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*.
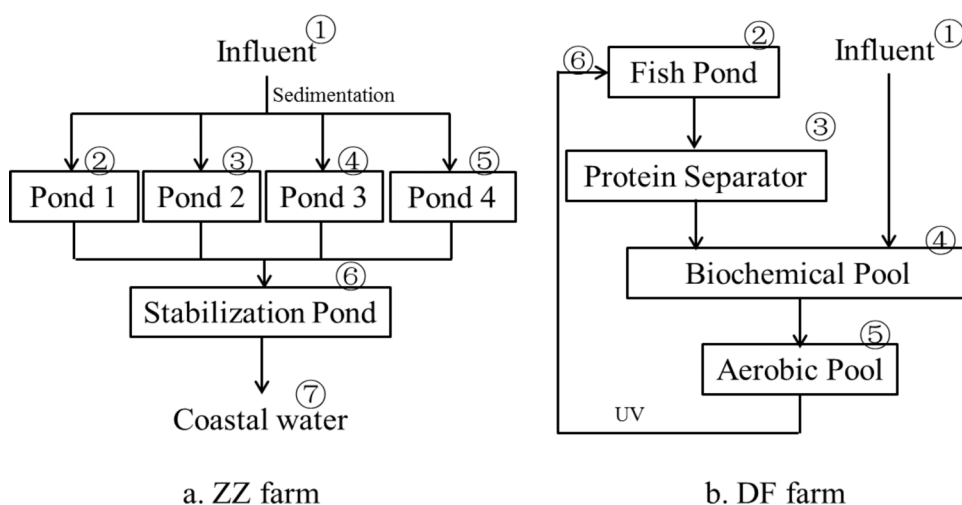
The recirculation ration (the proportion of the water reclaimed to the water needed for the breeding pond) is 90%. The numbers ①②③④⑤⑥⑦ denote the sampling points. The water samples collected from the sampling points of the conventional system (ZZ farm) were marked as ZZ1, ZZ2, ZZ3, ZZ4, ZZ5, ZZ6 and 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 ddH₂O, 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.

a. ZZ farm   b. DF farm

Table 1
Barcodes used in this study.

Sample	Barcode Sequence
ZZ1	AACGTGAT, GGTGCGAA
ZZ2	AACGTGAT, GTCGTAGA
ZZ3	AACGTGAT, TCTTCACA
ZZ4	AACGTGAT, TTCACGCA
ZZ5	AACGTGAT, AATCCGTC
ZZ6	AACGTGAT, AATGTTGC
ZZ7	AACGTGAT, AGCACCTC
DF1	AACGTGAT, ATAGCGAC
DF2	AACGTGAT, CCTCTATC
DF3	AACGTGAT, CGGATTGC
DF4	AACGTGAT, GAGTTAGC
DF5	AACGTGAT, GATGAATC
DF6	ACAGCAGA, GGTGCGAA

Note: In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water.

Table 2
Alpha diversity indices and OTUs number of all of the samples.

Snormal	OTUs	Observed Species	Shannon[a]	Chao 1[b]	Good's coverage[c]
ZZ1	1653	1496	6.297	1665.783	0.993
ZZ2	780	780	5.629	1139.841	0.993
ZZ3	645	476	3.985	649.067	0.996
ZZ4	657	528	4.133	620.286	0.996
ZZ5	589	464	3.247	674.348	0.995
ZZ6	824	693	5.950	783.533	0.996
ZZ7	982	856	5.747	969.286	0.996
DF1	1820	1732	7.694	1803.600	0.996
DF2	1332	1191	6.501	1284.531	0.996
DF3	878	749	5.926	854.940	0.996
DF4	900	780	4.639	857.020	0.996
DF5	1260	1104	6.702	1271.592	0.994
DF6	1522	1350	7.285	1483.155	0.994

Note: In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water.

[a] The Shannon index is used to calculated the diversity of bacteria community.

[b] The Chao1 estimator is used to estimate the community richness.

[c] The Good's coverage is used to evaluate the depth of sequencing.

Sample Preparation Kit (Illumina, USA) based on recommendations of manufacture. Index codes were added. The library quality was assessed on the Qubit 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. The library was finally sequenced on an Illumina HiSeq2500 platform and 250 bp paired-end reads were generated.

2.3. Data analysis

2.3.1. Data filtration

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH (V1.2.7). Quality filtering on the raw tags was performed by QIIME (V1.7.0) (Caporaso et al., 2010). The chimera sequences were removed by UCHIME Algorithm (Edgar et al., 2011), and the effective tags were obtained.

2.3.2. OTU cluster and species annotation

Sequences analysis was performed by Uparse software (Uparse v7.0.1001). Sequences with similarity ≥97% were assigned to the same OTUs (Armougom and Raoult, 2009). Representative sequence for each OTU was screened for further annotation. In terms of each representative sequence, the GreenGene Database was used based on RDP classifier (Version 2.2) (Wang et al., 2007) algorithm to annotate taxonomic information.

2.3.3. Phylogenetic relationship construction

Multiple sequence alignment was conducted using the MUSCLE software (Version 3.8.31) in order to study phylogenetic relationship of different OTUs as well as the difference of the dominant species in different samples (groups).

2.3.4. Data normalization

Abundance of OTUs was normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity was performed in the basis of the output normalized data.

2.3.5. Alpha diversity and beta diversity analysis

Alpha diversity was calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). Beta diversity analysis was performed by QIIME software (Version 1.7.0) and ggplot2 package in R software (Version 2.15.3).

The sequences derived from high-throughput sequencing were deposited in the National Center for Biotechnology Information (NCBI) database under accession number SRP128966.

