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High-throughput sequencing analysis of the microbial community in coastal intensive mariculture systems

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ABSTRACT

The conventional and recirculating mariculture systems are two typical intensive mariculture systems in the coastal zone. This study used high-throughput sequencing method to investigate the structural profiles of microbial communities in conventional and recirculating mariculture farms. The results showed that 13,842 OTUs (operational taxonomic units) were detected at a similarity level of 97%. Conventional and recirculating systems exhibited significant difference in microbialcommunity based on the results of the taxonomy and relative abundance of bacteria. Among the top 10 genera in coastal intensive mariculture systems, the predominant bacteria in the conventional intensive mariculture system were *Pseudofulvibacter*, *Polaribacter*, *Glaciecola*, *Crocinitomix*, *Colwellia*, *Oleispira,* and *Balneola*, while those in the recirculating systems were *Vibrio*, *Alteromonas,* and *Pseudoalteromonas*. Bacterial communities of different fish ponds suggested that the bacterial groups exhibited fish-specific or water treatment stage-specific features. Potential pathogens such as *Vibrio*, *Arcobacter*, *Pseudoalteromonas*, and *Shewanella* were readily accumulated as dominant bacteria in the recirculating mariculture system. The detection of similar potential pathogens in both the mariculture systems and the adjacent coastal water indicate that the mariculture farms without recirculating system could be the hotspots of pathogens and have great influences on the surrounding coastal environment. The wastewater treatment units used in recirculating farm could remove bacteria effectively, suggesting that the recirculating mariculture system may be more environmentally friendly than the conventional mariculture system.

1. Introduction

Aquaculture is one of the fastest growing food-producing sectors (FAO, 2009), with the products constituting an important food supply. The global aquaculture industry is dominated by Asian countries such as China that account for approximately 71% of the total global aquaculture production (Sapkota et al., 2008). Mariculture, one of the most important aquaculture in the coastal zone, has become more intensive in recent years (Gao et al., 2012). However, the rapid development in the mariculture industry has exerted significant environmental influence on coastal ecosystems. These influences include eutrophication (Holmer et al., 2005; Kalantzi and Karakassis, 2006), chemical pollutions (Antunes and Gil, 2004; Cabello, 2006; Sapkota et al., 2008), disturbed benthic fauna communities (Vezzulli et al. 2008; Tomassetti et al., 2009), and changes in the biodiversity and community structures of bacteria (Gao et al., 2012; Harnisz et al., 2015; Muziasari et al., 2017). The distribution of microorganisms in the aquaculture system is important for marine fish culture because both pathogens and probiotics can profoundly impact the development and physiological function of aquaculture organisms (Rungrassamee et al., 2016; Viljamaa-Dirks, 2016; Xiong et al. 2016). The wide use of antibiotics in the aquaculture leads to wide-spreading antibiotic resistant pathogens and the changes in structures of bacterial community in culture systems (Xiong et al., 2015; Muziasari et al., 2017). As a result, it is important to study the

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microbial community in the mariculture systems of the coastal zone and evaluate the environmental impact of the fish farms.

High-throughput sequencing is the most advanced technique to study microbial diversity and community at present (Xiong et al., 2015; Zheng et al., 2017) because it can detect most of the species in the tested sample without culturing. The high-throughput sequencing method based on 16S rDNA amplicon sequencing technology can make an in-depth analysis on the bacteria community and abundance (Leite et al., 2012). The amplicon sequencing approach has been widely used to characterize the microbial communities and abundances in various environments such as rivers (Zhou et al., 2017), aquaculture environment (Zheng et al., 2017), and soil (Hua et al., 2017).

The aquaculture industry covers a wide range of units from simple traditional systems to integrated aquaculture-agriculture systems (Heuer et al., 2009). The conventional (the flow-through system) and recirculating mariculture systems are two typical mariculture systems intensively used in the coastal zone. The mariculture wastewater is circulated and recycled after a systemic treatment in the recirculating system while the wastewater in the conventional system is discharged directly (or with simple treatment) into the sea (Su et al., 2011; Xiong et al., 2015). Mariculture is a major industry in global coastal zones and has led to concerns regarding the impact of fish farms on the coastal environment. Limited information is available on investigating the difference of the microbial community between the conventional and recirculating mariculture systems. The objectives of the present study are to compare the microbial communities of the conventional and recirculating mariculture systems using high-throughput sequencing technique, and evaluate the influences of the intensive mariculture on coastal aquatic environment based on the impacts of microbial community especially potential pathogen.

