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Comparative Analysis of Intestinal Bacterial Communities in Healthy and Diseased *Nibea albiflora*

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ABSTRACT

Background: The gut microbiota is an integral part of the host and plays an important role in both growth and development of host. The research on intestinal microbiota of *Nibea albiflora* and its relationship to fish disease have not been reported before. This study aimed to investigate the composition and differences of gut bacteria between healthy and diseased *Nibea albiflora*.

Methods: The intestines were collected from forty fish (twenty healthy fish and twenty diseased). Total DNA was extracted and then amplified by nested PCR. The PCR product was subjected to the DGGE test and performed at the IlluminaMiseq sequencing.

Result: The obtained results of both utilized techniques (DGGE and Next generation sequencing) showed that dominant bacteria could be grouped into four populations and the composition of intestinal bacteria differed significantly between healthy (NH) and diseased (ND) *Nibea albiflora*. NH has higher levels of γ-Proteobacteria and Firmicutes and with 46.91% *Photobacterium* supplied the dominant genus in NH. Fusobacteria and Bacteroidetes were higher in ND and *Cetobacterium* occupied 62.31% and was the dominant genus in ND. More probiotics were detected in NH, such as *Lactobacillus*, *Brevibacillus*, *Enterococcus and Lactococcus* (occupying 1.77% -19.76%), while less than 0.2% were detected for both in ND. More genera that belonged to Vibrionaceae, such as *Enterovibrio* (9.27%) and *Vibrio* (2.17%), were detected in ND and their abundances in NH were 0.79% and 0.03%, respectively.

Key words: 16S rRNA gene, DGGE, IlluminaMiseq sequencing, Intestinal bacteria, *Nibea albiflora*.

INTRODUCTION

The gut microbiota is an integral part of the fish and plays an important role in both growth and development of the host. Intestinal microbes can synthesize vitamins, essential growth factors *and* digestive enzymes (Nayak 2010). They can also effectively prevent the colonization and amplification of pathogens in fish by rapid growth, reproduction and secretion of antimicrobial substances (Austin 2006). A proper balance exists between endogenous microbiota of the intestine and the control mechanisms of the host. However, if this balance is disturbed, bacteria can increase the susceptibility of the host by damaging the intestinal lining *via* extracellular enzymes or toxins (Ringo *et al*. 2004, Nayak 2010) and consequently, several pathogens present in the transient state can then establish lethal infections. Studies have demonstrated that an apparent difference exists between intestinal microbial composition of diseased and healthy aquatic organisms (Xiong *et al*., 2015; Reveco *et al*. 2014). The variation of intestinal microbiota are closely related to the disease severity of aquatic organisms (Xiong *et al*. 2015). Therefore, deciphering the difference of intestinal microbiota between healthy and diseased fish is of great importance to understand the dynamic functions of this community and how these organisms affect their host's physiology (Ghanbari *et al*. 2015), which also provides clues for the identification of potential probiotics or pathogens in the intestine.

Molecular biotechnologies, such as Denaturing gradient gel electrophoresis (DGGE), fluorescence *in situ* hybridization and cloning library technologies have revealed the dominant and key microbial groups in the environment (Gilbride *et al*. 2006, Wu *et al*. 2010, Li *et al*. 2012). However, only the relative abundance of the predominant flora ranging above 1% of the total biomass can be analyzed (Ben Omar and Ampe 2000). The development of next-generation sequencing (NGS) provides a promising opportunity to further understand environmental microbes. NGS enables both rapid and cost-effective acquisition of substantial sequence data, which allows to theoretically detect all microorganisms in the environment (Sogin *et al*. 2006). During the past few years, these techniques have been applied to analyze the composition and functional properties of numerous fish microbial communities (Ghanbari *et al*. 2015).

Nibea albiflora (yellow drum) belongs to Sciaenidae and *Nibea*, mainly distributed throughout China, the Korean peninsula and the southern coast of Japan; it has high nutritional and medicinal value and is an important economic mariculture fish in China. During recent years, due to environmental degradation and intensive aquiculture, disease of *Nibea albiflora*is becoming increasingly severe.

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At present, the research on *Nibea albiflora* focuses on breeding and genetic diversity and studies of intestinal microbes have not been reported to date (Xu *et al*. 2012, Xie *et al*. 2014).