3. Results and discussion

3.1. Diversity and abundance of bacteria in coastal intensive mariculture farms

Total 925,227 reads with an average of 56,218 high-quality sequences per sample were obtained from all the 13 samples. These sequences clustered into 13,842 OTUs at a similarity level of 97%. The Good's coverages of all samples were greater than 0.99, suggesting that the sequencing depth of all samples were sufficient to represent bacterial community in the mariculture environments (Table 2). Shannon diversity index (Magurran, 1988; Chao and Bunge, 2002) showed variations of 3.247–7.694 in these samples (Table 2). The Chao 1 (Magurran, 1988; Chao and Bunge, 2002) indices of these samples were 620.286–1803.600 (Table 2). The relatively high Shannon and Chao 1 indices suggested that the bacterial diversity in the mariculture farms was high. The bacterial diversity analysis on fish farm sediment indicated that the Shannon index was about 4 and Chao 1 indices ranged from 250 to 2165 (Bissett et al., 2006). The study on bacterial community in the rearing water of Pacific white shrimp showed that the OTUs were in the range of 88–234, Shannon diversity indices were 2.05–3.90, and Chao 1 indices ranged from 107 to 350 (Zheng et al., 2017). Hua et al. (2017) investigated the bacterial community with different coastal reclamation histories in Jiangsu Province (Eastern China) to find that the Shannon indices and Chao 1 indices were in the ranges of 8–10 and 2000–20,000, respectively. All studies indicated that the bacterial diversity levels in the intensive fish farms were lower than those in other reclamation coastal ecosystems.

3.2. Distinct microbial community between conventional and the recirculating mariculture systems

The conventional intensive mariculture system (ZZ farm) and the
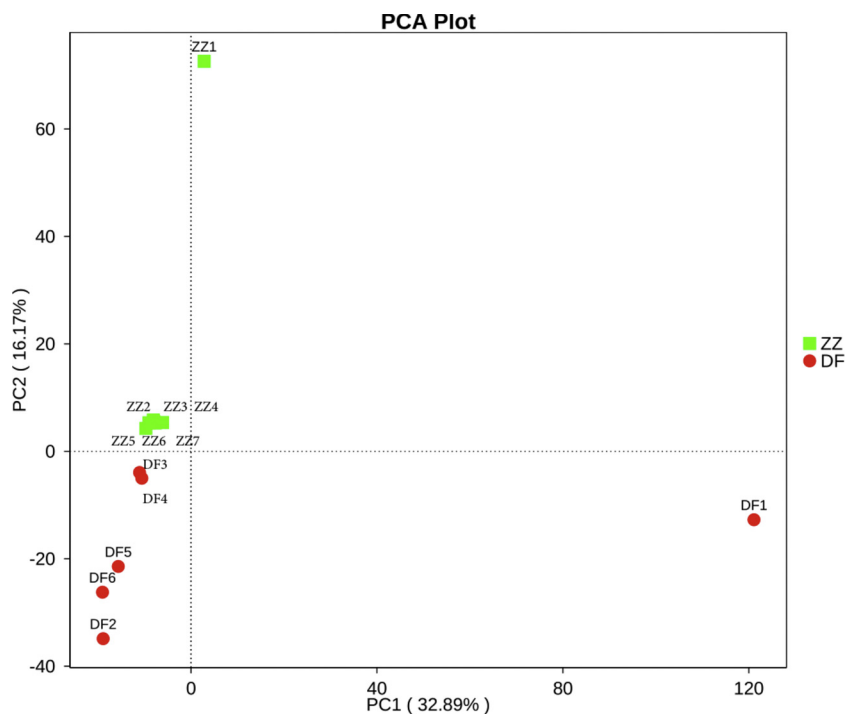
Fig. 2. The principal components analysis (PCA) based on OTU level. The red and green points represent ZZ and DF samples. ZZ refers to the conventional mariculture system while DF refers to the recirculating mariculture system. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
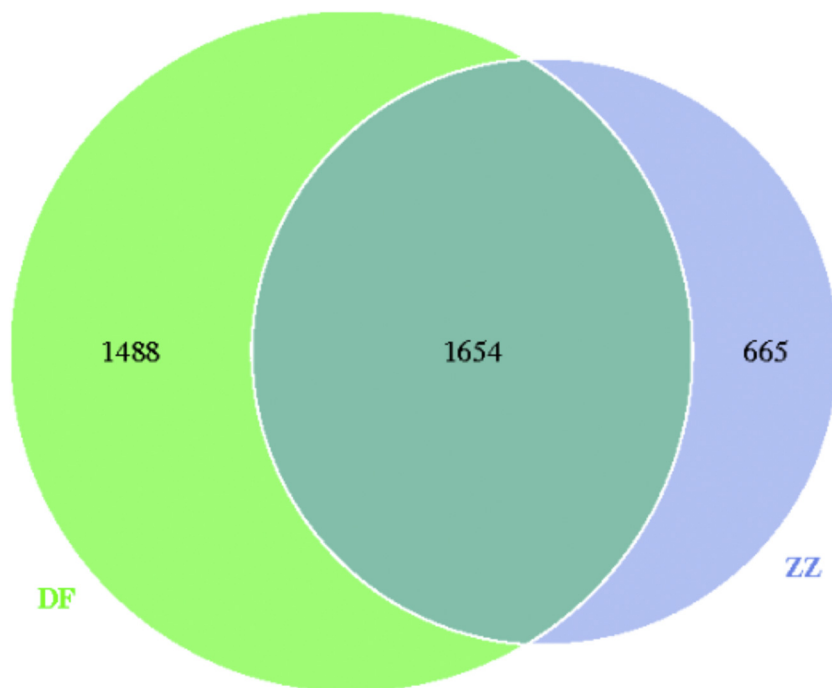

Fig. 3. The common and unique OTUs between ZZ and DF groups displayed by venn graph. Each circle in the figure represents a group: the left circle is for DF, and the right circle is for ZZ. The datum in the overlapped part shows the OTUs number shared by ZZ and DF. The data in the unoverlapped parts show the unique OTUs number in ZZ or DF group. ZZ refers to the conventional mariculture system while DF refers to the recirculating mariculture system.