2. Materials and methods

2.1. Sample collection

The water samples were collected from two intensive mariculture systems with or without recirculating units in Yantai City which is the most important mariculture city in China. The production of intensive seawater farming in Yantai City was 28,000 tons, accounting for 1/6 of China in 2015 (Municipal Work Report of Yantai, 2015). The mariculture systems and the sampling points were shown in Fig. 1. Fig. 1a showed the conventional intensive mariculture system (ZZ farm) without recirculating unit while Fig. 1b illustrated the recirculating mariculture system (DF farm). The Ponds 1, 2, 3, and 4 in ZZ farm are for *Penaeus vannamei*, *Scophthalmus maximus*, fish-fry of *S. maximus*, and *Platichthys stellatus*, respectively. Fish pond in DF farm is for *Salmo salar*. The recirculation ration (the proportion of the water reclaimed to the water needed for the breeding pond) is 90%. The numbers ①②③④⑤⑥⑦ denote the sampling points. The water samples collected from the sampling points of the conventional system (ZZ farm) were marked as ZZ1, ZZ2, ZZ3, ZZ4, ZZ5, ZZ6 and ZZ7, respectively. The samples ZZ1, ZZ6, and ZZ7 referred to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 referred to the sample of fish pond 1, 2, 3, and 4, respectively. The water samples collected from the sampling points of the recirculating system (DF farm) were marked as DF1 (from the influent), DF2 (from fish pond), DF3 (from protein separator), DF4 (from biochemical pool for wastewater treatment), DF5 (from biological aerobic pool for wastewater advanced treatment), and DF6 (from the recycled water), respectively. Water samples (30 L/point) were filtered through 0.22 μm micropore membrane and the filtered materials were kept at −80 °C for further study.

2.2. DNA extraction, library preparation and sequencing

2.2.1. Extraction of genome DNA

The filtered samples were transported on dry-ice to Novegene (Beijing, China) for DNA extraction and sequencing. The total genomic DNA was extracted by CTAB/SDS method (Armougom and Raoult, 2009). The concentrations and purity of DNA were monitored on 1% agarose gels. DNA was diluted to 1 ng/μL using sterile water according to the concentration.

2.2.2. PCR amplification

16S rRNA genes of distinct regions 16S V4-V5 were amplified using the universal forward 515 F (5′-GTGCCAGCMGCCGCGG-3′) and reverse 907R (5′−CCGTCAATTCMTTTRAGTTT-3′) (Armougom and Raoult, 2009) with the barcode (listed in Table 1). All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The PCR reaction system (25 μL) contained 17.2 μL ddH_2O, 2.5 μL 10× PCR buffer, 2.5 mM dNTP mixture, 1 μL of each primer, 1.5 U of Taq DNA polymerase, and 1 μL of template DNA. The PCR cycling procedures were the following: 2 min at 95 °C, followed by 25 cycles (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s) and a final extension at 72 °C for 8 min, and then held at 4 °C. The PCR products were checked on 2% agarose gel. Samples with bright main strip between 400–450 bp were chosen for further experiments. The target PCR products were mixed in equidensity ratios. Finally, mixed PCR products were purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

2.2.3. Library preparation and sequencing

Sequencing libraries were generated using TruSeq DNA PCR-free

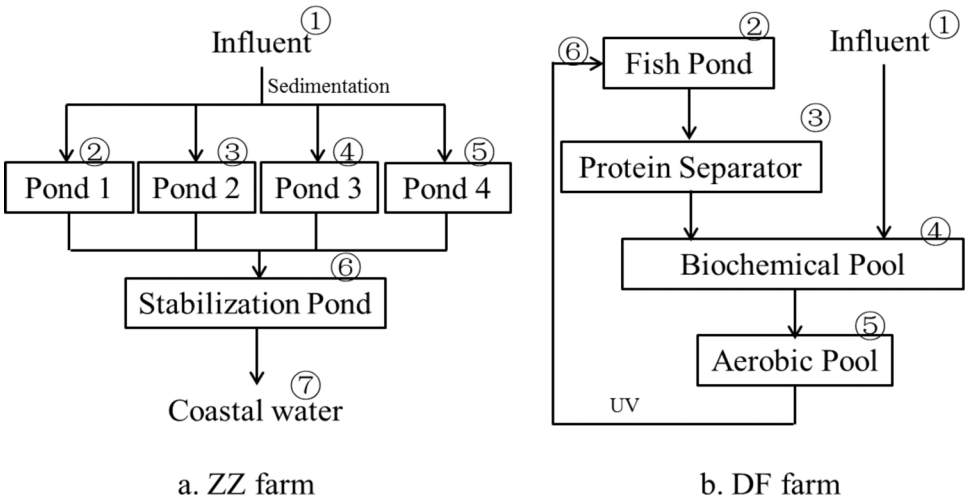

Fig. 1. Flowchart and sampling points of the two fish farms: (a) ZZ, a conventional mariculture system; (b) DF, a recirculating mariculture system. The pond 1 in ZZ farm is for *Penaeus vannamei*, pond 2 is for *Scophthalmus maximus*, pond 3 is for fish-fry of *S. maximus*, and pond 4 is for *Platichthys stellatus*. The fish pond in DF farm is for *Salmo salar*. The recirculation ration is 90%. The numbers ①②③④⑤⑥⑦ are for the sampling points.