In this study, DGGE and NGS were used to study bacterial communities of both healthy and diseased *Nibea albiflora*. The objectives of this study was to investigate the gut bacteria composition in *Nibea albiflora* and to compare the differences between gut bacteria of healthy and diseased fish.

MATERIALS AND METHODS

Sample collection

Samples were collected in June 2015 from the cage culture of Sanduao in Ningde City, Fujian Province, China. Healthy fish had normal diet and no obvious disease characteristics. The bellies of diseased fish were bloated, the color of the gills was darker and high mortality occurred. Twenty fish were randomly chosen from either healthy or diseased *Nibea albiflora* (resulting in two treatments with 40 fish), stored at ice boxes and carried to the laboratory. The intestines were aseptically removed from their abdominal cavity and the content of each fish was squeezed out and ten healthy or diseased samples were respectively harvested together. The 40 fish were divided into four intestinal samples: two healthy fish intestinal samples (NH) were labeled NH1 and NH2, while the other two diseased samples (ND) were labeled ND1 and ND2. Thereafter, the samples were centrifuged at 15,000 × g for 5 min and the pellet was collected and remained frozen until DNA extraction.

DNA extraction, PCR amplification and DGGE analysis

Total DNA was extracted using the QIAamp DNA Stool mini-Kit (Qiagen, Valencia, CA, USA). Nested PCR was performed from each sample to amplify the 16S rRNA gene fragment. The first amplification used the primers 27F-1492R (Miller *et al*., 2013) with 12.5 μL reaction containing 3.5 μL DNA, 5 μL 2×PhantaTM Master Mix and 1 μL of each 5 $\mu mol \cdot L^{-1}$ primer. The conditions were as follows: 94°C for 1 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 2 min at 72°C and a final extension step of 10 min at 72°C. The PCR product was used as the second amplification template. The reactions contained 1.2 μL of each 5 $\mu mol \cdot L^{-1}$ primer GM5F-518R (Muyzer *et al*., 1993), 1 μL DNA, 2.5 μL 10×PCR buffer (Mg^{2+}plus), 2 μL of 2.5 $\mu mol \cdot L^{-1}$ dNTPs and 0.2 μL rTaq enzyme (5 U/μL). The conditions were as follows: 94°C for 5 min, followed by 10 cycles of 1 min at 94°C, 1 min at 60°C, touchdown -0.5°C and 2 min at 72°C and then 25 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C and a final extension of the unfinished products for 30 min at 72°C. The PCR product was subjected to the DGGE test and DGGE bands were sequenced after purification.

The DGGE bands were analyzed *via* Quantity One-4.6.2 1-D Analysis Software and the Richness index, Shannon's index and Evenness index were calculated to evaluate the diversity of bacterial community (Zhong *et al*. 2015). The clusters of DGGE profiles were analyzed by Cluster software and the result of sequencing was compared *via* Blastn in the NCBI database to obtain information of species similarity.

IlluminaMiseq sequencing and data analysis

The primers 515F-907R with barcode were used to amplify the bacterial 16S rDNA fragments of V4-V5 (Armitag *et al*., 2012). The PCRs were conducted in 12.5μL reactions with 3.5 μL DNA, 5 μL 2xPhanta TM Master Mix (Vazyme Biotech), 5 μM of each primer and ddH_2O. The amplification program was as follows: 94°C for 2 min; followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, 2 min at 72°C and a final extension of 5 min at 72°C. Pyrosequencing was performed at the IlluminaMiseq sequencing (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China).

Raw sequences were screened and filtered according to standards for quality control. Chimeric sequences were moved *via* UCHIME (Edgar *et al.* 2011). A Venn diagram was analyzed *via* OTU with a similarity level of 97%. Chao, Ace, Coverage, Simpson and Shannon indexes were generated *via* Mothur. The principal component analysis (PCA) of the Fast UniFrac metric matrix was used to compare bacterial communities, based on the phylogenetic information (Lozupone *et al*. 2006). The sequences obtained in this study have been submitted to NCBI under the accession number SRP111146.