recirculating mariculture system (DF farm) are two typical aquaculture systems in the coastal zone. The bacterial communities from two different farms were compared at different taxonomic levels. The principal component analysis (PCA) based on OTUs significantly separated the ZZ samples from DF samples, indicating that each farm had its own specific characteristic microbial community, although the influents of two farms were both from the groundwater in the same coastal area (Fig. 2). The venn diagram (Fig. 3) directly showed the similarities and differences between ZZ and DF farms at OTU level. There were 1654 similar OTUs in the two farms, 1488 different OTUs in DF farm, and 665 different OTUs in ZZ farm. The results showed that the bacteria diversity in DF farm was richer than that in ZZ farm, which indicated that

recirculating mariculture system led to a greater accumulation of diverse bacteria. Additionally, the influences of environmental variables on the microbial communities of each mariculture system were quite different. Redundancy analysis (RDA) was performed to identify the correlations between taxonomic composition at OTU level and environmental variables (shown in Table 3) such as phosphate (P), total phosphorus (TP), ammonium (NH_3), nitrate (NO_3), nitrite (NO_2), silicate (Si), and total carbon (TOC) (Fig. 4). The first two axes explained 52.09% of the taxonomic information. RDA analysis illustrated that ZZ samples was positively correlated with P, Si and TOC while DF samples were positively correlated with NH_3, NO_3, NO_2 and TP, which confirmed the difference in the microbial community of the conventional

Table 3
Environmental parameters of each water sample.

Sample	P (mg/L)	NH$_3$ (mg/L)	NO$_2$ (mg/L)	NO$_3$ (mg/L)	TP (mg/L)	TN (mg/L)	TOC (mg/L)	Si (mg/L)
ZZ1	0.01	0.02	0.00	0.48	0.01	0.63	5.36	5.05
ZZ2	0.12	0.15	0.01	1.44	0.01	2.20	8.03	5.22
ZZ3	0.01	0.15	0.01	1.48	0.01	2.00	7.44	6.13
ZZ4	0.00	0.18	0.01	1.41	0.02	1.92	7.49	6.38
ZZ5	0.10	0.16	0.01	1.62	0.01	2.00	6.88	5.11
ZZ6	0.10	0.14	0.01	1.48	0.01	1.87	6.92	5.00
ZZ7	0.00	0.02	0.00	0.05	0.02	1.65	7.43	0.23
DF1	0.01	0.02	0.00	0.60	0.02	0.64	5.56	2.81
DF2	0.01	0.16	0.01	3.65	0.02	3.89	6.29	2.65
DF3	0.03	0.17	0.01	3.52	0.04	3.67	9.6	2.45
DF4	0.03	0.24	0.05	3.50	0.04	3.78	2.23	2.44
DF5	0.03	0.29	0.04	1.99	0.04	3.87	5.62	2.37
DF6	0.03	0.28	0.03	0.47	0.04	3.56	5.21	2.43

Note: In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water. P means phosphate.
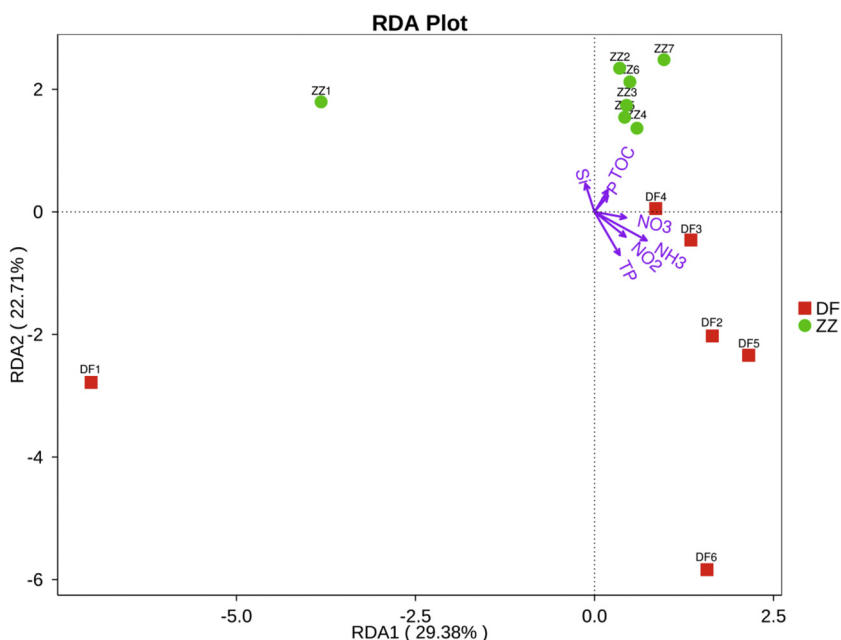

Fig. 4. Redundancy analysis shows the relationships between environmental variables and each sample based on OTU level. Abbreviations: Pphosphate; TPtotal phosphorus; NH3ammonium nitrogen; NO3nitrate nitrogen; NO2nitrite nitrogen; Sisilicate; TOCtotal organic carbon. In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water.

and the recirculating mariculture systems. The microbial community in the recirculating system (DF farm) relied on the nitrogen nutrient. Linear discriminant analysis Effect Size (LEfSe) was used to determine the potential discriminating taxa between DF and ZZ farms. The results showed that there were 10 bacterial taxa distinguishing DF from ZZ farm with LDA value greater than 4.0 (Fig. 5a). In ZZ farm, 1 phylum and 1 genus were enriched, including Bacteroidetes (phylum level) and *Pseudofulvibacter* (genus level) while 1 phylum, 1 order, 1 family, 3 genera and 2 species were enriched in DF farm, including Proteobacteria (phylum level), *Vibrionales* (from order to species levels), *Sulfitobacter* (from genus to species levels), and *Alteromonas* (genus level) (Fig. 5a and b).

