a supplement to improve cultures of early and advanced *Cherax quadricarinatus* juveniles (Viau et al., 2012).

Little is known about the effects of variations in microbial communities of biofilms and water quality when in the presence of different population density of freshwater shrimp. Biofilm microbial populations have been shown to have high activity, with the species and genera found to dominate biofilms varying depending on the conditions of the wastewater treatment plant or natural ecosystem environment (Schmidt et al., 2003). In addition, the study of pathogen on the biofilm could be important for growth of shrimps. *Vibrio parahaemolyticus* as a human foodborne pathogen can be formed on the biofilm (Han et al., 2016).

The Biolog Eco Plate method is a highly effective method to study the metabolism of bacterial community diversity, however there are some limitations to study the types of the bacteria. Recently, an increasing number of molecular biological techniques have been used for the analysis of microbial communities in various wastewater treatment systems. Denaturing gradient gel electrophoresis(DGGE), Phospholipid Fatty Acid (PLFA), Random amplified polymorphic DNA (RAPD),

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Amplified Fragment Length Polymorphism (AFLP), and Metagenome analysis were used to reveal the dynamics and evolution of microbial compositions in a wastewater treatment system (Yang et al., 2014; Jia et al., 2016). Analysis of 16S ribosomal RNA (rRNA) is an effective method of bacterial classification, obtaining highly accurate microbial community compositions via high-throughput sequencing (Sugiyama et al., 2014). To enhance understanding of microbial interactions on carrier biofilms, bacterial communities were analyzed in situ on the carrier biofilm using BioLog Eco plates and Metagenome. In this study, the physiological properties of bacterial communities were analyzed using the BioLog Eco plate community-level substrate utilization assay, while the composition of the communities was assessed by gene pyrosequencing (Sugiyama et al., 2014). The influence of shrimp populations on the water environment was investigated, with high-throughput sequencing technology and Biolog-Eco plates used to establish the relative abundance and diversity of biofilm microbial populations with and without the presence of shrimp species.

2. Materials and methods

2.1. Study site and sampling

The stable biofilm community was developed using a slightly adapted method of Viau et al. (2012) and Zhao et al. (2015). Field experiments were conducted in incubated aquaria in situ in Baiyangdian Lake, where biofilms were developed during a 15-day period prior to the start of the experiment, using plastic elastic packing (Yixing Hua ya environmental protection equipment Co., China) with the length of 1 m and a diameter of 0.12 m placed vertically in 70 L of natural water sourced from Baiyangdian Lake. Once a stable biofilm community had developed, six biofilms were placed in each plastic aquaria (0.07 m^2 bottom surface) and water was continuously aerated. A varying number of young *M. nipponensis* (mean weight 0.10 ± 0.05 g) were (0, 20, 40, or 60), added to incubation tanks, resulting in population densities of 0, 286, 572, 858 animals/m^2, respectively and representing absent (control), low (LM), medium (MM), and high (HM) population densities, respectively. Deionized sterile water was added to compensate for daily water loss via evaporation. All experiments were performed in triplicate. Water and biofilms were sampled every 15 days, where both water and biofilms were immediately transferred to the laboratory in a cool container and processed within 2 h 5 cm of plastic elastic packing were rinsed and placed into a plastic aquaria containing 200 ml sterile water. The water was agitated to suspend bacteria in solution, then following one hour' standing, supernatant was collected for the BioLog substrate utilization assay. The remaining supernatant water was filtered using a 0.22 μM filter and the filter membrane was sent to Novogene Bioinformatics Technology Co.Ltd (Beijing China) for analysis (Zhao et al., 2014).

2.2. Barcoded pyrosequencing and sequence analysis

DNA was extracted from samples using the E.Z.N.A.™ Soil DNA Kit, where DNA serves as a template for amplifying the 16S rRNA genes using the bacterial universal primer set (515F:806R). 515F: (5′-GTGC CAGCMGCCGCGGTAA-3′), 806R(5′-GGACTACHVGGGTWTCTAAT-3′). The primers were bound to the necessary adapters for analysis and PCR products were sent to Beijing Novartis grain source biological information technology co., LTD for sequencing on the Ilumina Miseq PE250. PCR products were measured according to the manufacturer's protocol. The raw sequences were trimmed, qualified and then clustered to operational taxonomic unit (OTUs) at the 97% similarity level, with presentative sequence from each OTU selected for taxonomic identification using BLAST with a 0.001 maximum-value.

2.3. Microbial community analysis using BioLog Eco plates

150 μL of supernatant was added to each well of the BioLog Eco plate, then incubated at 28 °C for 168 h. Absorbance was measured every 12 h at 590 nm and 750 nm, using a Spectraflour plus (TECAN) and data was analyzed using the average well color development (AWCD) method (Luo et al., 2016).

2.4. Determination of water temperature, DO, NH_4-N, TP, and TN concentrations

Water samples were analyzed for DO and temperature using a DO meter (JPB 607, Shanghai). The collected water samples were centrifuged at 3500 g for 3 min and supernatant measured for NH_4-N, total nitrogen (TN) and total phosphorus (TP) concentrations were measured by a Spectroquant testing kit (MERCK Corp., Germany) using Photolab 6100 (Zhao et al., 2014).

3. Results

3.1. Variation in water quality with different density shrimp populations

DO concentrations declined by varying degrees according to addition of different density shrimp populations, with a 17.4%; 16.4%; or18.7% reduction observed in LM, MM, or HM experimental groups by 75 days as compared with control groups (Fig. 1A). In the control and LM shrimp density experiment groups, DO concentrations increased during the first 30 days, then declined during the following 45 days of the experimental period. Conversely, DO concentrations initially increased for only 15 days in the MM and HM shrimp density experimental groups, then declined for the following 60 days.

The NH_4-N content of the biofilm environment was higher overall in experimental groups containing shrimp species, as compared with controls. NH_4-N concentrations in the low; middle; and high shrimp density experimental groups increased from 0.520 to 1.191; 1.164; and 1.298 mg/L, respectively (Fig. 1B), showing a 2.2-fold; 2.1-fold; and 2.5-fold increase, respectively. Conversely, in control groups, NH_4-N concentrations declined from 0.520 to 0.371 mg/L by 75 days, with declining NH_4-N concentrations during the first 45 days, followed by a slight increase during the following 30 days. In the low shrimp density experiment group, NH_4-N concentrations declined during the first 30 days, then increased; while in the middle shrimp density experiment group, NH_4-N concentrations remained relatively stable during the first 30 days, then declined for 15 days and increased again during the last 30 days; whereas in the high shrimp density experimental group, NH_4-N concentrations only marginally declined during the first 15 days, then increased for the rest of the experimental period.

TN concentrations showed a more consistent pattern, with TN concentrations increasing in groups containing shrimps, as compared with controls. By 75 days the TN concentrations had increased by 1.75-fold; 1.56-fold; and 1.93-fold in the LM, MM, and HM shrimp density experimental groups, respectively. During the first 15 days, TN concentrations in the control group declined significantly by 90.5%, recovering after 15 days culture when TN concentrations increased (Fig. 1C).

The concentration of TP increased during the first 45 d, then declined sharply from 45 d to 75 d (Fig. 1D). TP concentrations were significantly higher in groups containing shrimp populations, where a 3.63-fold increase was observed in the HM group at 60 days, as compared with controls (Fig. 1D).

3.2. Variation in microbial activity at different shrimp population densities

Average well color development (AWCD) was used to indicate microbial activity on elastic packing biofilms, showing the control and LM experimental groups had a faster rate of increase in AWCD as compared