RESULTS AND DISCUSSION

PCR-DGGE analysis

The Richness values of four samples (NH1, NH2, ND1 and ND2) were 9, 7, 9 and 8, respectively. Shannon values were 2.13, 1.85, 2.05 and 2.02. Evenness values were 0.97, 0.95, 0.93 and 0.97. The diversity of the microbial community did not significantly differ between ND and NH, analyzed *via* one-way ANOVA. Cluster analysis showed that ND and NH could be divided into two broad categories, with a similarity index of 0.45, indicating that the intestinal bacterial community structure differed between NH and ND. Although the NH and ND were clustered separately, the respective similarity within each cluster (ND or NH) was not high, indicating that the intestinal bacterial compositions of individual *N. albiflora* differed between both groups.

The result of phylogenetic analysis of sequences derived from DGGE bands are listed in the corresponding position of each band (Fig 1). Fifteen sequences were divided into four groups: γ-Proteobacteria, Fusobacteria, Firmicutes and Bacteroidetes. Bacteroidetes, Fusobacteria and γ-Proteobacteria were detected in both NH and ND; however, most of Fusobacteria were found in ND. Although Firmicutes were only detected in the NH1, the brightness was bright, indicating that it was also an important constituent in the healthy fish intestine. At the species level, the Prevotellaceae bacterium DJF_RP84 (B2), Uncultured *Cetobacterium* sp. (B9), *Photobacterium phosphoreum* (B5) and *Photobacterium damselae subsp. Damselae* (B6) were

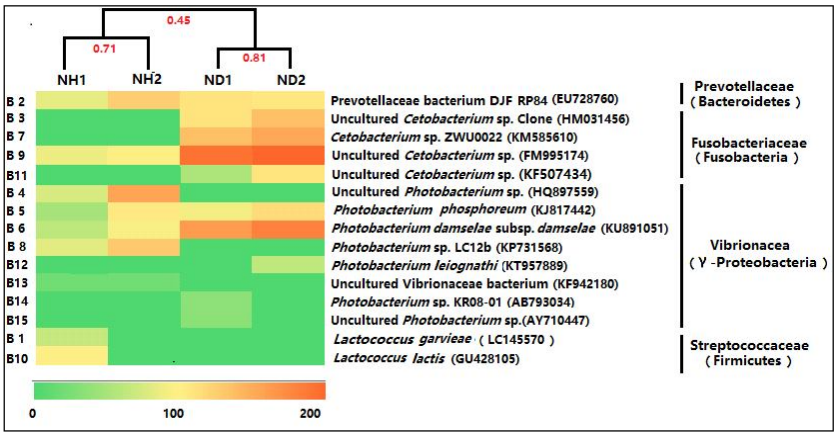

Fig 1: Relative abundance of bacteria in intestines of different *Nibea albiflora*.

common species in both NH and ND; however, the abundance of B5, B6 and B9 of ND were significantly higher than that of NH. Uncultured *Photobacterium* sp. (B4) derived from the gut of the *Atlantic salmon*, *Photobacterium* sp. LC12b (B8) derived from the intestine of *Paralichthys adspersus* and the Uncultured Vibrionaceae bacterium (B13) of the gulf sample were detected in NH, but were absent in ND. The Uncultured *Cetobacterium* sp. Clone (B3) derived from the intestine of *Hexagrammos otakii*, *Cetobacterium* sp. ZWU0022 (B7) derived from the intestine of the *zebra fish* and Uncultured *Cetobacterium* sp. (B11) of a seawater sample were detected in ND, but were absent in NH. In addition to these bacteria, several species of bacteria were only detected in individual samples, for example, *Lactococcus lactis* (B10) and *Lactococcus garvieae* (B1) were only found in NH1, but were absent in other samples. *Photobacterium leiognathi* (B12), derived from the light organ of *Gazzaminuta* was detected in ND2. *Photobacterium* sp. KR08-01 (B14) of the seawater sample and Uncultured *Photobacterium* sp. (B15) of the marsh sample were both detected in ND1.

Analyses of the pyrosequencing-derived dataset

A total of 198441 valid reads (mean 49610±1208 reads) were obtained from four samples *via* Illumina pyrosequencing analysis at a similarity level of 97%. To avoid effects of uneven sampling, 21210 reads were randomly selected from each sample for subsequent sequence analysis. The diversity analysis (Table 1) showed that the coverage of four samples was higher than 99%, indicating that the sequencing depth of the bacterial community in this study tended to saturate. The differences of OTUs, ACE, Chao, Shannon and Simpson values of each sample were significant; however, there no significant difference was found between the NH and ND (P>0.05).