The relative abundances of the top 10 bacteria at phylum and genus levels in the two farms were showed in Fig. 6a and b. Fig. 6a showed that the most dominant phyla were Proteobacteria and Bacteroidetes, with the total percentages of the two phyla exceeding 90% in both farms. The relative abundance of Proteobacteria phylum in DF farm was higher than that in ZZ farm while the relative abundance of Bacteroidetes phylum in ZZ farm was higher than that in DF farm. Many studies on the structure of microbial community in fish farms had

shown that Proteobacteria and Bacteroidetes were the most dominant phyla. Xiong et al. (2015) found that Proteobacteria and Bacteroidetes were the dominant phyla in aquaculture environment of Guangdong, China. Colombo et al. (2016) also found Proteobacteria was the dominant phylum in aquaculture facility. Comparing the genera with high relative abundance (top 10) in the two farms (Fig. 6b), *Pseudofulvibacter*, *Polaribacter*, *Glaciecola*, *Crocinitomix*, *Colwellia*, *Oleispira* and *Balneola* possessed higher abundance in ZZ farm while *Vibrio*, *Alteromonas* and *Pseudoalteromonas* showed higher abundance in DF farm. The *Pseudofulvibacter* and *Vibrio* were the most dominant genus in ZZ and DF farm, respectively, reconfirming the difference in the microbial community of the conventional and the recirculating mariculture systems. Zheng et al. (2017) found *Enterobacter* and some unclassified genera of *Enterobacteriaceae* were the most abundant genera in shrimp at all growth stages, followed by an unclassified genus of *Rhodobacteraceae*, and then genera *Ruegeria*, *Aquimarina*, and *Vibrio*. Iijima et al. (2009) isolated several strains of *Pseudoalteromonas*, *Vibrio* and *Halomonas* from fish farm sediments in Japan. *Bacillus*, *Vibrio*, *Acinetobacter*, *Pseudoalteromonas*, *Aeromonas*, *Pseudomonas*, *Flavobacterium*, *Edwardsiella* and *Hafnia* were also found in the mariculture
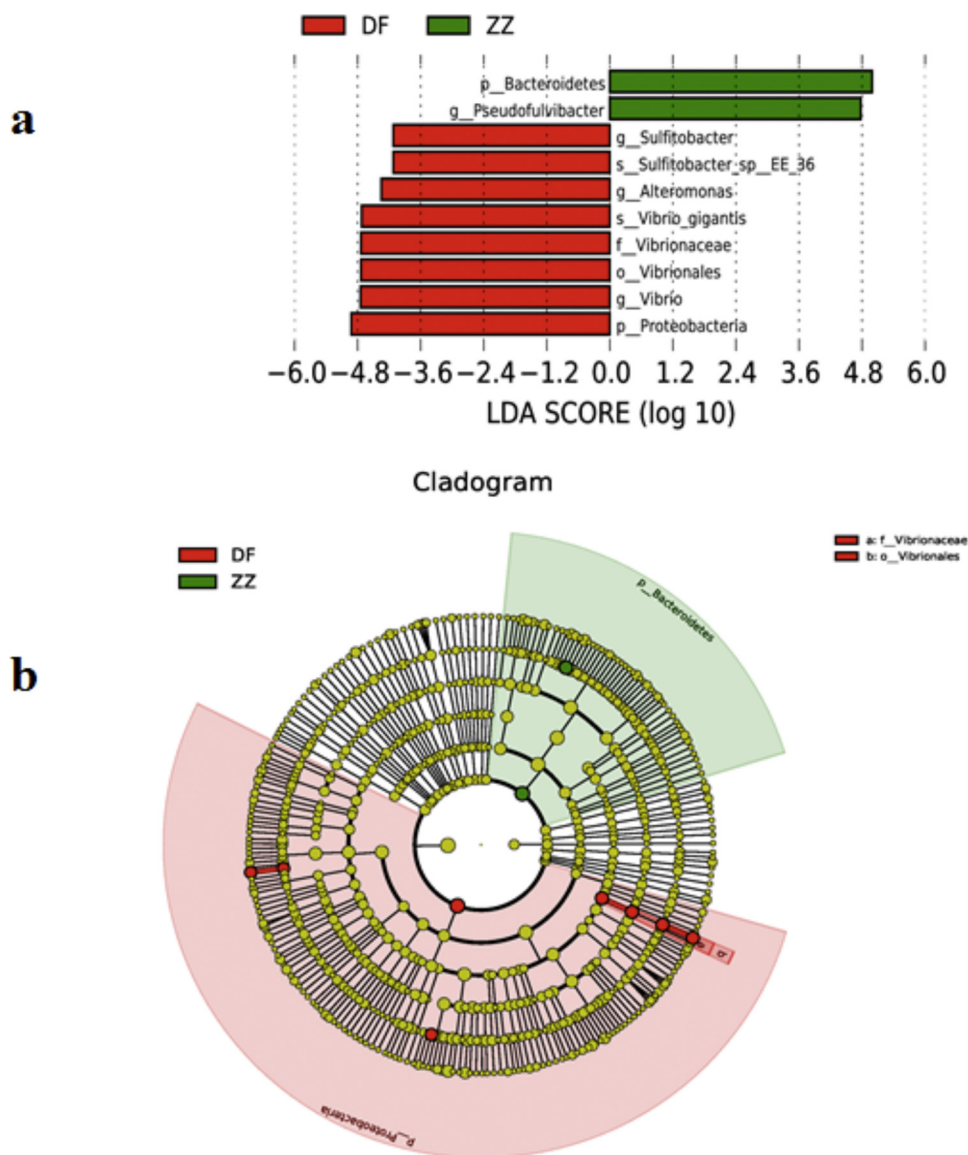
Fig. 5. (a) Linear discriminate analysis (LDA) value of the taxa in the conventional (ZZ farm) and recirculating (DF farm) mariculture systems. The length of the column represents the effect size of the bacterial lineages. (b) The cladograms of bacterial lineages with significant difference between ZZ and DF. The bacterial groups from phylum to species level are listed from center to outside. Each circle's diameter is proportional to the bacterial taxon's abundance. Green: bacterial taxa enriched in ZZ, Red: bacterial taxa enriched in DF. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

environment in China, Vietnam, and Australia (Akinbowale et al., 2006; Hoa et al., 2008; Gao et al., 2012).