The sequences of four samples were classified via the Silva database. All sequences were divided into six groups and other sparse groups (<1%) were merged into the "Others" group (Fig 2). γ-Proteobacteria was the most dominant group found in *Nibea albiflora* (41.12% of total sequence), while Fusobacteria and Firmicutes were sub-dominant groups (39.37%, 14.94%). Bacteroidetes, β-Proteobacteria and Tenericutes were 2.95%, 1.29% and 0.38% respectively and other groups accounted for 0.4%. The relative abundance of bacterial phyla in the intestinal content differed significantly between NH and ND. In NH, more γ-Proteobacteria was found than in ND (33.13% and 73.77% in NH, while 32.12% and 25.46% in ND). Fusobacteria (53.54% and 71.96% in ND, while 10.73% and 21.26% in NH) and Bacteroidetes (9.5% and 1.04% in ND, while 1.12% and 0.14% in NH) were richer in NH than in ND. Firmicutes were particularly abundant in NH1 (53.29% and 3.20% in NH, while 2.63% and 0.65% in ND). β-Proteobacteria accounted for 3.35% of NH1, while the remaining microbial supplied less than 1%. Tenericutes was at a very low abundance in all samples and only ND1 exceeded 1%, while others were less than 0.2%.

At the genus level, all sequences were divided into 16 groups and the other scarce groups (<1%) were merged into the group "Others". *Cetobacterium* was the most dominant group in *Nibea albiflora* (39.07% of total sequence), *Photobacterium* formed a sub-dominant group

Table 1: Diversity indices of intestinal bacterial communities of *Nibea albiflora*

Samples	Reads	OTUs	ACE	Chao	Coverage (%)	Shannon	Simpson
NH1	50667	65	60	63	99.98	2.30	0.1674
NH2	51147	81	123	92	99.91	1.16	0.5110
ND1	50623	96	93	97	99.96	2.10	0.2717
ND2	46004	27	21	19	99.99	1.38	0.3586

Note: Diversity indices were calculated *via* 21210 resampling sequences per sample.

(31.96%) and the abundances of other groups were below 10% (Fig 3). Many differences could be found between the intestinal bacterial composition of NH and ND. *Cetobacterium* was the most common dominant bacteria in ND (ND1: 52.87%; ND2: 71.74%), but was less prevalent in NH (NH1: 10.71%; NH2: 20.96%). *Photobacterium* was richer in NH (22.18% and 71.63% in NH, while13.22% and 20.83% in ND). *Lactococcus* was only present in NH, particularly in NH1 (NH1, 39.11%; NH2, 0.41%). Other groups, such as *Enterococcus*, *Acinetobacter* and *Lactobacillus* were mainly present in NH (accounting for 0.2%-5.8%) and remained below 0.3% in ND. *Brevibacillus*, unclassified *Pseudomonadales* and *Massilia*were only present in one sample of NH (1.45-4.38%). Other groups such as the Bacteroidales S24-7 group (ND1, 5.29%; ND2, 1.07%) and *Enterovibrio* (ND1, 13.85%; ND2, 4.68%) were more widespread in ND and the proportion of NH was below 1%. *Bacteroides*, *Vibrio, Escherichia-Shigella, Alloprevotella* and *Prevotellaceae*_UCG-001 were only present in one sample of ND with an abundance above 1%, while they were detected at little or no abundance in other samples.

To compare the composition of the intestinal bacterial communities, the principal component analysis was conducted of four samples at the OTU level. PC1 and PC2 accounted for 71.73% and 25.76% of the total variation,

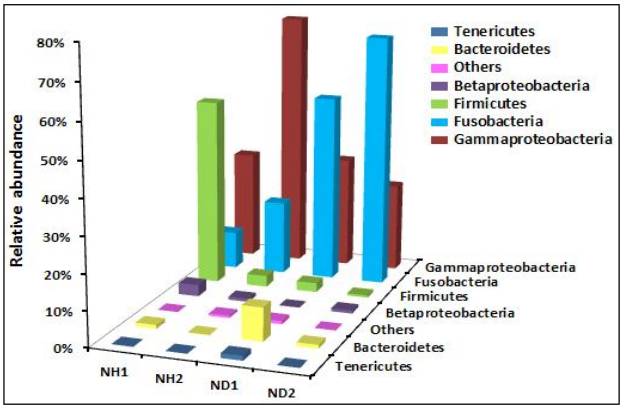

Fig 2: Taxonomic composition of intestinal bacteria of healthy and diseased *Nibea albiflora*.

respectively (Fig 4). Fig 4 shows that two samples of ND clustered together and were distant from the NH1 and NH2 *via* PC1, while NH1 were far from NH2 *via* PC2. This indicates a significant difference between the composition of intestinal bacterial communities of healthy and diseased *Nibea albiflora* and also between individuals of NH. The PCA results at the phyla and genus level were similar.

Venn diagrams were constructed to identify unique and shared OTUs of the four samples. The number of shared OTUs among the four samples were 10 (Fig 5). The shared OTUs mainly belonged to Fusobacteria and γ-Proteobacteria. Furthermore, the corresponding species were as follows: Uncultured or unclassified *Cetobacterium* (50.98% of shared OTUs of four samples), *Photobacterium kishitanii* (29.54%), unclassified *Photobacterium* sp. (10.96%), unclassified *Enterovibrio* (6.55%) and unclassidied *Vibrio* (1.40%), while the remaining accounted for less than 1%. Some OTUs were only present in NH or ND. Nine OTUs were only detected in NH and mainly belonged to Firmicutes and Proteobacteria. Corresponding species were as follows: Unclassified *Lactococcus* (59.64%), *Lactococcus garvieae* ATCC49156 (18.58%), unclassified *Enterococcus* (12.32%), unclassified *Lactobacillus* (6.64%), unclassified *Vagococcus* (1.17%), *Cupriavidus basilensis* (0.94%), unclassified *Photobacterium* (0.32%), *Photobacterium kishitanii* (0.37%) and unclassified *Cupriavidus* (0.01%). The two OTUs uncultured Bacteroidales (99.76%) and unclassified *Myroides* (0.24%), were only present in ND.

This study utilized PCR-DGGE and Illumina Miseq sequencing to detect the V3 and V4-V5 of the 16S rRNA of four samples, thus investigating the intestinal bacterial composition of healthy and diseased *Nibea albiflora*. As far as we know, this is the first such report for *Nibea albiflora.*

The intestinal content samples were dominated by four major phyla: γ-Proteobacteria, Fusobacteria, Firmicutes and Bacteroidetes, which have often been detected in the intestine of fish, such as *rainbow trout*, *grass carp* and *Atlantic cod* (Ringo *et al*. 2006, Desai *et al*. 2012, Li *et al*. 2013) and thus these belong to the core intestinal microbiota of fish (Wu *et al*. 2012, Wong *et al*. 2013). γ-Proteobacteria and Fusobacteria were also dominant bacteria of *zebra fish*

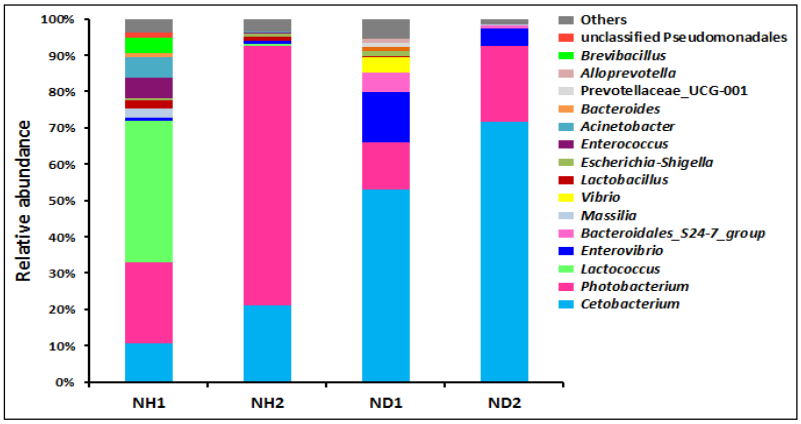

Fig 3: Intestinal bacterial composition at genus level of health and diseased *Nibea albiflora*.