3.3. Effects of different fish ponds and wastewater treatment units on the microbial communities

Bacterial communities along with different fish ponds were analyzed to study the variation among different mariculture animals and to find out the fish-specific groups. Compared with ZZ1, the relative abundance of bacteria genera *Pseudofulvibacter, Polaribacter, Glaciecola, Crocinitomix, Oleispira* and *Colwellia* decreased whereas that of the bacteria genera *Balneola, Phaeodactylibacter, Maritimibacter* and *Owenweeksia* increased in ZZ2 (Fig. 7a). It suggested that the aquaculture fish *P. vannamei* in Pond 1 could induce the propagation of *Balneola, Phaeodactylibacter, Maritimibacter* and *Owenweeksia,* and the fish pond might be suitable for the growth of these genera. Compared with ZZ1, the relative abundance of bacteria genera *Pseudofulvibacter, Polaribacter, Crocinitomix* and *Colwellia* increased in ZZ3, and the relative abundance of *Pseudofulvibacter, Polaribacter, Glaciecola* and *Colwellia* increased in ZZ4 and the relative abundance of *Pseudofulvibacter, Crocinitomix* and *Colwellia* increased in ZZ5. Similarly, it indicated that Ponds 2, 3 and 4 were suitable for the target bacteria. The relative abundance of bacteria including *Vibrio, Alteromonas, Pseudofulvibacter,*

Polaribacter, Pseudoalteromonas, Crocinitomix and *Sulfitobacter* in sample DF2 that was collected from Pond of *S. salar* increased by comparing the recycled water (DF6) of DF farm (Fig. 7b), which suggested that these bacteria were suitable for *S. salar* culturing. The dominant genera (the relative abundance of the bacteria over 1%) in ZZ2 (Pond for *P. vannamei*) were *Balneola, Phaeodactylibacter; Maritimibacter* and *Owenweeksia.* The dominant genera in ZZ3 (Pond for *S. maximus*) were *Pseudofulvibacter, Polaribacter, Crocinitomix, Oleispira* and *Colwellia.* The dominant genera in ZZ4 were *Pseudofulvibacter, Polaribacter, Glaciecola, Oleispira* and *Colwellia.* The dominant genera in ZZ5 were *Pseudofulvibacter, Crocinitomix, Oleispira* and *Colwellia* while those in DF2 were *Vibrio, Alteromonas, Polaribacter, Pseudoalteromonas, Colwellia,* and *Sulfitobacter* (Fig. 7). The investigation indicated that characteristics of the microbial community in the fish farms were dependent on the cultured animals. Study in Vietnamese shrimp ponds showed that *Vibrio* and *Bacillus* were the dominant bacteria (Hoa et al., 2008). Colombo et al. (2016) studied the bacteria community of water from a salmon farm in Italy and found *Bacillus, Synechococcus, Mycobacterium, Enterobacteria, Pseudomonas, Cellulophoga, Vibrio, Burkholderia, Escherichia,* and *Ralstonia* were the top 10 genera. Predominant bacteria in the salmonid fish-farm in Australia included *Sphyngopyxis, Rhodobacter, Nitrospira, Desulfosarcina, Colwellia, Halomonas, Moritella, Oceanospirillium, Photobacterium,* and *Pseudoalteromonas* (Bissett et al., 2005). Eight different
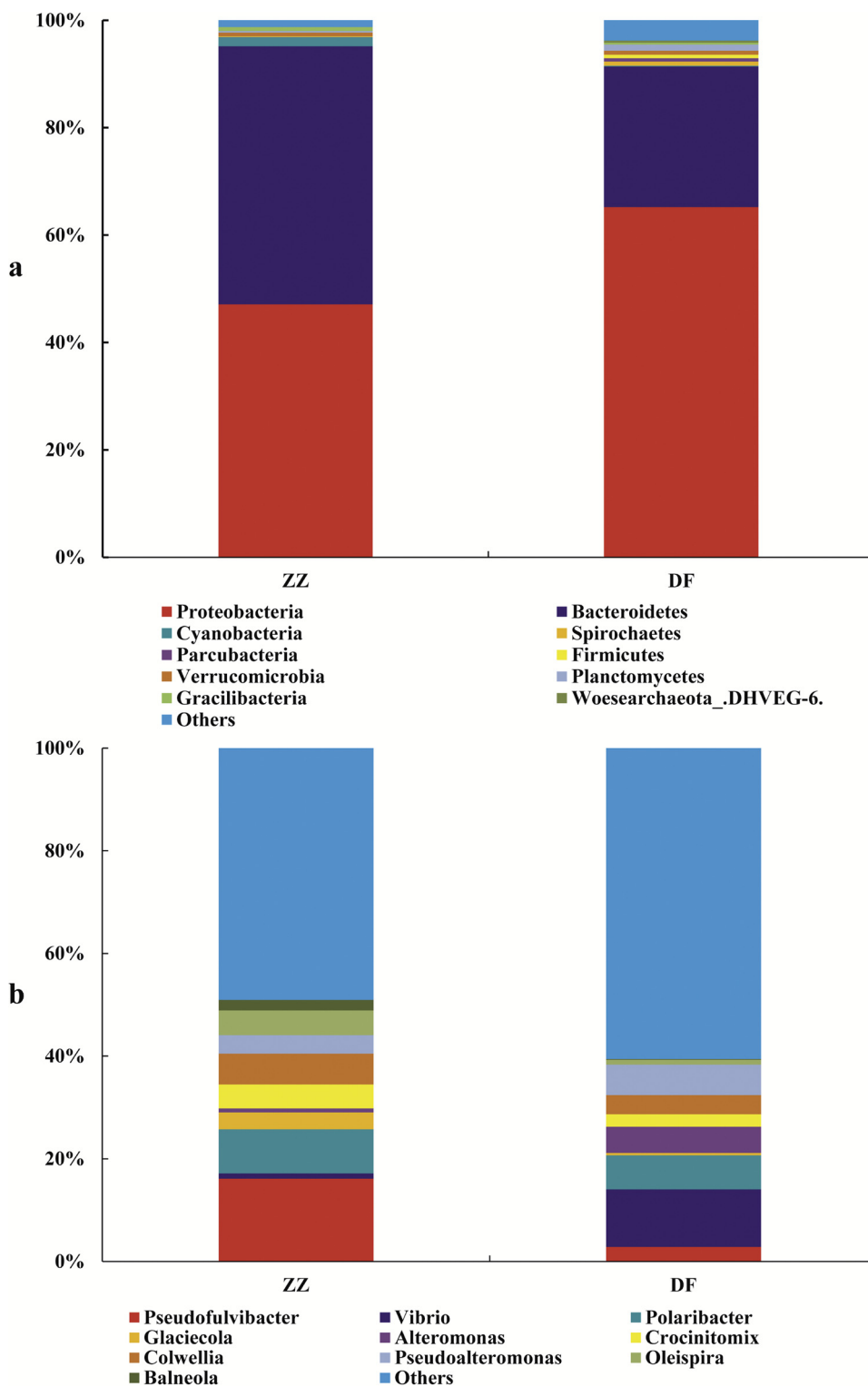
Fig. 6. Bar chart of the top 10 bacteria relative abundance of each group in (a) phylum and (b) genus level. The relative abundance is the percentage of the bacteria in total microorganism. ZZ refers to the conventional mariculture system while DF refers to the recirculating mariculture system.

genera including *Acinetobacter, Aeromonas, Chryseobacterium, Erwinia, Flavobacterium, Pseudomonas* and *Rheinheimera* were identified in the salmonid fish farm in Northern Ireland (Moore et al., 2014). Enger et al. (1989) isolated *Vibrio* from the sediments of fish farm on the western coast of Southern Norway. The bacterial groups also exhibited growth stage-specific features. Relative abundances of *Pseudofulvibacter* and *Crocinitomix* in ZZ3 (Pond for adult *S. maximus*) were higher than those in ZZ4 (Fig. 7a). However, relative abundances of *Polaribacter,*

Glaciecola, Oleispira and *Colwellia* in ZZ4 (Pond for fish-fry *S. maximus*) were higher than those in ZZ3. Zheng et al. (2017) also found that growth stages made effect on the bacterial communities of Pacific White Shrimp (*Litopenaeus vannamei*) in rearing water. *Rhodobacteraceae* was abundant in rearing water at all tested growth stages whereas its relative abundance displayed a decreasing trend at mysis and post-larva stages (Zheng et al., 2017). *Flavobacteriaceae* was abundant at zoea stage compared with that at mysis stage (Zheng et al., 2017). At
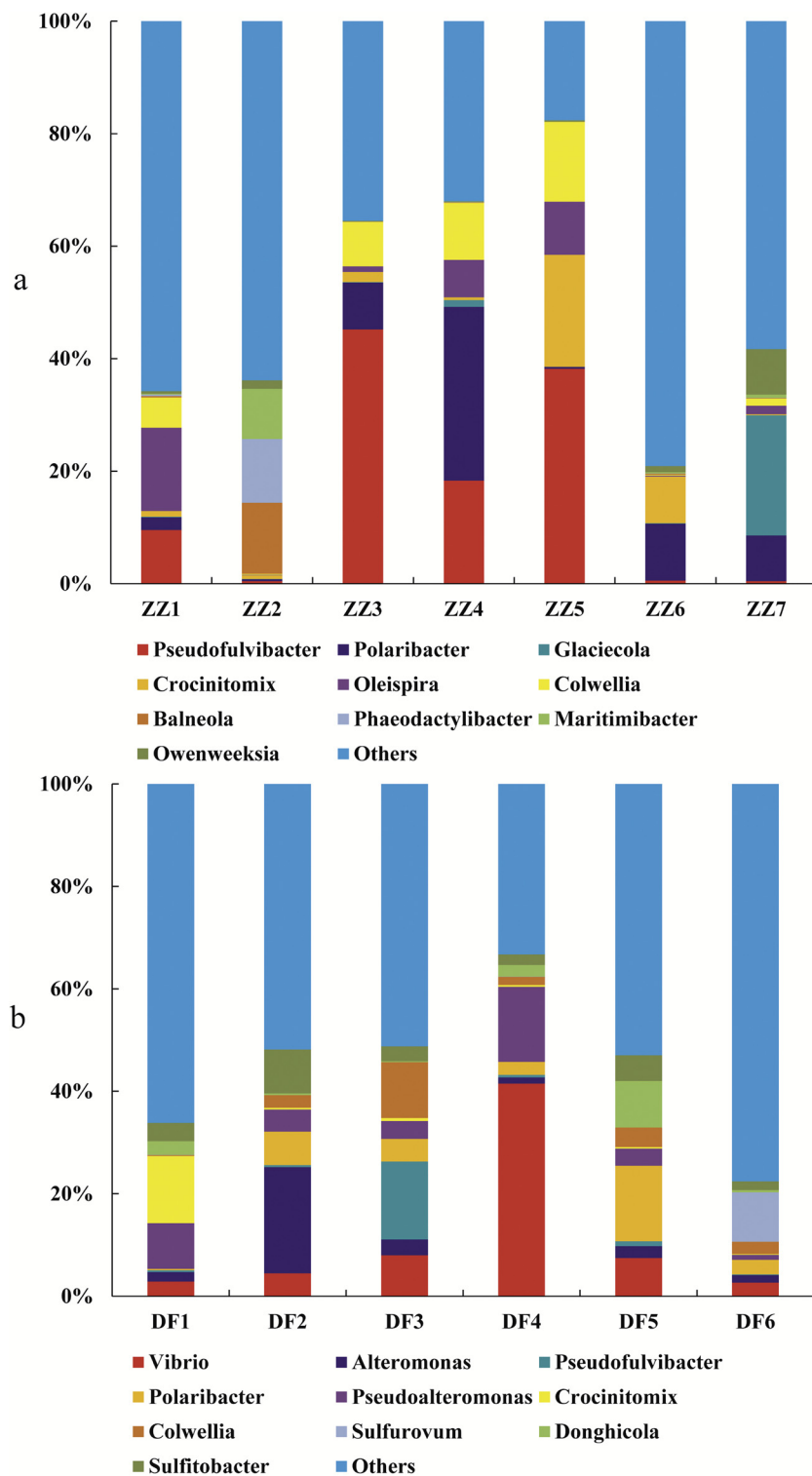
Fig. 7. Bar chart of the top 10 bacteria relative abundance of each sample from ZZ farm (a) and DF farm (b) in genus level. The relative abundance is the percentage of the bacteria in total microorganism. ZZ refers to the conventional mariculture system while DF refers to the recirculating mariculture system. In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water.