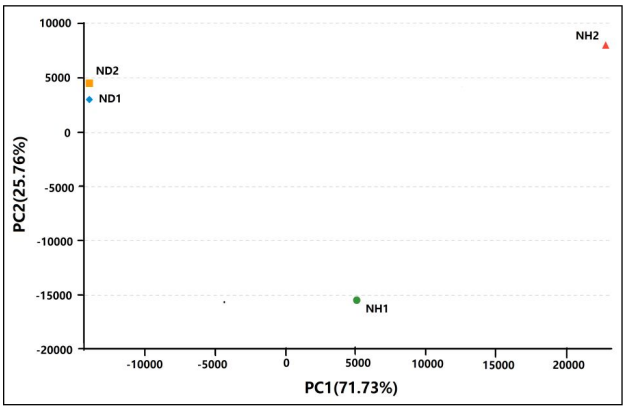

Fig 4: Principal component analysis (PCA) of the intestinal contents of the NH or ND individuals of *Nibea albiflora*. The percentages indicate the relative contribution of the two principal components (PC1-PC2).

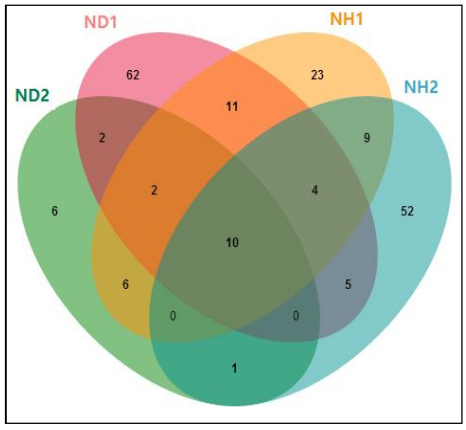

Fig 5: Venn diagram of unique and shared OTUs in different libraries for NH1, NH2, ND1 and ND2.

and *bighead carp* (Roeselers *et al*. 2011, Li *et al*. 2015). Previous studies indicated that Firmicutes and Bacteroidetes were dominant bacteria of both *Asian seabass* and *grass carp* (Wu *et al*. 2012, Xia *et al*. 2014). However, in this study, Firmicutes and Bacteroidetes were abundant in NH1 and ND1, respectively, while in other samples, Firmicutes and Bacteroidetes were not abundant. At the genus level, this study not only found several common taxa for the fish intestine (such as *Cetobacterium* and *Photobacterium*), but also detected several low abundance bacteria, such as *Massili, Escherichia-Shigella, Alloprevotella* (Nayak 2010).

Both of the DGGE clustering and PCA analysis of IlluminaMiseq sequencing showed obvious differences of intestinal microbiota between NH and ND within the same environment. At the phyla level, γ-Proteobacteria and Firmicutes were richer in NH, while Fusobacteria and Bacteroidetes were richer in ND. The bacteria belonging to Fusobacteria all were classified as uncultured or unclassfied bacteria of *Cetobacterium* and were more abundant in ND than in NH. Previous research has shown that *Cetobacterium* can produce vitamin B12, ferment peptides and carbohydrates (Li *et al*. 2015). Furthermore, *Cetobacterium* has been detected in many fish, such as the *zebra fish* (Stephens *et al*., 2016) and *Hexagrammos otakii* (GenBank description, accession number HM031456). About 75.66% of the bacteria of γ-Proteobacteria belonged to *Photobacterium* and were more common in NH than in ND. These bacteria were most similar to the uncultured clone sequence or strains isolated from the light organs of Leiognathidae fish (Boisvert *et al*., 1967). In addition to *Photobacterium*, two members of γ-Proteobacteria, *Enterovibrio* and *Vibrio*, were detected at higher abundance and more of them were found in ND compared to NH. Many marine vibrio are common aquatic pathogens and under the influence of extrensic factors (such as environmental pollution), they can lead to surface discoloration of fish and bleeding or inflammation of the liver, kidney, spleen and intestine of fish.

The abundance of Firmicutes was significantly higher in NH (94.59%) than in ND (5.41%). At the genus level, *Brevibacillus, Lactobacillus, Lactococcus*and *Enterococcus* supplied more than 1% in NH, but less than 0.3% in ND. At the OTU level, the bacteria that were only detected in NH and that had an abundance of more than 5% were the unclassfied *Enterococcus, Lactobacillus* and *Lactococcus,* which are commonly used probiotics (Nayak 2010). A previous study has shown that probiotics (*Lactobacillus, Streptococcus* and *Lactococcus*) provide disease prevention and immune repair for the host (Balcazar *et al*. 2006). Adding Brevibacillus to the feed can improve the activity of fish digestive enzymes (Shen *et al*. 2010). *Enterococcus faecalis* provides a good immune response to *Vibrio anguillarum* (Estrada *et al*. 2009). More probiotic bacteria that were detected in NH may be concerened with the healthy status of the *Nibea albiflora*. The abundance of Bacteroidetes was richer in ND than in NH. However, the Bacteroidetes found in this study were basically uncultured or unclassified bacteria and consequently, the relationship between these unknown species and the health status of *Nibea albiflora* requires further study.