postlarva stage, *Microbacteriaceae* (phylum *Actinobacteria*) increased to become the dominant bacterial group (Zheng et al., 2017).

Bacterial communities along with different wastewater treatment stages were analyzed to find out the stage-specific groups. Variation of bacterial community in the samples collected from DF farm was observed along with the wastewater treatment stages (Fig. 7b). The relative abundances of *Vibrio*, *Pseudofulvibacter*, *Crocinitomix* and *Colwellia* in sample DF3 were significantly higher than those in DF2, indicating the accumulation of these bacteria in the protein separation process. However, the relative abundances of *Alteromonas*, *Polaribacter*,

Pseudoalteromonas and *Sulfitobacter* in DF3 were much lower than those in DF2, suggesting that the protein separation process could remove these bacteria efficiently. The relative abundances of *Vibrio*, *Pseudoalteromonas* and *Donghicola* sharply increased in DF4 when compared with samples DF1 and DF3, indicating that these bacteria were accumulated in this biochemical water treatment process. It was found that the relative abundances of *Alteromonas*, *Pseudofulvibacter*, *Polaribacter*, *Colwellia*, *Donghicola* and *Sulfitobacter* accumulated in the aerobic process (DF5) when comparing samples DF5 and DF4. All of the bacteria (TOP 10 shown in Fig. 7b) in DF6 were reduced much in comparison

with DF5, indicating that UV could make removal effect on these bacteria. In addition, in contrast to DF1, it was found that many bacteria such as *Pseudofulvibacter*, *Sulfitobacter*, *Crocinitomix*, *Pseudoalteromonas* and *Donghicola* could be removed efficiently by the treatment processes in DF farm. These results indicated that the bacterial groups exhibited treatment stage-specific features, and the wastewater treatment systems used in the recirculating system (DF farm) could remove bacteria effectively. Disinfection process, such as chlorine disinfection and ultraviolet disinfection, was the main technique among many wastewater treatment methods to eliminate microbes especially bacteria (Shi et al., 2013; Wen et al., 2016). The microbial removal efficiency of other treatment processes including activated sludge process was relatively low (Yang et al., 2014; Wen et al., 2016).

3.4. Potential pathogens in the intensive mariculture systems and coastal waters

Some bacteria detected in fish farms or coastal waters were found as the pathogens such as *Aeromonas*, *Acinetobacter*, *Arcobacter*, *Clostridium*, *Flavobacterium*, *Vibrio*, *Yersinia*, *Pseudoalteromonas*, and *Pseudomonas*, and *Shewanella* (Enger et al., 1989; Viljamaa-Dirks, 2016; Xiong et al., 2015; Lamb et al., 2017). Potential pathogens including *Vibrio*, *Arcobacter*, *Pseudoalteromonas*, and *Shewanella* were detected in the

mariculture systems (Fig. 8). Worst of all, *Vibrio*, *Arcobacter*, *Pseudoalteromonas*, and *Shewanella* were found as the dominant genus in the recirculating mariculture (DF) farm, indicating that potential pathogens could become the majority of microorganisms in the intensive mariculture systems even in the absence of recorded fish diseases. The *Vibrio* spp. was common pathogen which occurred only in sea farms (Viljamaa-Dirks, 2016). It was reported that high abundance of Gamma-proteobacteria presented in diseased shrimps was attributed to *Vibrio* (Rungrassamee et al., 2016). In the mariculture farm of Pacific White Shrimp (*Litopenaeus vannamei*), *Vibrio* was also found as one of the dominant genera in shrimp (Zheng et al., 2017). Another study on Vietnamese shrimp ponds reported that *Vibrio* was a common antibiotic resistance pathogen (Hoa et al., 2008). Xiong et al. (2015) found that fish ponds were reservoirs of pathogens related to human health such as *Acinetobacter*, *Arcobacter*, and *Clostridium*.