CONCLUSION

In summary, a comprehensive understanding of composition changes of intestinal bacterial communities in both healthy and diseased *Nibea albiflora* plays an important role for the promotion of a healthy and sustainable development of the artificial breeding industry. The results of both DGGE and NGS technologies regarding the composition and difference of intestinal microbiota between NH and ND were similar. Although, DGGE can only detect the bacteria that more than 1% of the total biomass, this technique also reflected the dominant group and main differences of intestinal microbes between NH and ND. To reduce the difference of intestinal microbes among different individuals, each intestinal sample utilized in this experiment was a mixture of 10 individuals; however, the difference among samples was still considerable.

Subsequent studies should increase the number of samples, isolates and culture the differentiated microbial populations to conduct functional studies, that would better reveal the relationship between intestinal microbiota and the health status of the fish host.

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REFERENCES

Armitage, D.W., Gallagher, K.L., Youngblut, N.D., Buckley, D.H. and Zinder, S.H. (2012). Millimeter-scale patterns of phylogenetic and trait diversity in a salt marsh microbial mat. Frontiers in Microbiology. 3: 293.

Austin, B. (2006). The bacterial microflora of fish, revised. The Scientific World Journal. 6: 931-945.

Balcazar, J.L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Muzquiz, J.L. (2006). The role of probiotics in aquaculture. Veterinary Microbiology. 114: 173-186.

Ben Omar, N. and Ampe, F. (2000). Microbial community dynamics during production of the Mexican fermented maize dough pozol. Applied Environmental Microbiology. 66: 3664-3673.

Boisvert, H., Chatelain, R. and Bassot, J.M. (1967). Study on a photobacterium isolated from the light organ of the leiognathidae fish. Annales De Linstitut Pasteur. 112(4): 521.

Desai, A.R., Links, M.G., Collins, S.A., Mansfield, G.S., Drew, M.D., Van Kessel, A.G. and Hill, J.E. (2012). Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 350: 134-142.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27: 2194-2200.

Estrada, U.R., Satoh, S., Haga, Y., Fushimi, H. and Sweetman, J. (2009). Effects of single and combined supplementation of *Enterococcus faecalis*, mannan oligosaccharide and polyhydroxybutyrate acid on growth performance and immune response of rainbow trout *Oncorhynchus mykiss*. Aquaculture Science. 57: 609-617.

Ghanbari, M., Kneifel, W. and Domig, K.J. (2015). A new view of the fish gut microbiome: Advances from next-generation sequencing. Aquaculture. 448: 464-475.

Gilbride, K.A., Lee, D.Y. and Beaudette, L.A. (2006). Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens and real-time process control. Journal of Microbiological Methods. 66: 1-20.

Li, S., Sun, L., Wu, H., Hu, Z., Liu, W., Li, Y. and Wen, X. (2012). The intestinal microbial diversity in mud crab (*Scylla paramamosain*) as determined by PCR-DGGE and clone library analysis. Journal of Applied Microbiology. 113: 1341-1351.

Li, T., Long, M., Gatesoupe, F., Zhang, Q., Li, A. and Gong, X. (2015). Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. Microbial Ecology. 69: 25-36.

Li, X., Yan, Q., Xie, S., Hu, W., Yu, Y. and Hu, Z. (2013). Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio L.*). PLoS One. 8: e64577.

Lozupone, C., Hamady, M. and Knight, R. (2006). UniFrac - An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics. 7: 371.

Miller, C.S., Handley, K.M., Wrighton, K.C., Frischkorn, K.R., Thomas, B.C. and Banfield, J.F. (2013). Short-read assembly of full-length 16S amplicons reveals bacterial diversity in subsurface sediments. PLoS One. 8: e56018.

Muyzer, G., de Waal, E.C. and Uitterlinden, A.G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied Environmental Microbiology. 59: 695-700.

Nayak, S.K. (2010). Role of gastrointestinal microbiota in fish. Aquaculture Research. 41: 1553-1573.

Reveco, F.E., Overland, M., Romarheim, O.H. and Mydland, L.T. (2014). Intestinal bacterial community structure differs between healthy and inflamed intestines in Atlantic salmon (*Salmo salar* L.). Aquaculture. 420: 262-269.

Ringo, E., Jutfelt, F., Kanapathippillai, P., Bakken, Y., Sundell, K., Glette, J., Mayhew, T.M., Myklebust, R. and Olsen, R.E. (2004). Damaging effect of the fish pathogen *Aeromonas salmonicida* ssp salmonicida on intestinal enterocytes of Atlantic salmon (*Salmo salar* L.). Cell and Tissue Research. 318: 305-312.

Ringo, E., Sperstad, S., Myklebust, R., Refstie, S. and Krogdahl, A. (2006). Characterisation of the microbiota associated with intestine of Atlantic cod (*Gadus morhua* L.) - The effect of fish meal, standard soybean meal and a bioprocessed soybean meal. Aquaculture. 261: 829-841.

Roeselers, G., Mittge, E.K., Stephens, W.Z., Parichy, D.M., Cavanaugh, C.M., Guillemin, K. and Rawls, J.F. (2011). Evidence for a core gut microbiota in the zebrafish. The ISME Journal. 5: 1595-1608.

Shen, W., Fu, L., Li, W. and Zhu, Y. (2010). Effect of dietary supplementation with Bacillus subtilis on the growth, performance, immune response and antioxidant activities of the shrimp (*Litopenaeus vannamei*). Aquaculture Research. 41: 1691-1698.

Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch. D., Huse, S.M., Neal, P.R., Arrieta, J.M. and Herndl, G.J. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of National academy of Science of the United States of America. 103: 12115-12120.

Stephens, W., Burns, A., Stagaman, K., Sandi, W., Rawls, J., Guillemin, K. *and* Bohannan, B. (2016). The composition of the zebrafish intestinal microbial community varies across development. The ISME Journal. 10: 644-654.

Wong, S., Waldrop, T., Summerfelt, S., Davidson, J., Barrows, F., Kenney, P.B., Welch, T., Wiens, G.D., Snekvik, K., Rawls, J.F. and Good, C. (2013). Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. Applied Environmental Microbiology. 79: 4974-4984.

Wu, S., Gao, T., Zheng, Y., Wang, W., Cheng, Y. and Wang, G. (2010). Microbial diversity of intestinal contents and mucus in yellow catfish (*Pelteobagrus fulvidraco*). Aquaculture. 303: 1-7.

Wu, S., Wang, G., Angert, E.R., Wang, W., Li, W. and Zou, H. (2012). Composition, diversity and origin of the bacterial community in grass carp intestine. PLoS One. 7: e30440.

Xia, J.H., Lin, G., Fu, G.H., Wan, Z.Y., Lee, M., Wang, L., Liu, X.J. and Yue, G.H. (2014). The intestinal microbiome of fish under starvation. BMC Genomics. 15: 266.

Xie, Z., Wang, F., Liu, H., Guo, S., Shi, H., Zhan, W. and Lou, B. (2014). Effect of dietary taurine levels on growth performance and taurine content of *Nibea albiflora* larvae. Aquaculture International. 22: 1851-1862.

Xiong, J., Wang, K., Wu, J., Qiuqian, L., Yang, K., Qian, Y. and Zhang, D. (2015). Changes in intestinal bacterial communities are closely associated with shrimp disease severity. Applied Microbiology and Biotechnology. 99: 6911-6919.

Xu, D., Lou, B., Shi, H., Geng, Z., Li, S. and Zhang, Y. (2012). Genetic diversity and population structure of *Nibea albiflora* in the China Sea revealed by mitochondrial COI sequences. Biochemical Systematics and Ecology. 45: 158-165.

Zhong, F., Wu, J., Dai, Y., Yang, L., Zhang, Z., Cheng, S. and Zhang, Q. (2015). Bacterial community analysis by PCR-DGGE and 454-pyrosequencing of horizontal subsurface flow constructed wetlands with front aeration. Applied Microbiology and Biotechnology. 99: 1499-1512.