Mariculture is a major industry in global coastal zones and has led to concerns about the impact of fish farming on marine environments. In order to evaluate the effect of the mariculture farm on the coastal waters, the microbial community of the sample ZZ7 collected from coastal marine near a lot of fish farms including ZZ and DF farms was investigated. Comparing ZZ7 with samples collected from ZZ and DF farms, the potential pathogens genera such as *Vibrio*, *Arcobacter*, *Pseudoalteromonas*, and *Shewanella* detected in the coastal mariculture
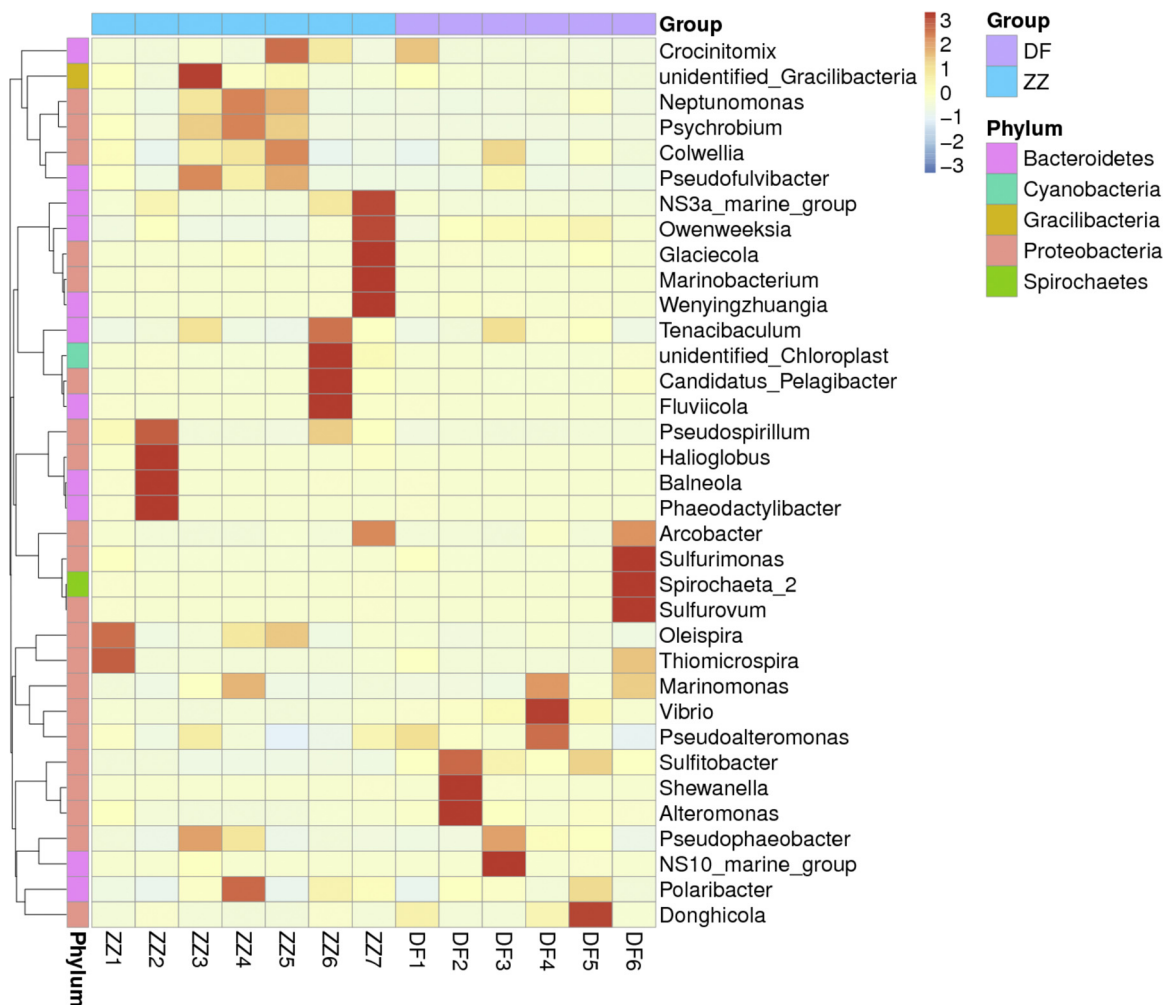

Fig. 8. Clustering heat map of the relative abundance of the top 35 bacteria in genus level of each sample. ZZ refers to the conventional mariculture system while DF refers to the recirculating mariculture system. In the conventional mariculture system (ZZ farm), samples ZZ1, ZZ6, and ZZ7 refer to the influent, the stabilization and coastal water sample, respectively while ZZ2, ZZ3, ZZ4, and ZZ5 refer to the sample of fish pond 1, 2, 3, and 4, respectively. In the recirculating mariculture system (DF farm), samples DF1, DF2, DF3, DF4, DF5, and DF6 refer to the influent, fish pond, protein separator, biochemical pool for wastewater treatment, biological aerobic pool for wastewater advanced treatment, and the recycled water.

systems were also found in the adjacent coastal water (ZZ7) (Fig. 8). Among these potential pathogens, *Arcobacter* was found as a dominant genus in the coastal waters near the mariculture farms (Fig. 8). The comparison results suggested that the discharge of tail water from intensive mariculture system could affect the microbial community of coastal waters and bring potential harmful bacteria (Fig. 8). Harnisz et al. (2015) studied the impact of a freshwater fish farm and found that the fish farm affected water quality and increased the diversity of tetracycline-resistance bacteria in the river near the farm. The detection of potential pathogens suggested that the coastal intensive mariculture systems were the hotspot of pathogens which might exert the potential risks to both human and fish health.

4. Conclusions

The structural profiles of microbial community in the typical conventional and recirculating mariculture farms were investigated using high-throughput sequencing technology. Each farm had its own microbial community characteristics. Among the top 10 genera in coastal intensive mariculture systems, the predominant bacteria in conventional intensive mariculture system were the *Pseudofulvibacter*, *Polaribacter*, *Glaciecola*, *Crocinitomix*, *Colwellia*, *Oleispira* and *Balneola* while those in the recirculating systems were *Vibrio*, *Alteromonas* and *Pseudoalteromonas*. The bacteria community structure in the fish pond relied on the mariculture animals. Potential pathogen such as *Vibrio*, *Arcobacter*, *Pseudoalteromonas*, and *Shewanella* were readily accumulated as dominant bacteria in the recirculating mariculture system (Fig. 8). The detection of similar potential pathogens in the mariculture systems and the adjacent coastal water indicated that the mariculture farms without recirculation system might be the hotspot of pathogens and have great influences on the surrounding coastal waters. The discharge of tail water from mariculture farm had an impact on the microbial community of coastal waters. The wastewater treatment processes used in the recirculating mariculture system displayed a high removal efficiency on bacteria, indicating that the recirculating mariculture system may be more environmentally friendly than the conventional mariculture system.

Acknowledgements

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